RESEARCH ARTICLE

Acaricidal activity of zinc oxide nanoparticles against *Hyalomma* spp. *in vitro*

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ABSTRACT

Objective(s): *Hyalomma* spp. is responsible for transmission of protozoan, bacterial, rickettsial and viral diseases in humans and animals. Recently, there was a wide number of attempts to evaluate and application of nanoparticles for the control of ticks.

Methods: The object of this study was to appraise the acaricidal activity of zinc oxide nanoparticles (ZnO NPs) size 15 nm against *Hyalomma* spp. *in vitro*. The arcaricidal activity of Zn NPs were evaluated at concentrations of 50, 125 and 250 mg/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.

Results: The results of this study revealed all concentrations of Zn NPs had acaricidal activity and concentration of 125 mg/ml after 30 minutes exposure and concentration of 250 mg/ml at all exposure times had highest acaricidal effect (100%). The median lethal concentration (LC50) values were 50 mg/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.

Conclusions: The findings of present study showed that Zn NPs had potent acaricidal effect and recommended as an effective acaricidal agent. However, further *in vivo* studies are required to evaluate the efficacy of this nanoparticle.

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INTRODUCTION

The ticks, which are an important hematophagous ectoparasites of vertebrate animals and humans that causes huge economic loss through anorexia, anemia, toxicosis, general stress, decrease in productivity and the quality of animal's products, depression of immune function, transmission of protozoan, bacterial, rickettsial and viral pathogens and treatment costs [1].

Most tick-borne diseases are caused by microbes which fall in to four general categories: bacteria, rickettsia, viruses and protozoa. The rickettsia category is the largest of the four, containing at least 20 different diseases caused by bacteria in the genus Rickettsia. The bacteria category includes anaplasmosis, ehrlichiosis, Lyme disease, and tularemia. Diseases caused by viruses include Colorado Tick Fever, Crimean-Congo Hemorrhagic Fever, Powassan, and Tick-borne Encephalitis. Babesiosis is caused by protozoa. A disease known not caused by a microbe is Tick Paralysis, which is caused by a toxin [2].

The use of pesticides has decreased the level of diseases. However, pests usually rapid development of resistance in target species, toxicity, effects on public health and also the environmental hazards, and it is therefore necessary to search continuously for Eco-friendly pesticides currently available pesticides [3]. At the present time, green pesticides are claimed as one of the beneficial tools for

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ectoparasites control [4-7].

Now, synthetic acaricides have been widely applied for treating and controlling of the ticks, and obtained the relative good treatment effectiveness, including Ivermectin, and Cypermethrin, etc [8]. The sparing and contact methods with maximum efficiency and minimum efforts as well as are ease of use and flexible.

Recently, there was a global trend to evaluate and present new safe and effective agents as alternative option due to being inexpensive, easy available, low environmental contaminations, low side effects, toxicity and resistance. New functionalities and properties of matter are observed in a wide range of applications. Nanotechnology provides important new tools expected to have 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. For example, silver is an important accessible metal, and its NPs are superior to other nanosized metal particles for their anti-tick effects. However, their stability is a serious problem with polar terminal groups like hydroxyl groups or amine are usually used for their stabilization [9].

Zinc nanoparticles are used in the different field of industry and health. It was used as antifungal antibacterial, anti-corrosive, UV protection agent, catalyst and also used in nanosensors, electronic/nano-optical devices, sunscreens, cosmetic products, food additive and etc [10].

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 Zn NPs with two spraying and contact methods against *Hyalomma* spp. *in vitro*.

MATERIAL AND METHODS

The ticks Collection

The female ticks were randomly gathered 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.

Zn NPs synthesis and characterization

The nano-zinc is metal grey powder. Zn NPs average particle size was 15 nm (10-15 nm) and bulk density was about 0.20 - 0.40 g/m^3. Zn NPs was purchased from the Intelligent Materials Pvt. Ltd., Nanoshel LLC, Wilmington, DE, USA. Nanoshel Zn NPs is produced by evaporation process. Zn NPs were characterized by transmission electron microscopy (TEM, Leo 906, Zeiss 100 KV, Germany). The BET specific surface area was reported about 30-50 m^2/g. The zinc oxide nanoparticles used in this experiment possessed analytical grade with the highest purity.

Evaluation of the acaricidal activity of Zn NPs by contact and spraying methods

In an experiment *in vitro*, it was studied the anti-tick activity of Zn NPs at the 3 concentrations of 50, 125 and 250 mg/ml. For contact method, under optimal conditions (temperature = 25 ± 1 C°, humidity = 55 ± 5%), the circular filter papers of 4.8 cm in diameter (approximate area of 18 cm2) were treated with the provided concentrations of Zn NPs (50, 150 and 250 mg/ml). After drying for 2-3 minutes under a fume hood, the dried filter papers were putted 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 dished were covered with their lids and sealed with parafilm [8]. For spraying method, firstly the filter papers without any treatment were placed in to Petri dishes and groups of 10 ticks transferred on filter papers, after which different concentrations of Zn NPs were sprayed directly on to the ticks and finally the dishes were immediately covered and sealed tightly. With the same manners, Cypermethrin (Cypermethrin 10%, Hacker, Iran) at similar concentration were prescribed as the positive control. Distilled water was administrated as negative control. Three replications were considered for each dilution for the two methods. Subsequently, all Petri dishes were left for a period of 10, 30 and 60 minutes to monitor the acaricidal activity of Zn 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 to be dead [11].

Statistical analysis

The data were analyzed by the GraphPad Prism program version 5 and presented as a mean ± SD. Data were analyzed by two-way ANOVA, then by Student's two-tailed t-test.

RESULTS AND DISCUSSION

The results showed that Zn NPs showed the anti-tick effects against to *Hyalomma* spp. at all test times and concentrations, especially for concentration of 125 mg/ml for 30 minutes and concentration of 250 mg/ml at all exposure times had highest acaricidal effect (100%).

Acaricidal effects of Zn NPs at the dose of 50 mg/ml after 10 minutes of application was lower than others concentrations (14.3%). The median lethal concentration (LC50) values were 50 mg/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. The mortality rate of ticks after exposure to different concentrations of the Zn NPs in various exposure times is presented in Table 1. Fig. 1 illustrates the TEM image of Zn NPs and Fig. 2 showed UV spectrum of this nanoparticle. The optical transmission spectra of ZnO were registered using a UV-VIS spectrophotometer (Hitachi, U-3010).

Newly, nanoparticles have been introduced as novel pesticides against arthropod vectors and pests [12, 14]. The carbon nanoparticles, metal and metal oxide and carbon nanoparticles were reported extremely effective against vectors and arthropod pests [14]. The most parts of these investigations focused on mosquitoes [15], however, only few investigations prescribed nanoparticles on other pests [16-18], beetles [19], blowflies [20], hematophagous flies, sheep biting [21] and harmful mites [22]. Some recent investigations studied on the toxicity of metal nanoparticles on tick vectors that are important in public health. Several studies have anti-tick effects of silver, titanium, nickel and zinc on ticks, such as Marimuthu et al. (2011) used 50 mg of TiO_2 NPs on *Rhipicephalus microplus* larvae. The findings of this study indicated TiO_2 Nano Particles were extremely stable and showed acaricidal property against the larvae of Rhipicephalus *microplus* significantly [23].

Jayaseelan et al. (2012) investigated the efficacy of silver nanoparticles (AgNPs) on the larvae of *Hyalomma marginatum* and *Hyalomma anatolicum*. The highest efficacy was seen in the AgNPs against *H. a. anatolicum* and *H. m. isaaci* with LC_{50} and LC_{90} of 0.78 and 1.00 mg/L, and 1.51 and 1.68 mg/L, respectively [24].

Zahir *et al.* (2003) investigated the efficacy of synthesised Ag nanoparticles (NPs) against the cattle tick *Haemaphysalis bispinosa*. These results suggest that Ag NPs can be used as an eco-friendly pesticide against *Haemaphysalis bispinosa* [25].

Rajakumar and Rahuman. (2012) evaluated the

Table 1. The acaricidal activity of different concentrations of the Zn NPs against *Hyalomma* spp. after various exposure times

	Times	Positive control	Spraying method	Contact method	Negative control
50 mg/ml	10 minutes	100.00	28.5±0.00	14.3±0.00	00.00
	30 minutes	100.00	42.9±1.61	35±1.89	00.00
	60 minutes	100.00	57.2±0.00	42.9±1.61	00.00
125 mg/ml	10 minutes	100.00	78±1.89	64±1.89	00.00
	30 minutes	100.00	100±0.00	71.4±0.00	00.00
	60 minutes	100.00	100±0.00	71.4±0.00	00.00
250 mg/ml	10 minutes	100.00	100±0.00	85.7±0.00	00.00
	30 minutes	100.00	100±0.00	86.7±9.89	00.00
	60 minutes	100.00	100±0.00	87.7±0.00	00.00

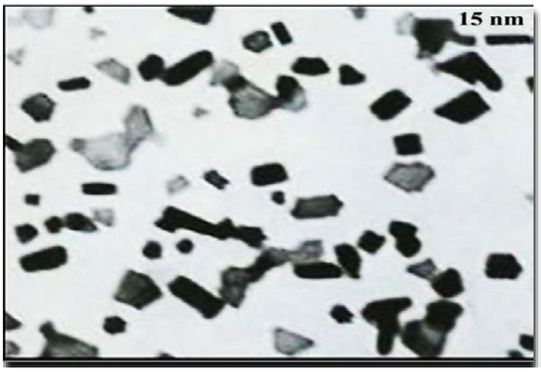

Fig. 1. TEM image of 15 nm Zn-NP

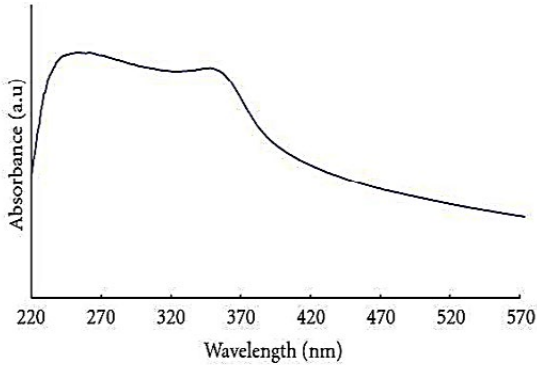

Fig. 2. UV- visible spectrum of the Zn-NPs (Hitachi, U-3010).

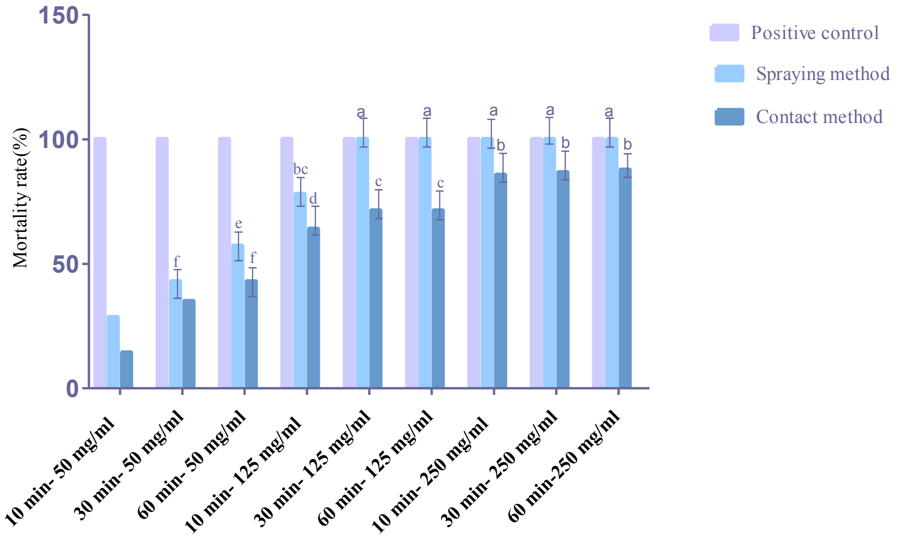

Fig. 3. Acaricidal effects of different concentrations of the Zn NPs against *Hyalomma* spp. after various exposure times

effect of silver nanoparticles (AgNPs) to control *Rhipicephalus (Boophilus) microplus* and the results revealed that LC_{50} of 16.72 and 3.44 mg/L; r^2 = 0.856 and 0.783), respectively [26].

Santhoshkumar *et al.* (2012) determined the efficacies of antiparasitic activities of synthesized silver nanoparticles (Ag-NPs) on the larvae of cattle tick, *Rhipicephalus* (*Boophilus*) *microplus.* They showed that Ag-NPs was an ecofriendly and inexpensive pesticide to the control of *R.* (*B.*) *microplus* [27].

Kirthi *et al.* (2011) determine the efficacies of zinc oxide nanoparticles (ZnO NPs) against the larvae of cattle tick *Rhipicephalus* (*Boophilus*) *microplus*. LC_{50} of ZnO NPs was 13.41 mg/L [28]. Avinash *et al.* (2017) investigated acaricidal activity of green Ag nanoparticles on deltamethrin resistance *Rhipicephalus (Boophilus) microplus.* Deltametrin-Ag NPs had significant acaricidal activity against *Rhipicephalus (Boophilus) microplus.* [29]. Rajakumar *et al.* (2013) evaluated the anti-parasitic effects of nickel nanoparticles against the larvae of cattle ticks *Hyalomma anatolicum* (*a.*) *anatolicum* and *Rhipicephalus (Boophilus) microplus.* The results showed that Ni NPs have ideal larvicidal and anti-parasitic activity [30].

The findings of this study indicated that all concentration of Zn NPs have statistically significant difference in the acaricidal activity with different dilutions ($p>0.05$) and this NPs recommended as a powerful acaricidal agent in control of ticks furthermore, further studies are required to evaluate the efficacy of Zn NPs *in vivo*.

CONCLUSIONS

The "green pesticides" can be used as one of the beneficial approach for controlling ectoparasites, therefore necessary to search continuously for Eco-friendly pesticides currently available pesticides. Our results suggest that Zn NPs showed the anti-tick effects against to *Hyalomma* spp. at all test times and concentrations and is suitable for the control of *Hyalomma* spp..

CONFLICTS OF INTEREST

None of authors had conflict of interests.

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