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Research paper

Acaricidal efficacy of deltamethrin-zinc oxide nanocomposite on *Rhipicephalus* (*Boophilus*) *annulatus* tick

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ABSTRACT

In the veterinary field, there is a great concern about the issue of acaricides resistance in cattle ticks. A comparative study was designed to evaluate the *in vitro* acaricidal efficacy of imidacloprid nano-emulsion, zinc oxide nanoparticles (ZnO NPs), silver nanoparticles (Ag NPs), deltamethrin, deltamethrin-ZnO NPs, and deltamethrin-Ag NPs. In addition, the novel formulations of deltamethrin (deltamethrin-ZnO NPs and deltamethrin-Ag NPs) against *Rhipicephalus* (*Boophilus*) *annulatus* were also evaluated by *in vivo* animal efficacy trials. Nanoparticles and their loaded forms, deltamethrin-Ag NPs and deltamethrin-ZnO NPs, were characterized by Transmission Electron Microscope (TEM) and Fourier-Transform Infrared Spectrum (FTIR). The adulticidal activity of deltamethrin-ZnO NPs at different concentrations; 2 mL/L, 1 mL/L, 0.5 mL/L and 0.25 mL/L induced a significant *($P \leq 0.05$)* lethal effect on adult ticks compared to deltamethrin-Ag NPs at the same concentrations. The larvicidal efficacy of deltamethrin-ZnO NPs resulted in a complete larval mortality within 24 h of exposure, while deltamethrin and deltamethrin-Ag NPs exhibited 100% immobility of larvae 48 h post-exposure. Furthermore, the *in vivo* experiments showed a considerable reduction in the tick's survival after using deltamethrin-ZnO NPs. There was no significant effect of different treatments on liver or kidney function tests at pre- and post-treatment of animals. In conclusion, deltamethrin-ZnO nanocomposite was the most effective adulticide and larvicide against *R.* (*B.*) *annulatus*. To the best of our knowledge, this is the first report for using deltamethrin-ZnO NPs as an acaricide.

1. Introduction

Ticks cause substantial losses in cattle production; reduced productivity, fertility and often death as well as being one of the most important vectors of infectious diseases of livestock (Eyo et al., 2014; Lorusso et al., 2013). A wide range of pathogenic bacteria can be transmitted by ticks making them one of the most important vectors of diseases affecting animals (Yiwombe, 2013). Ticks also transmit different protozoal infection, *Babesia bigemina*, *Babesia bovis* and *Anaplasma marginale,* that result in a drastic economic loss of livestock (De Campos-Pereira et al., 2008).

The resistance of ticks to different acaricides has spread dramatically. The intensive use and/or abuse of chemical acaricides play an essential role in the development of resistance in ticks (Rodríguez-Vivas et al., 2006). Accordingly, current acaricide control programs recommend continuous changing of the acaricides used for tick control periodically to avoid the emergence of resistant strains (Khalaf-Allah, 1996). The synthetic pyrethroids, deltamethrin and flumethrin constitute the most predominant insecticides used for tick control (Mathivathani et al., 2011; Sharma et al., 2012). Recently, resistant *R.* (*B.*) *annulatus* tick populations were reported in two localities in Beni-Suef province, Egypt (Aboelhadid et al., 2018). The mode of action of pyrethroids is through the interaction with the sodium channel thus preventing its transition from an active to an inactive state (Davies et al., 2007). As a result, the excitable cell membranes of tick become persistently depolarized with subsequent paralysis and death (Field et al., 2017). Meanwhile, imidacloprid, a neonicotinoid insecticide, recommended for insect control (Cox, 2001), acts selectively on the insect nervous system by blocking postsynaptic acetylcholine receptors (Matsuda et al., 2001; Tomizawa and Casida, 2005). Its selective toxicity results from its high affinity to the insect nicotinic acetylcholine receptors (Zhang et al., 2000).

In the veterinary field, the effective control strategy of *R.* (B.) *annulatus* is still representing a major challenge to cattle production.

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Novel acaricides from herbal products and green synthesized nanoparticles are considered promising safe agents to control ticks in livestock (Banumathi et al., 2018). Moreover, the use of metal, metal oxide and nanoparticles such as carbon, zinc oxide, and silver improved the efficacy of acaricides against a wide variety of arthropod pests and vectors (Avinash et al., 2017; Benelli et al., 2017).

Recent studies are geared toward the evaluation of the possibility of using mixtures of more than one acaricide in a synergistic formulation to control resistant *R.* (B.) *annulatus* ticks. Furthermore, a combination of acaricides could enhance the performance of certain classes of acaricides against ectoparasites with a subsequent increased in its efficacy (Barré et al., 2008; Rodríguez-Vivas et al., 2013).

Therefore, the objective of the current study was to evaluate the *in vitro* efficacy of imidacloprid nano-emulsion and novel formulations of deltamethrin, loaded on both Ag NPs and ZnO NPs, on the larvae and adults of *R. (B) annulatus,* as a new method to control acaricide-resistant ticks. Furthermore, *in vivo* evaluation of the acaricidal activity of these formulations and their effects on the animal health was also screened.

2. Material and methods

2.1. Study area and tick collection

Cattle herds of small dairy farms located in Beni-Suef province, Egypt were used in this study. The animals were naturally infested with ticks of a previous history of acaricide resistance. Moreover, animals received acaricides within 14 days, were not used for the tick collection. Animals treated previously with the macrocyclic lactones and any other acaricides were not used for the study. The experiments were conducted from October 2017 until July 2018. Tick samples were collected from naturally infested cattle and identified under a stereomicroscope. The collected ticks were washed with distilled water and dried prior to use.

2.2. In vitro acaricidal efficacy against Rhipicephalus (Boophilus) annulatus

2.2.1. Acaricides

Formulated deltamethrin (Butox®, EC; 5% active ingredient, Arab Company for Chemical Ind.) and imidacloprid (CAS No. 138261-41–3, Jiangzoue Agrostar Company, China) were used for the synthesis of the nanocomposites. A 35% stock solution of imidacloprid nano-emulsion was prepared by Cairo Company for Chemicals Inc., Egypt. A 10% imidacloprid nano-emulsion (w/w) was used to evaluate the acaricidal activity. The acaricidal efficacy of deltamethrin, imidacloprid nano-emulsion, metal nanoparticles (silver 400 mg/L and zinc oxide 8gm/L) and its loaded forms (deltamethrin-ZnO NPs and deltamethrin-Ag NPs) were evaluated against larvae and adult ticks. The different treatments of nanocomposite (deltamethrin-ZnO NPs and deltamethrin-Ag NPs) were tested at different concentrations (0.25, 0.5, 1.0 and 2.0 mL/L).

2.2.2. Synthesis and characterization of nanoparticles and different nanocomposites

Silver nanoparticles (Ag NPs) were synthesized according to Šileikaitė et al. (2009) using chemical reduction method. Meanwhile, zinc oxide nanoparticles (ZnO NPs) were synthesized by the hydrothermal method as described by Aneesh et al. (2007). Both Ag NPs and ZnO NPs were characterized by Fourier-Transform Infrared Spectrum (FTIR) and Transmission Electron Microscope (TEM) (JEOL-JEM-100CX II) in National Research Center (NRC), Egypt. Meanwhile, imidacloprid was synthesized using the nano-emulsion method described by Wang et al. (2009). This method depended on the diffusion of molecules of the imidacloprid (disperse phase) on different sizes of the big droplets, with continuous mixing under pressure, and a lower concentration of the used surfactant (olive oil). NPs were characterized by using their Zeta Potential to show the hydrodynamic diameter of imidacloprid.

Deltamethrin loaded on both Ag NPs and ZnO NPs capping agent was prepared as described by Ahmed et al. (2016) with some modifications. Briefly, deltamethrin concentrations (0.25, 0.5, 1.0 and 2.0 mL/L) were added to 400 mg/L of Ag NPs. The same doses of deltamethrin added to ZnO NPs (8gm/L). In order to avoid agglomeration of nanoparticles, all formulations were shaken continuously for 4 h using magnetic stirrer, then centrifuged at 3000 rpm for 30 min and washed with distilled water several times. These formulations were characterized by both FTIR spectrum and TEM.

2.2.3. In-vitro bioassays

2.2.3.1. Adult immersion test. Engorged female ticks were immersed in 10 mL of each concentration for 2 min, then dried and incubated under Biochemical Oxygen Demand (BOD) at 26–28 °C and 80% relative humidity in Petri dishes. Five replicates, 10 ticks per replicate, for each tested concentration were used. The control group was treated by immersion of the ticks in 10 mL distilled water for 2 min. All the biological parameters including females' weight, egg mass, and egg hatchability were recorded for each treatment as described by Bennett (1974). The index of egg laying (IE) and percent inhibition of fecundity (IF) were calculated (FAO, 2004). Death of female ticks was confirmed by black coloration of the cuticle, complete immobility even by stimulation *via* blowing and absence of egg mass deposition.

2.2.3.2. Larval packet test. Seven to Fourteen-day old larvae were subjected to the different treatments described above. Briefly, a filter paper was placed in a Petri dish prior to addition of 1 mL of the prepared treatment suspensions, then the impregnated papers were allowed to dry. The treated papers were folded to form a packet and approximately 100 larvae were transferred in the packet by using a brush. Packets were subsequently sealed using bulldog clips. The treated packets were kept in BOD at 26–28 °C and 80% relative humidity under observation for 7 days. In the control group, the filter paper was impregnated with 1 mL distilled water (Pirali-Kheirabdi et al., 2007). Three replicates of each treatment were conducted. Death of larvae was confirmed by its complete immobility after examination under the stereo-microscope.

2.3. In vivo acaricidal efficacy of different treatments against Rhipicephalus (Boophilus) annulatus

2.3.1. Animals and counting of ticks

In vivo trials were conducted on a total of 18 tick-infested-cattle of Baladi-Holstein cross breed in a small dairy cattle farm. The naturally infested animals were randomly assigned to three groups (6 animals/group). Animals were kept in partially sheltered yards throughout the study period of the trial. Ticks were collected and identified under a stereomicroscope. The half body tick counts were doubled to obtain the whole-body tick burden according to Walker (2003).

2.3.2. Assessment of acaricidal efficacy

Animals that were infested with at least 100 ticks were separated in groups and treated with deltamethrin (1 mL/L), deltamethrin-ZnO NPs (0.5 mL/L) and deltamethrin-Ag NPs (1 mL/L), for groups 1–3 respectively. Animals were sprayed with the different treatments twice at 2 weeks intervals. The animals in each group were examined for counting and collection of ticks at 0, 3, 7, 10, 21 days post-treatment. The collected ticks were incubated in the laboratory for checking its reproductive indices and vitality. The eggs deposited from the treated ticks were collected, weighed, and incubated for monitoring the egg hatchability. The efficacy of different acaricides was calculated as follow: Efficacy (%) = 100 × (TC pre – TC post) / TC pre, where TC pre is the mean number of live ticks on cattle before the treatment, and TC post is the mean number of live ticks on cattle in the treated groups.

2.3.3. Evaluation of *in vivo* toxicity

Liver and kidney function tests viz., alanine aminotransferase (ALT), aspartate aminotransferase (AST), blood urea nitrogen and creatinine were measured to evaluate the possible *in-vivo* toxicity. Blood samples were collected from the treated animals in each group (0, 3, 7, 10, and 21 days post-treatment). Both ALT and AST were measured according to Reitman and Frankel (1957). Meanwhile, blood urea nitrogen (BUN) and creatinine were evaluated using standard diagnostic kits and analytical grade reagents [Biosystems Egyptian Company for biotechnology, Egypt] according to manufacturer's instructions (Uboh et al., 2012). The tested parameters were measured by using a Konelab20-fully automated biochemical analyzer (Thermo Scientific, Japan).

2.4. Statistical analysis

The data were subjected to one-way analysis of variance (ANOVA) test, accompanied by Duncan multiple range test using Statistical Package for Social Science (SPSS for Windows (IBM), version 22; SPSS Inc., Chicago, Illinois, USA). A probability $\leq$ *0.05* was considered statistically significant in all tests.

3. Results

3.1. Characterization of nanoparticles and different nanocomposites by TEM and FTIR spectrum

The TEM photograph of Ag NPs clarified the morphological shape and diameter of nanoparticles (Fig. 1a–b). The Ag NPs particles appeared spherical, and the diameter of such particles ranged from 36.3–48.5 nm. On the other hand, ZnO NPs in TEM photograph showed hexagonal and rectangular nanoparticles, while the size of such NPs ranged from 72.6–95.4 nm (Fig. 2a–b). Deltamethrin-Ag NPs exhibited a spherical shape with sizes that ranged from 27.2–55.3 nm (Fig. 3). Finally, the TEM image of deltamethrin-ZnO NPs showed a hexagonal and elongated shape, with diameter ranging from 65.8–90.1 nm (Fig. 4).

The FTIR spectrum of silver nanoparticles revealed characteristic peaks of Ag NPs at 1625 and 1638 cm^{-1}. The strong broad band observed at 3470 cm^{-1} was related to the stretching vibrations of the H-bond of the OH group (ν O−H) in all nanostructures. Moreover, the FTIR spectrum of deltamethrin exhibited the fingerprint region between 600 and 2200 cm^{-1} while that of deltamethrin-Ag NPs was revealed at 2480 cm-1 (Fig. 5). The FTIR spectrum of the ZnO NPs was acquired in the range of 400–4000 cm^{-1}. The band from 450-500 cm^{-1} was correlated to the metal oxide bond (ZnO). From the FTIR spectra, it was observed that the peaks in the range of 1400-1500 cm^{-1} correspond to the C=O bonds. Meanwhile, the new peak of deltamethrin-ZnO nanocomposite that appeared at 2300 cm^{-1} indicated the successful loading of deltamethrin on ZnO NPs (Fig. 6). The FTIR spectrum of imidacloprid nano-emulsion showed peaks in an area ranging from 1577 to 1567 cm^{-1}. Moreover, its characteristic peak at 1900 cm^{-1} and a broad peak at 3400 cm^{-1} were observed as shown in (Fig. 7).

3.2. *In-vitro* acaricidal efficacy of different treatments against adult *Rhipicephalus (Boophilus) annulatus* ticks

3.2.1. Adult immersion test

The adulticidal efficacy of the different commercial deltamethrin concentrations; 0.25 mL/L, 0.5 mL/L, 1 mL/L, and 2 mL/L were 53.3, 60, 60, and 66.6%, respectively. There was no significant difference noticed with increasing the deltamethrin dosage. The adulticidal efficacy of deltamethrin-Ag NPs at different concentrations; 0.25 mL/L, 0.5 mL/L, 1 mL/L, and 2 mL/L were 50, 60, 60 and 70%, respectively. There was a significant difference *($P \leq 0.05$)* noticed only at the highest concentration (2 m/L). On the other hand, treatment with deltamethrin-ZnO NPs at the same concentrations exhibited 100% mortality of adult ticks. In contrast, the adulticidal efficacies of imidacloprid nano-emulsion (100 mg/mL), ZnO NPs (8gm/L), and Ag NPs (400 mg/L) were non-significant *($P > 0.05$)* when compared with the control (Table 1). The egg mass per females was significantly reduced to 0% by deltamethrin treatment at ≥0.5 mL/L, while at 0.25 mL/L about 130 mg eggs per 10 females were recorded. Groups treated with deltamethrin-Ag NPs exhibited similar findings of ticks treated with deltamethrin. However, no eggs were noticed after treatment with any concentrations of deltamethrin-ZnO. On the contrary, there were no significant differences *($P > 0.05$)* in the egg mass laid by ticks treated with ZnO NPs at 8gm/L, Ag NPs at 400 mg/L, and imidacloprid nano-emulsion at 100 mg/mL when compared with the control (Table 1). Moreover, egg masses collected from ticks treated with the different chemicals hatched.

3.2.2. Larval packet test

The larvicidal efficacy of the variable deltamethrin treatments was significantly high *($P \leq 0.05$)*. Deltamethrin alone or loaded on Ag NPs showed 100% mortality of larvae after 48 h of treatment. Moreover, deltamethrin-ZnO NPs treated larvae showed a complete immobility within 24 h of treatment. On the contrary, non-deltamethrin treatments (imidacloprid nano-emulsion 100 mg/mL, ZnO NPs 8gm/L, and Ag NPs 400 mg/L groups) exhibited no significant *($P > 0.05$)* difference when compared to the control.

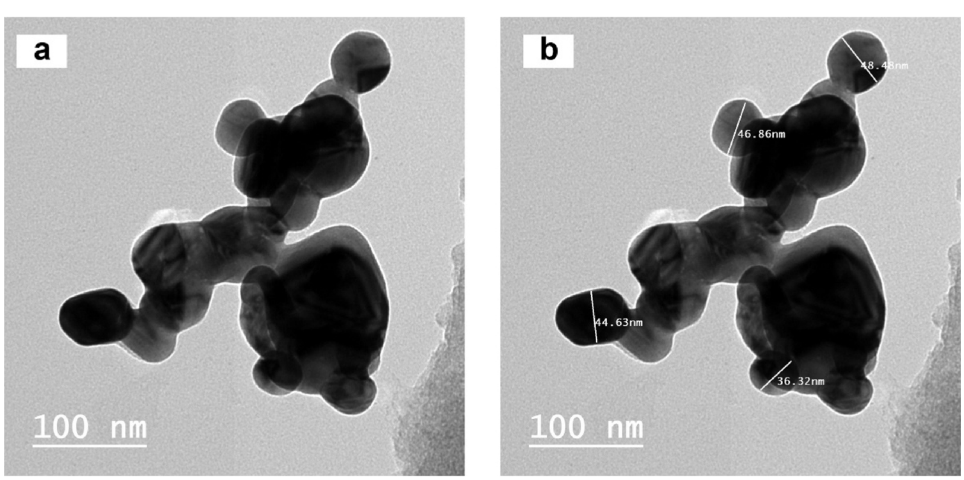

Fig. 1. (a and b). TEM photograph of silver nanoparticles (Ag NPs).

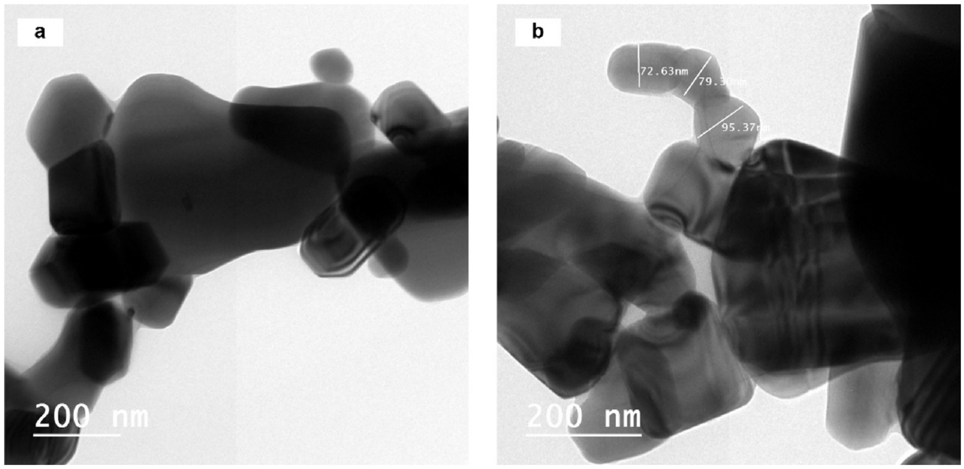

Fig. 2. (a and b). TEM photograph of zinc oxide nanoparticles (ZnO NPs).

3.3. *In vivo acaricidal efficacy*

No significant difference was observed between tick numbers of infested animals before and after treatments with deltamethrin and deltamethrin-Ag NPs (Table 2 and Fig. 8F–I). However, the reduction percentage of ticks was significant *($P \leq 0.05$)* in animals treated with deltamethrin-ZnO NPs when treated at 0.5 ml/L and the effect was observed at 7 days post-treatment. The tick reduction percentages of animals treated with deltamethrin-ZnO NPs at 0.5 mL/L were 63.33, 81.66 and 95.0% at 7, 10, and 21 days post-treatment, respectively (Table 2 and Fig. 8A–D). Furthermore, the egg mass per female in these treated animals (Fig. 8E) was significantly *($P \leq 0.05$)* reduced when compared to treatment with deltamethrin-Ag NPs 1 mL/L and deltamethrin alone (Table 2 and Fig. 8J and O). The egg masses collected from the different treatments were incubated and found to be hatched. It is worth noticing that animals treated with deltamethrin-ZnO NPs showed an improvement in their hair and coat appearance.

The *in vivo* toxicity of different treatments on the animal health revealed that using deltamethrin-ZnO NPs at a concentration of 0.5 mL/L did not induce significant alterations in the biochemical findings of animals over a period of three weeks post treatment (Table 3). Moreover, the treatment with both deltamethrin and deltamethrin-Ag NPs at a concentration of 1 mL/L did not exhibit significant elevation in the serum enzyme levels as compared to the pre-treatment status.

4. Discussion

In the current study, the adulticidal and larvicidal efficacy of different types of insecticides (deltamethrin and nano-emulsion of imidacloprid), metal nanoparticles (zinc oxide and silver) and its loaded forms were evaluated against *Rhipicephalus* (*Boophilus*) *annulatus* ticks. The adulticidal efficacy of deltamethrin at different concentrations showed that individuals of this tick species were resistant to deltamethrin even at the highest concentration (2 mL/L). Meanwhile, the adulticidal activity of deltamethrin in combination with Ag NPs capping agent at a concentration of 2 mL/L was slightly higher than that observed with deltamethrin treatment. In this case, the conjugation of deltamethrin to the surface of a silver nanoparticles was confirmed by FTIR spectrum and TEM photograph and the size of NPs in nanocomposite ranged from 27.2–55.3 nm. In addition, a characteristic peak of deltamethrin-Ag NPs was found at 2480 cm^{-1} that was in agreement with the findings of Sooresh et al. (2011). The increased resistance of ticks to deltamethrin might be due to its widespread use as a drug of choice in the management and control of pest in agricultural settings worldwide (Kumar et al., 2013; Ahanger et al., 2015). The results generated through this study agree with the extended use of deltamethrin to control tick infestations in our current area of the study (Aboelhadid et al., 2018). Thus, its excessive use led to the appearance of resistant tick strains with decreased susceptibility to the acaricide,

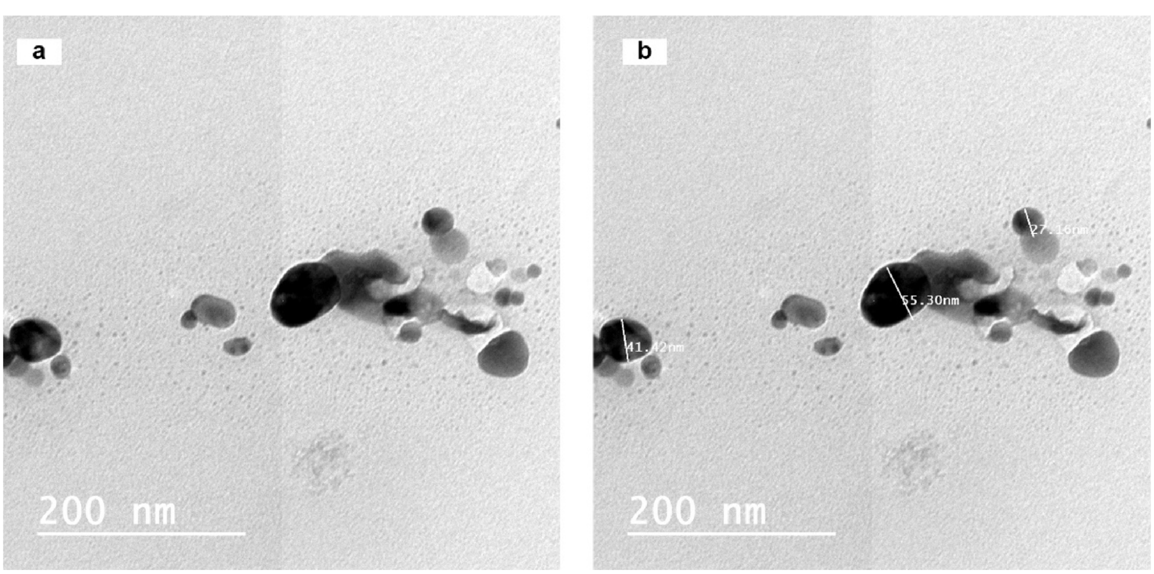

Fig. 3. (a and b). TEM photograph of deltamethrin loaded on silver nanocomposite (deltamethrin-Ag NPs).

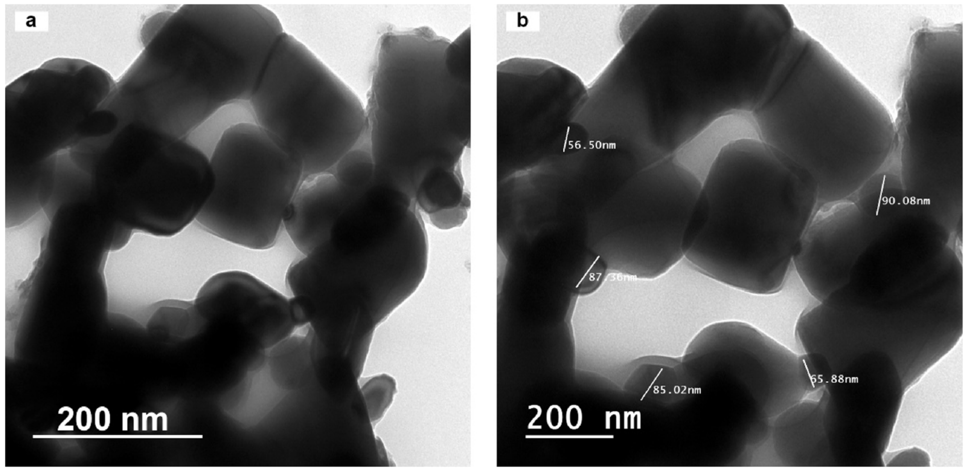

Fig. 4. (a and b). TEM photograph of deltamethrin loaded on zinc oxide nanocomposite (deltamethrin-ZnO NPs).

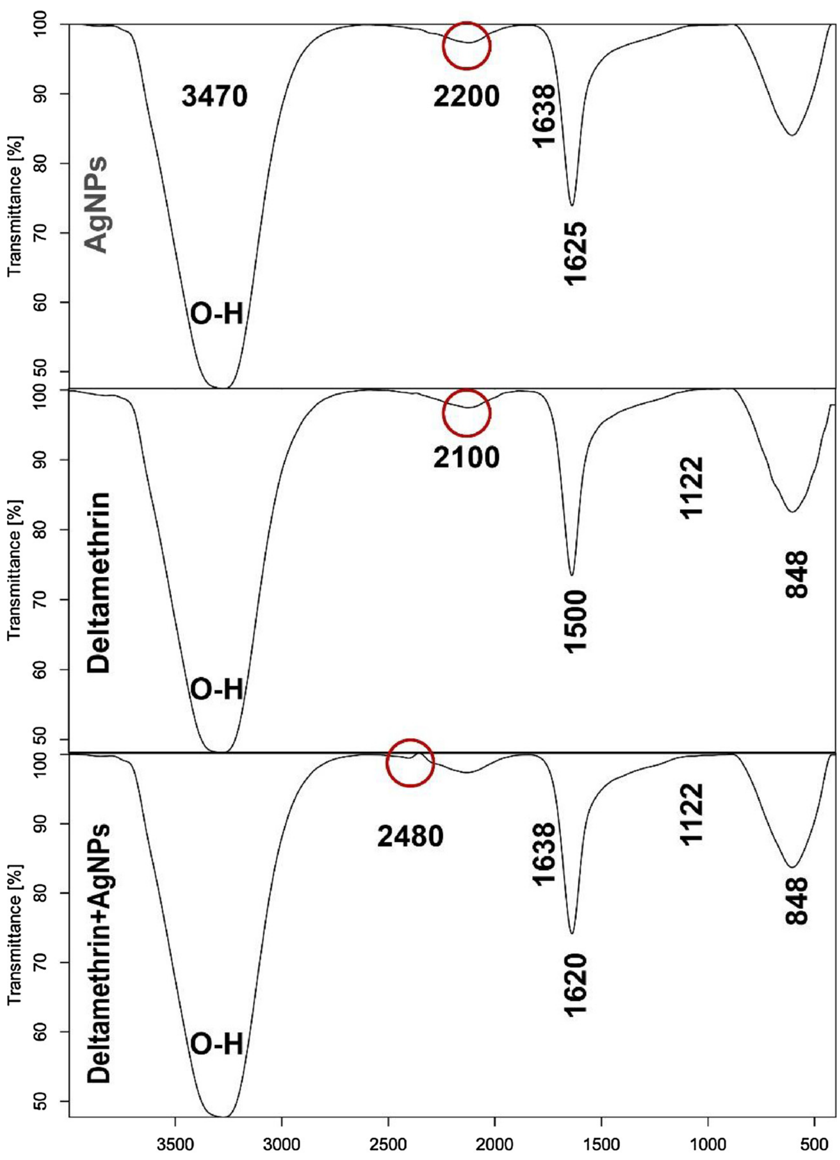

Fig. 5. FTIR spectrum of deltamethrin loaded on silver nanoparticles (Ag NPs).

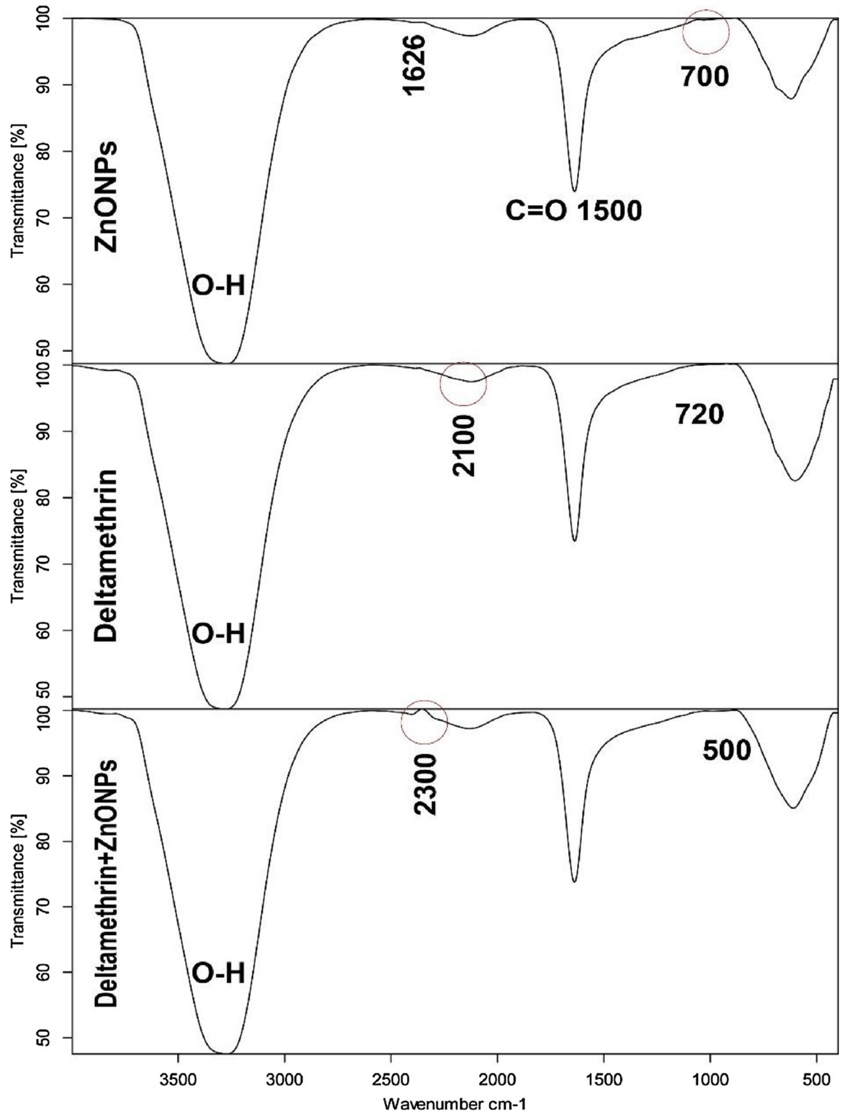

Fig. 6. The FTIR spectrum of the deltamethrin loaded on zinc oxide nanocomposite (deltamethrin-ZnO NPs).

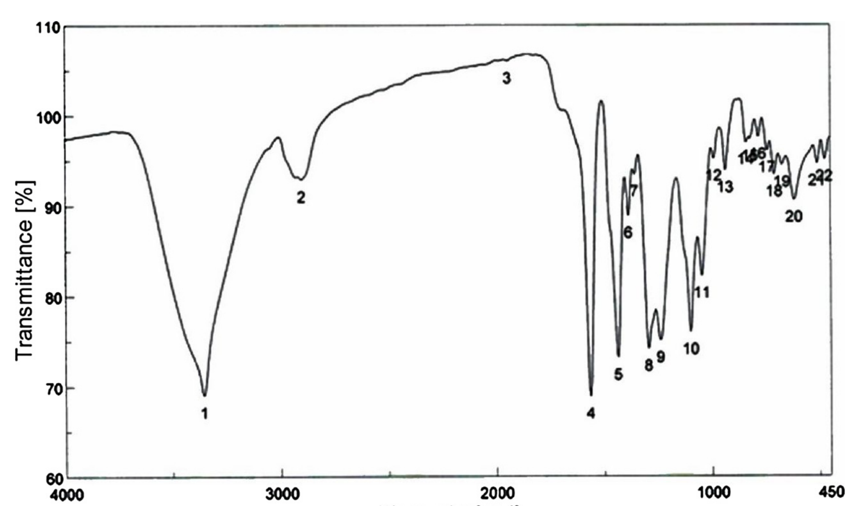

Fig. 7. The FTIR spectrum of imidacloprid nano-emulsion.

Table 1
In vitro acaricidal efficacy of different treatments against *R.* (*B*) *annualatus* adult ticks.

Tested compound (Conc.)	Number of dead tick/10 (Mean ± SE)	Number of live tick/10 (Mean ± SE)	Egg mass weight (mg/ 10 female ticks) Mean ± SE))	Female weight (gm/ 10 female ticks) (Mean ± SE)	Dead tick percent	Index of fecundity (Mean ± SE)	Percent reduction of oviposition
Deltamethrin	5.33 ± 0.3^{b}	4.66 ± .33^{b}	130.00 ± 5.77^{b}	.72 ± .060	53.30 ± 3.0^{b}	0.18 ± 0.057^{b}	20.34
0.25 mL/L	6.00 ± 2.0^{b}	4.00 ± 2.00^{a}	.00 ± 0.0^{a}0	.72 ± .040	60.00 ± 20.0^{b}	0.0 ± 0.0^{a}	100
0.5 mL/L	6.00 ± 2.0^{b}	4.00 ± 0.0^{a}	.00 ± 0.0^{a}0	.72 ± .060	60.00 ± 20.0^{b}	0.0 ± 0.0^{a}	100
1 mL/L	6.66 ± 2.4^{b}	3.33 ± 2.40^{a}	.00 ± 0.0^{a}0	.67 ± 0.050	66.60 ± 24.0^{b}	0.0 ± 0.0^{a}	100
2 mL/L							
Deltamethrin- Ag NPs	5.00 ± 0.0^{b}	5.00 ± 0.00^{b}	130.00 ± 5.77^{b}	.69 ± 0.050	50.00 ± 0.0^{b}	0.19 ± 0.0^{b}	16.87
0.25 mL/L	6.00 ± 2.0^{b}	4.00 ± 2.00^{a}	.00 ± 0.0^{a}0	.72 ± 0.060	60.00 ± 20.0^{b}	0.0 ± 0.0^{a}	100
0.5 mL/L	6.00 ± 2.0^{b}	4.00 ± 200^{a}	.00 ± 0.0^{a}0	.71 ± 0.070	60.00 ± 20.0^{b}	0.0 ± 0.0^{a}	100
1 mL/L	7.00 ± 2.1^{c}	3.00 ± 2.08^{a}	.00 ± 0.0^{a}0	.74 ± 0.030	70.00 ± 21.0^{c}	0.0 ± 0.0^{a}	100
2 mL/L							
Deltamethrin-ZnO NPs	10.00 ± 0.0^{c}	.00 ± 0.0^{a}0	.00 ± 0.0^{a}0	.73 ± .070	100.00 ± 0.0^{c}	0.0 ± 0.0^{a}	100
0.25 mL/L	10.00 ± 0.0^{c}	.00 ± 0.0^{a}0	.00 ± 0.0^{a}0	.71 ± 0.020	100.00 ± 0.0^{c}	0.0 ± 0.0^{a}	100
0.5 mL/L	10.00 ± 0.0^{c}	.00 ± 0.0^{a}0	.00 ± 0.0^{a}0	.73 ± .0.070	100.00 ± 0.0^{c}	0.0 ± 0.0^{a}	100
1 mL/L	10.00 ± 0.0^{c}	.00 ± 0.0^{a}0	.00 ± 0.0^{a}0	.75 ± 0.030	100.00 ± 0.0^{c}	0.0 ± 0.0^{a}	100
2 mL/L							
Imidacloprid nano-emulsion 100 mg/mL	2.00 ± 0.0^{a}	8.00 ± 0^{c}	140.0 ± 5.77^{b}	.67 ± .050	20.00 ± 0^{a}	0.21 ± 0.057^{b}	7.81
Ag NPs 400 mg/L	2.00 ± 0.0^{a}	8.00 ± 0.0^{c}	130.0 ± 5.77^{b}	.72 ± 0.040	20.00 ± 0.0^{a}	0.18 ± 0.057^{b}	20.34
ZnO NPs 8gm/L	2.00 ± 0.0^{a}	8.00 ± 0.0^{c}	130.0 ± 5.77^{b}	.72 ± 0.060	20.00 ± 0.0^{a}	0.18 ± 0.057^{b}	20.34
Control	0.66 ± 0.6^{a}	9.33 ± 0.66^{d}	170.0 ± 30.0^{b}	.75 ± 0.0350	6.60 ± 6.0^{a}	0.23 ± 0.030^{b}	0.0

-Superscript of the same letter in cells of the same column is non-significant with the zero day before treatment. Superscript of different letters in cells of the same column is significant ($P \le 0.05$).

Table 2
In vivo acaricidal efficacy of different treatments against *R.* (*B*) *annualatus* infested cattle.

Group /days post treatment (dpt)		Reduction% (Mean ± SE)	Female tick weight (mg)/15 (Mean ± SE)	Mean of egg mass(mg)/ female
Deltamethrin-	3dpt	20.0 ± 5.77^{a}	14.00 ± 0.67^{c}	2.00 ± 0.00^{a}
ZnO NPs	7dpt	63.33 ± 3.33^{b}	13.00 ± 0.17^{c}	2.00 ± 0.04^{a}
(0.5 mL/L)	10dpt	81.66 ± 1.66^{c}	7.60 ± 0.47^{b}	1.00 ± 0.00^{a}
	21dpt	95.00 ± 2.88^{d}	3.50 ± 0.57^{a}	0.00 ± 0.00^{a}
	3dpt	18.33 ± 1.66^{a}	21.10 ± 0.34^{e}	14.60 ± 0.00^{b}
Deltamethrin-	7dpt	20.00 ± 0.00^{a}	28.70 ± 0.55^{f}	12.70 ± 0.05^{b}
Ag NPs	10dpt	18.33 ± 1.66^{a}	21.60 ± 0.57^{e}	8.80 ± 0.57^{b}
(1 mL/L)	21dpt	18.33 ± 1.66^{a}	30.00 ± 0.23^{f}	15.50 ± 0.55^{b}
	3dpt	18.33 ± 1.67^{a}	17.20 ± 1.57^{d}	12.0 ± 0.57^{b}
	7dpt	18.33 ± 1.67^{a}	42.70 ± 2.57^{g}	10.9 ± 0.55^{b}
	10dpt	18.33 ± 1.67^{a}	42.20 ± 1.50^{g}	10.13 ± 0.53^{b}
Deltamethrin	21dpt	15.67 ± 1.67^{a}	28.20 ± 0.30^{f}	10.12 ± 0.57^{b}
(1 mL/L)				

-Superscript of the same letter in cells of the same column is non-significant with the zero day before treatment. Superscript of different letters in cells of the same column is significant ($P \le 0.\ 05$).

causing only 66% efficacy at the highest concentration of 2 mL/L. It was of interest to explain that, eggs deposited by deltamethrin treated ticks hatched and the larvae were active for not less than 14 days.

Interestingly, the *in vitro* study showed that, the adulticidal efficacy of deltamethrin-ZnO NPs against tick *R.* (B.) *annulatus* was very promising, with 100% mortality at all tested concentrations. Moreover, it showed a complete larval immobility within 24 h compared to other treatments that exhibited 100% mortality of larvae after 48 h. Furthermore, TEM photograph showed that the NPs size of deltamethrin-ZnO NPs nanocomposite ranged from 65.8–90.1 nm and FTIR spectrum recorded the appearance of new peaks of deltamethrin-ZnO NPs that existed at 2300 cm^{-1}, indicating the successful loading on ZnO NPs (Ghosh, 2012). Findings of the current study confirmed that the performance of deltamethrin was increased through successful loading on ZnO NPs at different concentrations (0.25, 0.5, 1.0 and 2.0 mL/L). The acaricidal efficacy of ZnO NPs depends upon the adhesion of NPs aggregates to the exoskeleton of the parasites causing physical effects and/or loss of motility (Baun et al., 2008). In addition, deltamethrin targets the insect nervous system; thus, the combination of the neurological effect of the drug and the mechanical damage induced by the particle will have an increased acaricidal effect on ticks. Currently, the nano synthesis of different metals and their oxides, using the green nano-synthesis fabrication routes, were found to be highly effective against different pests and vectors especially of zoonotic importance (Avinash et al., 2017; Benelli et al., 2017). Nanoparticles have been proposed as novel pesticides to arthropod vectors and pests of public health importance (Rai et al., 2009). Also, synthesized ZnO NPs showed promising acaricidal, pediculicidal and larvicidal activity against the larvae of *R. microplus* and other species at a concentration of 50 mg/L (Kirthi et al., 2011).

Capping of deltamethrin with silver nanoparticle resulted in a significant acaricidal effect on ticks as compared to uncapped nanoparticles (Ag NPs 400 mg/L and ZnO NPs 8gm/L. This result could be attributed to the surface reactivity facilitated by the capping agent that enabled these functionalized nanoparticles as promising candidates for various pharmaceutical, biomedical, and environmental applications (Marimuthu et al., 2011). On the contrary, imidacloprid nano-emulsion at 100 mg/ml was ineffective against *R.* (B.) *annulatus* ticks. Nanoemulsions are often said to be metastable as they may possess a relatively high kinetic (meta-) stability for several years, (Gutierrez et al., 2008). However, flocculation may be a possible breakdown mechanism for nano-emulsions formulated with mixed nonionic-ionic surfactants (Wang et al., 2009). Furthermore, using of imidacloprid was ineffective for tick control in veterinary practice (Cruthers et al., 1999; Coles and Dryden, 2014). Moreover, Jawahar and Meyyanathan, (2012) showed that the ineffectiveness of imidacloprid could be occurred due to aggregation of its particle size that confirmed by the zeta- sizer of nano-emulsion. *In vivo*, the efficacy trial confirmed that deltamethrin-ZnO NPs at a concentration of 0.5 mL/L can be used as a promising alternative acaricide to reduce tick numbers on infested cattle, as early as 7 days post-treatment, reaching the maximum percent reduction (95.0%) on the 21st day post-treatment. Besides, egg mass per female was significantly reduced compared to other treatment groups. The mechanisms for cellular cytotoxicity of ZnO NPs depend on the generation of reactive oxygen species (ROS), and the release of toxic cations causing

Fig. 8. *In vivo* acaricidal efficacy of deltamethrin and its loaded forms; deltamethrin-ZnO NPs, deltamethrin-Ag NPs against adult ticks. (A–D) deltamethrin-ZnO NPs at 3,7,10, and 21 days post-treatment respectively, and (E) egg mass per female ticks at 21 days post-treatment. (F–I) deltamethrin-Ag NPs at 3,7,10, and 21 days post-treatment respectively, and (J) egg mass per female ticks at 21 days post-treatment. (K–N) deltamethrin group at 3,7, 10, and 21 days post-treatment respectively, and (O) egg mass per female ticks at 21 days post treatment.

lysosomal damage, and inflammation (Nel et al., 2009).The soluble fraction (Zn2+ ion) of ZnO NPs exerts the toxic actions, while its insoluble form exerts a higher toxic effect as compared to that of the same amount of $Zn2^{+}$. The toxic action of nanoparticles can be linked to stress and/or a chemical effect or stimuli caused by the peculiar physical characteristics of the nano-state (Manzo et al., 2010).

Moreover, all treated animals didn't show any signs of toxicity and/or mortality, that was further evidenced by absence of significant differences in the liver and kidney function profiles pre- and up to 21 days post-treatment. However, these observations disagree with Najafzadeh et al. (2013) who recorded increased creatinine levels in lambs after oral administration of ZnO NPs. In addition, other studies found that oral administration of deltamethrin and Ag NPs resulted in a deterioration of both liver and kidney functions (Sarhan and Hussein, 2014; Sharma et al., 2014). Thus, the current study suggests that the cutaneous application of deltamethrin-ZnO NPs composite is safe at the dose of 0.5 ml/L.

5. Conclusions

The current study showed that, deltamethrin-ZnO NPs at a concentration of 0.5 mL/L exhibited a significant reduction in both tick numbers on animals and egg mass deposition per female tick. On the contrary, imidacloprid nano-emulsion was ineffective against *R.* (B.) *annulatus* ticks. Therefore, the current study highlights the value of using nanoparticles to improve the efficacy of the acaricides. Further studies on the efficiency of deltamethrin-ZnO NPs against a wide range of ectoparasites of veterinary and medical importance are needed.

Conflict of interest

The authors declare that they have no conflict of interest.

Table 3
Serum biochemical levels (Mean ± SE) of treated cattle pre- and post-treatment.

Group / days Post treatment		GPT U/L (ALT)	GOT U/L (AST)	Urea mg/dL	Creatinine mg/dL
Deltamethrin-ZnO NPs (0.5 mL/L)	Zero day	26.0 ± 0.55^{a}	68.0 ± 0.55^{b}	37.0 ± 0.52^{a}	1.67 ± 0.03^{b}
	3 days post treatment	24.0 ± 0.58^{a}	67.0 ± 0.57^{b}	38.0 ± 0.0^{a}	1.42 ± 0.01^{a}
	7 days post treatment	25.0 ± 0.55^{a}	73.0 ± 1.1^{b}	32.0 ± 4.0^{a}	1.25 ± 0.1^{a}
	10 days post treatment	27.0 ± 0.55^{a}	58 ± 0.57^{a}	35.0 ± 3.0^{a}	1.28 ± 0.1^{a}
	21 days post treatment	24.0 ± 0.55^{a}	62 ± 3.0^{b}	36.0 ± 4.0^{a}	1.37 ± 0.01^{a}
Deltamethrin-Ag NPs (1 mL/L)	Zero day	27.0 ± 1.53^{a}	66.6 ± 3.8^{b}	36.0 ± 0.50^{b}	1.20 ± 0.20^{a}
	3 days post treatment	29.67 ± 2.91^{a}	74.67 ± 0.3^{b}	37.0 ± 0.57^{b}	1.29 ± 0.03^{a}
	7 days post treatment	27.0 ± 0.55^{a}	73.0 ± 1.1^{b}	35.0 ± 4.0^{a}	1.27 ± 0.1^{a}
	10 days post treatment	29.0 ± 0.55^{a}	58 ± 0.57^{a}	37.0 ± 3.0^{a}	1.29 ± 0.1^{a}
	21 days post treatment	25.0 ± 0.55^{a}	62 ± 3.0^{b}	36.0 ± 4.0^{a}	1.37 ± 0.01^{a}
Deltamethrin (1 mL/L)	Zero day	30.0 ± 0.55^{a}	52.0 ± 0.57^{a}	29.0 ± 0.0^{a}	1.68 ± 0.04^{b}
	3 days post treatment	27.00 ± 0.58^{a}	54.6 ± 2.6^{a}	42.0 ± 0.0^{a}	1.72 ± 0.02^{b}
	7 days post treatment	28.0 ± 0.55^{a}	68.0 ± 4.1^{b}	35.0 ± 4.0^{a}	1.27 ± 0.1^{a}
	10 days post treatment	27.0 ± 0.55^{a}	58 ± 0.57^{a}	37.0 ± 3.0^{a}	1.29 ± 0.1^{a}
	21 days post treatment	26.0 ± 0.55^{a}	62 ± 3.0^{b}	36.0 ± 4.0^{a}	1.37 ± 0.01^{a}

-Superscript of the same letter in cells of the same column is non-significant with the zero day before treatment. Superscript of different letters in cells of the same column is significant ($P \leq 0.05$).

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References

Aboelhadid, S.M., Arafa, W.M., Mahrous, L.N., Fahmy, M.M., Kamel, A.A., 2018. Molecular detection of *Rhipicephalus (Boophilus) annulatus* resistance against deltamethrin in middle Egypt. Vet. Parasitol. Reg. Stud. Reports 13, 198–204.
Ahanger, R.R., Godara, R., Katoch, R., Yadav, A., Bhutyal, A.D.S., Katoch, M., Singh, N.K., Bader, M.A., 2015. Deltamethrin resistance in field populations of *Rhipicephalus (Boophilus) microplus* (Acari: ixodidae) in Jammu and Kashmir, India. Exp. Appl. Acarol. 67, 467–475.
Ahmed, S., Saifullah, Ahmad M., Swami, B.L., Ikram, S., 2016. Green synthesis of silver nanoparticles using *Azadirachta indica* aqueous leaf extract. J. Rad. Res. Appl. Sci. 9, 1–7.
Aneesh, P.M., Vanaja, K.A., Jayaraj, M.K., 2007. In: In: Gaburro, Zeno, Cabrini, Stefano (Eds.), Synthesis of ZnO Nanoparticles by Hydrothermal Method NanoPhotonic Materials IV Vol. 6639 Proc. of SPIE 66390J.
Avinash, B., Venu, R., Alpha Raj, M., Srinivasa Rao, K., Srilatha, C., Prasad, T.N., 2017. In vitro evaluation of acaricidal activity of novel green silver nanoparticles against deltamethrin resistance Rhipicephalus (Boophilus) microplus. Vet. Parasitol. 237, 130–136.
Banumathi, B., Vaseeharan, B., Malaikozhundan, B., Ramasamy, P., Govindarajan, M., Alharbid, N.S., Kadaikunnan, S., Canale, A., Benelli, G., 2018. Green larvicides against blowflies, *Lucilia sericata* (Diptera, Calliphoridae): screening of seven plants used in Indian ethno-veterinary medicine and production of green-coated zinc oxide nanoparticles. Physiol. Mol. Plant Pathol. 101, 214–218.
Barré, N., Li, A.Y., Miller, R.J., Gaia, H., Delathiére, J.M., Davey, R.B., 2008. *In vitro* and *in vivo* evaluation of deltamethrin and amitraz mixtures for the control of *Rhipicephalus (Boophilus) microplus* (Acari: ixodidae) in New Caledonia. Vet. Parasitol. 155 (1-2), 110–119.
Baun, A., Hartmann, N.B., Grieger, K., Kusk, K.O., 2008. Ecotoxicity of engineered nanoparticles to aquatic invertebrates: a brief review and recommendations for future toxicity testing. Ecotoxicology 17, 387–395.
Benelli, G., Maggi, F., Romano, D., Stefanini, C., Vaseeharane, B., Kumarf, S., Higuchi, A., Alarfaj, A., Mehlhorni, H., Canale, A., 2017. Nanoparticles as effective acaricides against ticks-a review. Ticks Tick Borne Dis. 8 (6), 821–826.
Bennett, G.F., 1974. Oviposition of *Boophilus microplus* (Canestrini) (Acarida Ixodidae). I. Influence of tick size on egg production. Acarologia 16, 52–61.
Coles, T.B., Dryden, M.W., 2014. Insecticide/acaricide resistance in fleas and ticks infesting dogs and cats. Parasit. Vectors 7, 8.
Cox, C., 2001. Insecticide factsheet: imidacloprid. J. Pestic. Reform. 21, 15–21.
Cruthers, L., Guerrero, J., Robertson-Plouch, C., Slone, R., 1999. Evaluation of the speed of kill of fleas and ticks with fipronil or imidacloprid. Proceedings, 24th World Small Animal Veterinary Association Congress, 23rd-26th September.
Davies, T.G.E., Field, L.M., Usherwood, P.N.R., Williamson, M.S., 2007. DDT, pyrethrins, pyrethroids and insect sodium channels. IUBMB Life 59 (3), 151–162.
De Campos-Pereira, M., Bahia-Labruna, M., Szabó, M., Marcondes-Klafe, G., 2008. *Rhipicephalus* (*Boophilus*) *microplus* Biología, controle e resistência. Primeira edição. São Paulo. Editorial MedVet 192 p.
Eyo, J.E., Ekeh, F.N., Ivoke, N., Atama, C.I., Onah, I.E., Ezenwaji, N.E., Ikele, C.B., 2014. Survey of tick infestation of cattle at four selected grazing sites in the tropics. Glob. Vet. 12 (4), 479–486.
FAO, 2004. Guidelines Resistance Management and Integrated Parasite Control in Ruminants. Rome.
Field, L.M., Emyr, Davies, T.G., O'Reilly, A.O., Martin, S., Williamson, M.S., Wallace, B.A., 2017. Voltagegated sodium channels as targets for pyrethroid insecticides. Eur. Biophys. J. 46, 675–679.
Ghosh, S.P., 2012. Synthesis and Characterization of Zinc Oxide Nanoparticles by Sol-gel Process. National Institute of Technology, Rourkela-769008, Orissa, India. http://ethesis.nitrkl.ac.in/3259/1/projectfinaal.pdf.
Gutierrez, J.M., Gonzalez, C., Maestro, A., Sole, I., Pey, C.M., Nolla, J., 2008. Nano-emulsions: new applications and optimization of their preparation. Curr. Opin. Colloid Interfaces Sci. 13, 245–251.
Jawahar, N., Meyyanathan, S.N., 2012. Polymeric nanoparticles for drug delivery and targeting: a comprehensive review. Int. J. Health Allied Sci.1 4, 217–223.
Khalaf-Allah, S., 1996. Acaricidal efficacy of cypermethrine (a new synthetic pyrethroid) against *Boophilus annulatus* ticks in cattle. DTW. Dtsch Tierarztl Wochenschr. 103 (11), 463–464.
Kirthi, A.V., Abdul Rahuman, A., Rajakumar, G., Marimuthu, S., Santhoshkumar, T., Jayaseelan, C., Velayutham, K., 2011. Acaricidal, pediculocidal and larvicidal activity of synthesized ZnO nanoparticles using wet chemical route against blood feeding parasites. Parasitol. Res. 109 (2), 461–472.
Kumar, R., Nagar, G., Sharma, A.K., Kumar, S., Ray, D.D., Chaudhuri, P., Ghosh, S., 2013. Survey of pyrethroids resistance in Indian isolates of *Rhipicephalus (Boophilus) microplus*: identification of C190A mutation in the domain II of the para-sodium channel gene. Acta Trop. 125, 237–245.
Lorusso, V., Picozzi, K., de Bronsvoort, B.M., Majekodunmi, A., C, Dongkum, G, Balak, Welburn, S.C., 2013. Ixodid ticks of traditionally managed cattle in central Nigeria: where Rhipicephalus (Boophilus) microplus. Parasit. Vectors 6 (6), 171.
Manzo, S., Rocco, A., Carotenuto, R., DeLuca, P.F., Miglietta, M.L., Rametta, G., Di Francia, G., 2010. Investigation of ZnO nanoparticles' ecotoxicological effects towards different soil organisms. Environ. Sci. Pollut. Res. Int. 18 (5), 756–763.
Marimuthu, S., Rahuman, A.A., Rajakumar, G., Santhoshkumar, T., Kirthi, A.V., Jayaseelan, C., Bagavan, A., Zahir, A.A., Elango, G., Kamaraj, C., 2011. Evaluation of green synthesized silver nanoparticles against parasites. Parasitol. Res. 108 (6), 1541–1549.
Mathivathani, C., Basith, S.A., Latha, B.R., Raj, G.D., 2011. In vitro evaluation of synthetic pyrethroid resistance in Rhipicephalus sanguineus ticks of Chennai. J. Vet. Parasitol. 25, 56–58.
Matsuda, K., Buckingham, S.D., Kleier, D., Rauh, J.J., Grauso, M., Satelle, D.B., 2001. Neonicotinoids: insecticides acting on insect nicotinic acetylcholine receptors. Trends Pharmacol. Sci. 22, 573–580.
Najafzadeh, H., Ghoreishi, S.M., Mohammadian, B., Rahimi, E., Afzalzadeh, M.R., Kazemivarnamkhasti, M., Ganjealidarani, H., 2013. Serum biochemical and histopathological changes in liver and kidney in lambs after zinc oxide nanoparticles administration. Vet. World 6 (8), 534–537.
Nel, A.E., Madler, L., Velegol, D., Xia, T., Hoek, E.M., Somasundaran, P., 2009. Understanding bio-physicochemical interactions at the nano-bio interface. Nat Matters 8, 543–557.
Pirali-Kheirabdi, K., Haddadzadeh, H., Razzaghi-Abyaneh, M., Bokaie, S., Zare, R., Ghazavi, M., Shams-Ghahfarokhi, M., 2007. Biological control of *Rhipicephalus* (*Boophilus*) *annulatus* by different strains of *Metarhizium anisopliae*, *Beauveria bassiana* and *Lecanicillium psalliotae* fungi. Parasitol. Res. 100, 1297–1302.
Rai, M., Yadav, A., Gade, A., 2009. Silver nanoparticles as a new generation of antimicrobials. Biotechnol. Adv. 27, 76–83.
Reitman, S., Frankel, S., 1957. A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. Am. J. Clin. Pathology 28, 56–63.

Rodríguez-Vivas, R.I., Alonso-Díaz, M.A., Rodríguez-Arevalo, F., Fragoso-Sanchez, H., Santamaria, V.M., Rosario-Cruz, R., 2006. Prevalence and potential risk factors for organophosphate and pyrethroid resistance in *Boophilus microplus* ticks on cattle ranches from the state of Yucatan, Mexico. Vet. Parasitol. 136 (3-4), 335–442.

Rodríguez-Vivas, R.I., Li, A.Y., Ojeda-Chi, M.M., Trinidad-Martinez, I., Rosado-Aguilar, J.A., Miller, R.J., 2013. *In vitro* and *in vivo* evaluation of cypermethrin, amitraz, and piperonyl butoxide mixtures for the control of resistant *Rhipicephalus (Boophilus) microplus*(Acari: Ixodidae) in the Mexican tropics. Vet. Parasitol. 197 (1-2), 288–296.

Sarhan, O.M.M., Hussein, R.M., 2014. Effects of intraperitoneally injected silver nanoparticles on histological structures and blood parameters in the albino rat. Int. J. Nanomed. 9, 1505.

Sharma, A.K., Kumar, R., Kumar, S., Nagar, G., Singh, N.K., Rawat, S.S., Dhakad, M.L., Rawat, A.K., Ray, D.D., Ghosh, S., 2012. Deltamethrin and cypermethrin resistance status of *Rhipicephalus (Boophilus) microplus* collected from six agro-climatic regions of India. Vet. Parasitol. 188 (3-4), 337–345.

Sharma, P., Singh, R., Jan, M., 2014. Dose-dependent effect of deltamethrin in testis, liver, and kidney of wistar rats. Toxicol. Int. 21 (2), 131–139.

Šileikaitė, A., Puišo, J., Prosyčevas, I., Tamulevičius, S., 2009. Investigation of silver nanoparticles formation kinetics during reduction of silver nitrate with sodium citrate. Mater Sci. (Medžiagotyra) 15 (1), 21–27.

Sooresh, A., Kwon, H., Taylor, R., Pietrantonio, P., Pine, M., Sayes, C.M., 2011. Surface functionalization of silver nanoparticles: novel applications for insect vector control. ACS Appl. Mater. Interfaces 3, 3779–3787.

Tomizawa, M., Casida, J.E., 2005. Neonicotinoid insecticide toxicology: mechanisms of selective action. Annu. Rev. Pharmacol. Toxicol. 45, 247–268.

Uboh, F.E., Ebong, B.E., Akpan, H.D., Usoh, I.F., 2012. Hepatoprotective effect of vitamins C and E against gasoline vapor-induced liver injury in male rats. Turk. J. Biol. 36, 217–223.

Walker, A.R., 2003. Ticks of Domestic Animals in Africa: a Guide to Identification of Species. Bioscience Reports Edinburgh.

Wang, L., Dong, J., Chen, J., Eastoe, J., Li, X., 2009. Design and optimization of a new self nano-emulsifying drug delivery system. J. Colloid Interface Sci. 330, 443–448.

Yiwombe, K., 2013. An Investigation to Determine the Resistance of the *Boophilus* tick (Blue Tick) to Amitraz in Selected Areas of Zimbabwe. Dissertation for Bachelor's Degree. Department of Biological Sciences. Faculty of Science and Technology, Midlands State University. http://ir.msu.ac.zw:8080/jspui/bitstream/11408/512/3/karen_dissertation-final.pdf.

Zhang, A., Kayser, H., Maienfisch, P., Casida, J.E., 2000. Insect nicotinic acetylcholine receptor: conserved neonicotinoid specificity of [3H] imidacloprid binding site. J. Neurochem. 75, 1294–1303.