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Original Article

Acaricidal effect of Iron nanoparticles against *Hyalomma* spp. *in vitro*

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Summary

Hyalomma spp. is responsible for the transmission of bacterial, protozoan, rickettsial and viral pathogens in animals and humans. The aim of this study was to evaluate the acaricidal activity of iron oxide nanoparticles (Fe2O3 and Fe3O4 NPs) size 15 nm against *Hyalomma* spp. *in vitro*. The acaricidal activity of Fe-NPs was evaluated at concentrations of 50, 125, and 250 µg/ml and controls (distilled water and Cypermethrin) following 10, 30, and 60 min of exposure in triplicate and the experiments were performed two spraying and contact methods.

The results of this study showed that all concentrations of Fe-NPs had acaricidal activity, and a concentration of 250 µg/ml at an exposure time of 10 min had the highest acaricidal effect (85.7%). The median lethal concentration (LC50) values were 50 µg/ml in 60 min, and (LC99) values were 150 mg/ml in 30 min for *Hyalomma* spp.. The results showed that the spray method was more effective than the contact method. Statistically, there was no difference between the acaricidal effect of trivalent iron (Fe2O3) and quadrivalent (Fe3O4) iron nanoparticles.

The findings of the present study showed that Fe-NPs had potent acaricidal activity. However, further *in vivo* studies are required to evaluate the efficacy of this nanoparticle.

Keywords: Acaricide, Iron nanoparticles, *Hyalomma* spp., *in vitro*.

Introduction

The ticks, known as the most important ectoparasites of vertebrate animals and humans, cause decreasing the productivity and quality of animal's products, anorexia, anemia, toxicosis, general stress, transmission of protozoan, bacterial, rickettsial and viral diseases (De La Fuente et al., 2008).

The use of pesticides has decreased the level of diseases. However, pests usually develop rapid resistance to target species, toxicity, effects on human health, and the environmental hazards, and it is, therefore, necessary to search continuously for Eco-friendly pesticides currently available pesticides (Al-Rajhy et al., 2003). The so-called, "green pesticides" are currently proposed as one of the helpful tools for controlling ectoparasites (Benelli, 2015; Salam et al., 2012; Adhikari et al., 2013; Marimuthu et al., 2011). Now, chemical acaricides have been obtained the relatively good treatment effectiveness, including Ivermectin, and Cypermethrin (Abbas et al., 2008) and the sparing and contact methods with maximum efficiency as well as are ease of use and flexible.

Recently, there was a global trend to evaluate new agents that are safe, effective, inexpensive, easily available, low environmental contaminations, low resistance. Nanotechnology provides important new tools expected to have the most impact on many areas in medical sciences. The polymer-coated metal NPs have recently appeared as an active and novel field of advanced researches. However, metal NPs stability is a severe problem with polar terminal groups like hydroxyl groups or amine are usually used for their stabilization (Prasad, 2008).

Iron, which well known as one of the most plentiful element in the Earth's crust, is the structural backbone of our modern infrastructure. It is therefore ironic that as a nanoparticle, iron has been somewhat neglected in favor of its own oxides, as well as other metals like nickel, gold, cobalt, and platinum. Iron, however has a great deal to provide at the nanoscale, comprising very potent magnetic and catalytic properties (Huber, 2005).

During recent years the use of nanoparticles has been attracted the attention of researchers in the world and Iran. This study was undertaken for the first time to evaluate the acaricidal activity of Fe-NPs with two spraying and contact methods against *Hyalomma* spp. *in vitro*.

Materials and methods

Fe-NPs characterization

The nano-iron is metal orang and brown powder. Fe2O3 and Fe3O4 NPs average particle size was 15 nm (10-15 nm), and bulk density was about 0.20 - 0.40 g/m^3. Fe-NPs was purchased from the Intelligent Materials Pvt. Ltd., Nanoshel LLC, Wilmington, DE, USA. Nanoshel. Fe-NPs particle is produced by the evaporation process and was characterized by Scanning Electron Microscopy (SEM, Leo 906, Zeiss 100 KV, Germany). The Fe-NPs used in this experiment possessed an analytical grade with the highest purity.

Collection of ticks

The ticks were randomly collected from naturally infected sheep and cattle. Firstly, ticks were collected and placed in Petri dishes. Then, Petri dishes were examined under a stereomicroscope, and the species of ticks were determined in the laboratory.

Evaluation of the acaricidal activity of Fe-NPs by contact and spraying methods

In an experiment *in vitro*, it was studied the anti-tick activity of Fe-NPs at the concentrations of 50, 125, and 250 µg/ml. For the contact method, the circular filter papers of 4.8 cm in diameter (approximate area of 18 cm^2) were treated with the provided concentrations of Fe-NPs (50, 150, and 250 µg/ml). After drying for 2-3 minutes under a fume hood, the dried filter papers were put into Petri dishes. Ten live newly adult ticks were transferred on treated filter papers, water-soaked cotton was placed into Petri dishes to supply the humidity, and finally, Petri dishes were covered with their lids and sealed with parafilm (Abbas, 2014). For the spraying method, firstly, the filter papers without any treatment were placed into Petri dishes and groups of 10 ticks transferred on filter papers, after which different concentrations of Fe-NPs were sprayed directly on to the ticks. Finally, the Petri dishes were immediately covered and sealed tightly. With the same manners, Cypermethrin (Cypermethrin 10%, Hacker, Iran) at similar concentrations were used as positive control, and distilled water was used as a negative control. Three replications were considered for each dilution for the two methods and two iron capacities (Fe2O3 and Fe3O4). Subsequently, all Petri dishes were left for 10, 30, and 60 minutes to monitor the acaricidal activity of Fe-NPs preparations. After 10, 30, and 60 min, the legs of ticks were agitated with an entomological pin under a loop; if the legs did not move, the tick was considered dead (Kim et al., 2016).

Statistical analysis

The data obtained were analyzed using the GraphPad Prism software program version 5 and expressed as a mean ± SD.

Results

The results showed that Fe-NPs showed the anti-tick effects against *Hyalomma* spp. at all test times and concentrations, especially for the concentration of 250 µg/ml at an exposure time of 10 min had the highest acaricidal effect (85.7%). Acaricidal effects of Fe-NPs at the concentration of 50 µg/ml after 10 minutes of the application was lower than others concentrations (14.3%). The median lethal concentration (LC50) values were 50 µg/ml in 60 min, and (LC99) values were 150 µg/ml in 30 min for *Hyalomma* spp..

The results showed that the spray method was more effective than the contact method, and there was no difference between the acaricidal effect of trivalent iron (Fe2O3) and quaternary (Fe3O4) nanoparticles .

The mortality rate of ticks after exposure to different concentrations of the Fe-NPs in various exposure times is presented in Table 1, Figures 1 and 2. Figure 1 shows the SEM image of Fe-NPs.

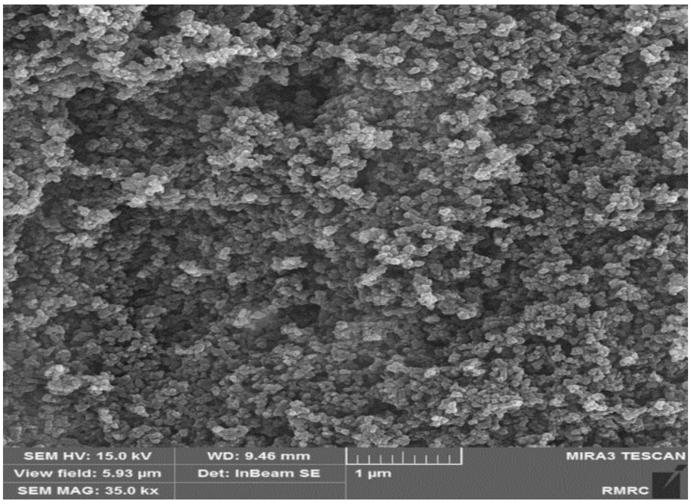

Fig. 1. The SEM image of 15 nm Fe-NP

Table 1. Mortality rate (Mean± SD%) of the Fe-NPs against *Hyalomma* spp. in different concentrations after various exposure times

	Times	Positive control	Spraying method	Contact method	Negative control
50 µg/ml	10 min	100.00	14.3±0.00	14.3±0.00	00.00
	30 min	100.00	21±1.61	21±1.89	00.00
	60 min	100.00	28.5±0.00	28.5±1.61	00.00
125 µg/ml	10 min	100.00	64±1.89	42±1.89	00.00
	30 min	100.00	71.4±0.00	49.5±0.00	00.00
	60 min	100.00	71.4±0.00	75.4±0.00	00.00
250 µg/ml	10 min	100.00	85.7±0.00	64±0.00	00.00
	30 min	100.00	85.7±0.00	71.5±9.89	00.00
	60 min	100.00	85.7±0.00	71.5±0.00	00.00

Discussion

The nanoparticles have been suggested as novel pesticides toxic to arthropod vectors and pests of public health importance (Rai et al., 2009; Santhoshkumar et al., 2011; Athanassiou et al., 2018). Growing evidence proposed that the metal oxide nanoparticles were highly effective against arthropod pests and vectors of economic importance (Athanassiou et al., 2018). However, most of these studies focused on mosquitoes (Benelli, 2016), *Pediculus humanus* **(**Jayaseelan et al., 2011; Marimuthu et al., 2012), *Aedes aegypti* (Roni et al., 2015), beetles (Elango et al., 2016), blowflies

(Banumathi et al., 2017), *Hippobosca maculata* (Velayutham et al., 2012), *Bovicola ovis* (Velayutham et al., 2012), harmful mites (Pavela et al., 2017), and *Hyalomma* spp. (Norouzi et al, 2019).

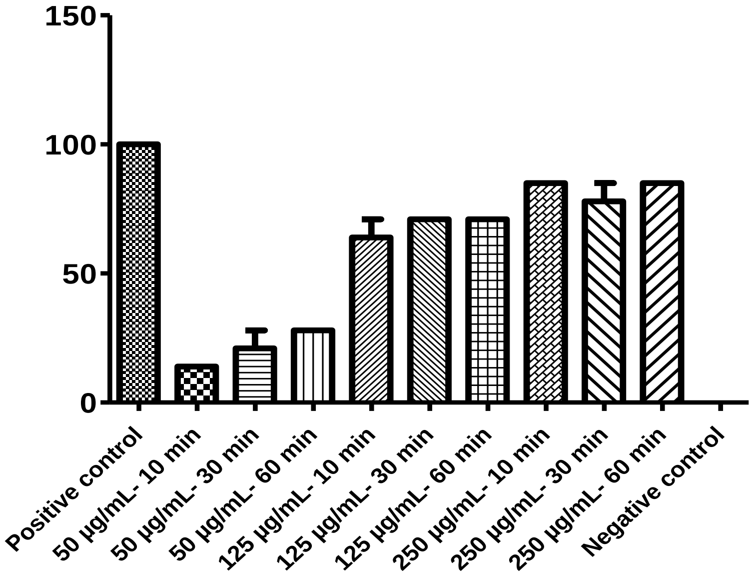

Fig. 2. The acaricidal activity of different concentrations of the Fe-NPs against *Hyalomma* spp. after various exposure times by spraying method.

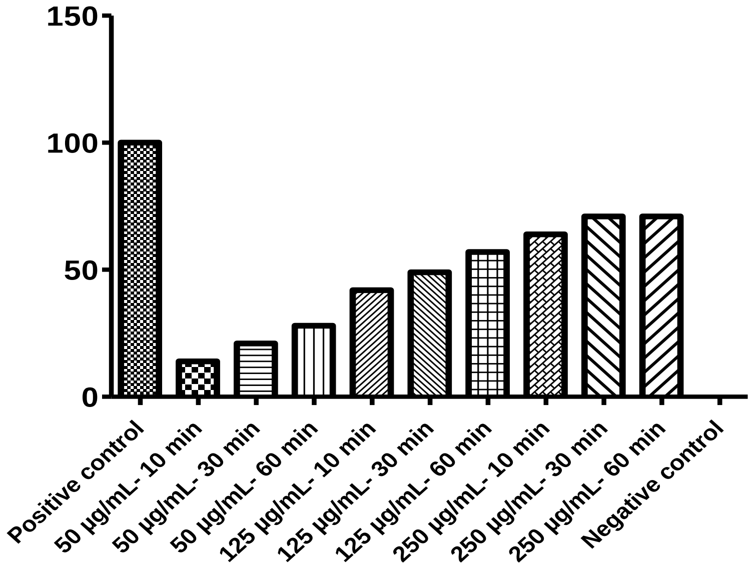

Fig. 3. The acaricidal activity of different concentrations of the Fe-NPs against *Hyalomma* spp. after various exposure times by contact method.

Several studies have anti-tick effects of silver, titanium, nickel and zinc on ticks, such as Norouzi *et al.* (2019) determined the efficacies Marimuthu et al. (2011) used 50 mg of TiO_2 NPs on *Rhipicephalus microplus* larvae. The findings of this study indicated TiO_2 NPs were highly stable and had significant acaricidal activity against the larvae of *R. (B.) microplus* (Marimuthu et al., 2013).

Jayaseelan et al. (2012) determined the efficacy of synthesized silver nanoparticles (AgNPs) against the larvae of *Hyalomma anatolicum* and *Hyalomma marginatum*. The maximum efficiency was observed in the synthesized AgNPs against *H. anatolicum* and *H. isaaci* with LC_{50} and LC_{90} values of 0.78 and 1.00 mg/L, and 1.51 and 1.68 mg/L, respectively (Jayaseelan et al., 2012).

Zahir *et al.* (2012) investigated the efficacy of synthesized Ag nanoparticles (NPs) against the adult cattle tick *Haemaphysalis bispinosa*. These results suggest that the green synthesis of Ag NPs have the potential to be used as an ideal eco-friendly approach for the control of *H. bispinosa* (Zahir et al., 2012).

Rajakumar and Rahuman. (2012) evaluated the effect of plant synthesized silver nanoparticles (AgNPs) to control *Rhipicephalus* ***(****Boophilus****)*** *microplus* and the results showed the LC_{50} values of 16.72 and 3.44 mg/L; r^2 = 0.856 and 0.783), respectively (Rajakumar and Rahuman, 2012) of zinc oxide nanoparticles (ZnO NPs) against the *Hyalomma* spp.. The ZnO NPs showed the LC_{50} 50 mg/mL (Norouzi et al., 2019).

Santhoshkumar *et al.* (2012) determined the efficacies of anti-parasitic activities of synthesized silver nanoparticles (Ag-NPs) against the larvae of the cattle tick, *Rhipicephalus* (*Boophilus*) *microplus.* The results showed that Ag-NPs were an ideal eco-friendly and inexpensive approach for the control of *R.* (*B.*) *microplus* (Santhoshkumar et al., 2012).

Kirthi *et al.* (2011) determined the efficacies of synthesized zinc oxide nanoparticles (ZnO NPs) against the larvae of cattle tick *R.* (*B.*) *microplus.* The synthesized ZnO NPs showed the LC_{50} 13.41 mg/L (Kirthi et al., 2011). Avinash *et al.* (2017) investigated the first time, in vitro acaricidal activity of green silver nanoparticles on deltamethrin resistance *R. (B.) microplus.* These results showed that the DN-Ag NPs had significant acaricidal activity against *R. (B.) microplus* (Avinash et al., 2017). Rajakumar *et al.* (2013) assessed the anti-parasitic activities of nickel nanoparticles (Ni NPs) against the larvae of cattle ticks *R.* (*B.*) *microplus* and *H. a. anatolicum.* The findings revealed that synthesized Ni NPs possess excellent larvicidal parasitic activity (Rajakumar et al., 2013).

Here, the present data indicated that all concentrations of Fe-NPs have a statistically significant difference in the acaricidal activity with different dilutions ($p > 0.05$), and further studies are required to evaluate the efficacy of Fe-NPs *in vivo*.

Conclusions

The "eco-friendly pesticides" are as one of the valuable tools for controlling ectoparasites, therefore necessary to search continuously. Our results suggest that Fe-NPs showed the anti-tick effects against *Hyalomma* spp. at all test times and concentrations and maybe is suitable for the control of *Hyalomma* spp..

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Ethical approval

No applicable.

Conflicts of interest

The author declared no potential conflicts of interest for publication of this paper.

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