RESEARCH ARTICLE

In vitro acaricidal activity of nano-SiO2 against *Hyalomma* spp. ticks and its toxicity

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ARTICLE INFO

Article History:
Received 15 September 2021
Accepted 20 December 2021
Published 01 January 2022

Keywords:
Acaricide
Hyalomma spp.
nano-SiO2
Toxicity
MTT assay

ABSTRACT

Objective(s): The *Hyalomma* species of ticks is hematophagous ectoparasites and responsible for bacterial, protozoan, rickettsial and viral infection transmission in vertebrate. Nanobiotechnology is considered as a novel approach for the control of parasites. The purpose of this investigation is for evaluation acaricidal activity of nanocristobalite (nano-SiO2) against the *Hyalomma* spp. in vitro and to determine its toxicity by MTT assay.

Methods: The acaricidal activities of the SiO2-NPs were tested at concentrations of 50, 125 and 250 mg/ml and controls (distilled water and Cypermethrin) at exposure times of 10, 30 and 60 min, were performed each with two replications and two spraying and contact methods. Then the toxicity of each concentration was evaluated by MTT assay. Mortality percentages and LC50 values were calculated. Data were analysed by Graph Pad Prism 5 software.

Results: The results revealed all experimental concentrations of SiO2-NPs had acaricidal effects. The concentration of 250 mg/ml of SiO2-NPs had the highest acaricidal effect (90%) at 60 min exposure time. The experiment results indicated that the spray application method was more effective than the contact application method and the results of MTT toxicity test showed that nano-SiO2 have very little toxicity in all concentrations. The median lethal concentration (LC50) values were obtained at 125 mg/ml in 60 min.

Conclusions: The results indicated that SiO2-NPs contained potent acaricidal activity and might provide new acaricidal compounds for the effective control of *Hyalomma* spp.

How to cite this article
Norouzi R., Irani S., Katiraee F., Hejazy M. In vitro acaricidal activity of nano-SiO2 against Hyalomma spp. ticks and its toxicity . Nanomed Res J, 2022; 7(1): 93-98. DOI: 10.22034/nmrj.2022.01.009

INTRODUCTION

Ticks are hematophagous ectoparasites of vertebrates. They accelerate transmission of some pathogen microorganisms such as protozoan, bacterial, rickettsial, and viral pathogens [1]. Infestation with ticks in animals leads to weight loss and reduced meat and milk production, and anorexia, severe blood loss, toxicity and general stress [1, 2]. *Hyalomma* spp., is an important tick specie that attacks livestock and can cause great economic loss to such livestock. This species transmitted protozoan diseases, such as theilerioses, babesiosis, anaplasmosis as well as Crimean-Congo hemorrhagic fever (CCHF) virus in Iran [4].

The use of chemical acaricidal agents reduces the population of ticks, but these chemicals cause resistance to ticks and the accumulation of residues in food sources, groundwater and the environment causes problems. Therefore, it is necessary to search for green pesticides that have low harm to humans and the environment, and the use of nanoparticles is one of these trends [5,6]. Currently, nanobiotechnology is considered as a novel method in the control and fight of the ticks [7]. Recently, nanoparticles have emerged as new field in advanced research. Silver, zinc, nickel, and iron are important metals that are available, and

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their NPs have been suggested in research as anti-tick agents [6]. So far, the acaricidal effect of SiO2-NPs has not been studied in the world and in Iran.

The applications of SiO2-NPs are different. SiO2-NPs increases bone density, reduces bleeding and porosity, improves the bond between the cement matrix and the aggregates [8]. Recently, SiO2-NPs has developed medical applications and can be used to store drugs [9]. Another useful application of nano silica is to obtain hydrophobic coatings [10]. The purpose of this experiment is to evaluate acaricidal effects of nanocristobalite (nano-SiO2) against the *Hyalomma* spp. *in vitro* and to determine its toxicity by MTT assay.

METHODS

Ticks Collection

In 2020, cluster random sampling was performed in the villages around East Azerbaijan province with the consent of livestock owners. In this study, the female hard ticks of three domestic animals (goats, sheep and cows) were collected by Forceps sampling method and sent to the laboratory with information such as animal name, collection date and place of capture. Finally, ticks were identified under a stereomicroscope (Olympus, Okayama-shi, Okayama, Japan) based on the detection key [11].

Characterization of SiO2-NPs

The Silicon Dioxide (SiO2) amorphous nanopowder is white powder. SiO2-NPs characterized with Purity: 99+%, Average Particle Size (APS): 30-50 nm, Bulk Density; <0.10 g/cm3, True Density: 2.4 g/cm3 and Specific Surface Area: 180-600m2/g. SiO2-NPs was purchased from the Pishgaman Iranian Nanomaterial Company (Iran). SiO2-NPs were characterized by Transmission Electron Microscopy (Leo 906, Zeiss 100 KV, Germany) and Scanning Electron Microscope (Tescan vega co, Czech). The Silicon dioxide

Investigation on the acaricidal effect of SiO2-NPs by contact methods

The acaricidal activities of the SiO2-NPs were evaluated at of 50, 125 and 250 mg/ml at exposure times of 10, 30 and 60 min, were performed each with two replications and two spraying and contact methods.

For contact method, under optimal conditions, the circular filter papers (diameter:4.8 cm) were exposed with the concentrations of SiO2 nanoparticles (50, 150 and 250 mg/ml). The papers dried at room temperature for 2-3 minutes and 10 live ticks were transferred to the dried filter paper. Moisture was provided by a wet cotton putting in petri dish, and at the end, the petri dishes were sealed with parafilms [12].

Investigation on the acaricidal effect of SiO2-NPs by spraying methods

For spraying method, firstly 10 live ticks transferred into petri dishes, then different doses of SiO2-nanoparticles were sprayed on to the ticks, directly. Similar concentration of Cypermethrin 10% (Hacker, Iran) were applied as the positive control. Distilled water was applied as negative control. Two replications for each experiment was applied. Finally, all Petri dishes were left for 10, 30 and 60 minutes to evaluation the acaricidal activity of SiO2 nanoparticles. After exposure time, under a loop, the legs of ticks were agitated with an entomological pin, if the movement was not seen, the tick was categorized as dead [13].

Toxicological effects of SiO2-NPs on Hyalomma spp.

The cytotoxicity of the SiO2-NPs was evaluated by the MTT method. To evaluate cytotoxicity, cells Vero cell line were cultured in a 96-well plat. *Vero* cells were cultured in 50 to 500 mg/ml, respectively four replicates and incubated again for 48 hours. Then, 20 µl of MTT solution at a concentration of 5 mg/ml were added to each well and incubated for 3 hours. The absorbance was read at 570 nm using a spectrophotometer (Aqua Labo, UVILINE 9600, France). Percentage of cell viability was acquired according following formula:

cell viability percentage = (OD negative control / OD of tested sample)100%

Statistical analyses

Data were statistically analyzed by two-way ANOVA test followed by Student's two-tailed t-test using Graph Pad Prism program version 5. The lethal concentration LC_{50} values were calculated by applying regression equation.

RESULTS AND DISCUSSION

The expriment results showed that all concentrations of SiO2-NPs had acaricidal activity and concentration of 250 mg/ml of SiO2-NPs had the highest acaricidal effect (90%) at 60 min exposure time. The dose of 50 mg/ml for 10

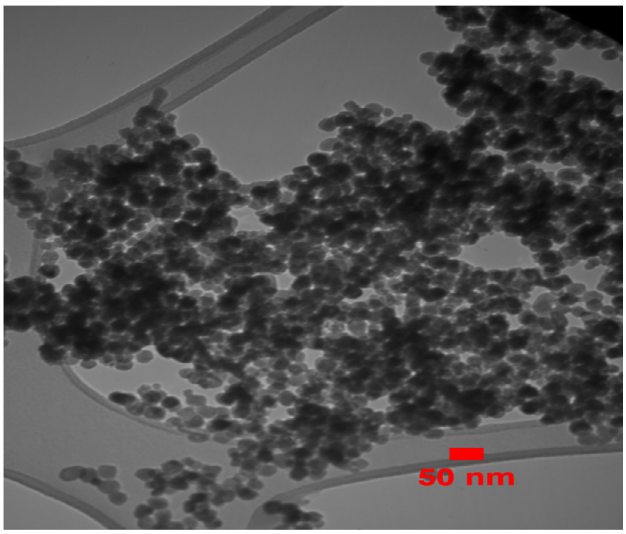

Fig. 1. TEM image of 50 nm SiO2-NPs

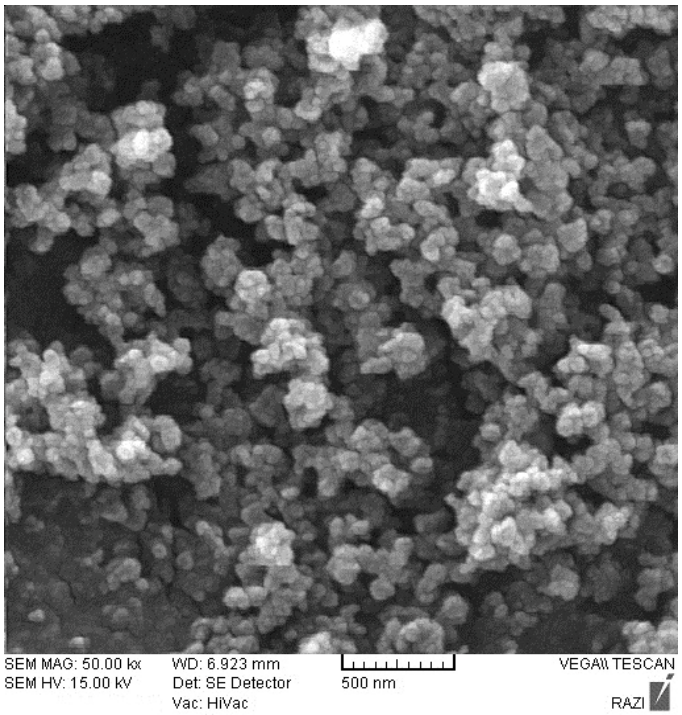

Fig. 2. SEM image of 50 nm SiO2-NPs

minutes had the lowest level of toxicity (10.61%). The median lethal concentration (LC50) values were obtained at 125 mg/ml in 60 min. The results showed that the spray method of exposure is more potent and most effective than the contact exposure method. Fig. 1 and 2 illustrates the TEM and SEM image of SiO2-NPs, respectively.

Tick rate mortality rate after exposure to different doses of the SiO2-NPs in different exposure times is showed in Table 1 and Fig. 3. Different concentrations of all treatments (SiO2-NPs and Cypermethrin) had a significant difference in the two methods of evaluating (P <0.0001). The results of MTT toxicity test showed that SiO2-

Table 1. The acaricidal activity of SiO2-NPs against *Hyalomma* spp. *in vitro*

	Times	Positive control	Sparing method	Contact method	Negative control
50 mg/ml	10 min	100 ± 0.0	14.3 ± 0.0	10.61 ± 4.76	0.0 ± 0.0
	30 min	100 ± 0.0	35 ± 4.76	22.81 ± 4.89	0.0 ± 0.0
	60 min	100 ± 0.0	70 ± 4.89	42 ± 0.0	0.0 ± 0.0
125 mg/ml	10 min	100 ± 0.0	64 ± 0.0	43.81 ± 4.89	0.0 ± 0.0
	30 min	100 ± 0.0	71.39 ± 4.62	50.24 ± 4.76	0.0 ± 0.0
	60 min	100 ± 0.0	75.81 ± 4.89	71 ± 0.0	0.0 ± 0.0
250 mg/ml	10 min	100 ± 0.0	78 ± 0.0	62.81 ± 4.89	0.0 ± 0.0
	30 min	100 ± 0.0	80.81 ± 4.89	78 ± 3.57	0.0 ± 0.0
	60 min	100 ± 0.0	90 ± 4.76	85 ± 0.0	0.0 ± 0.0

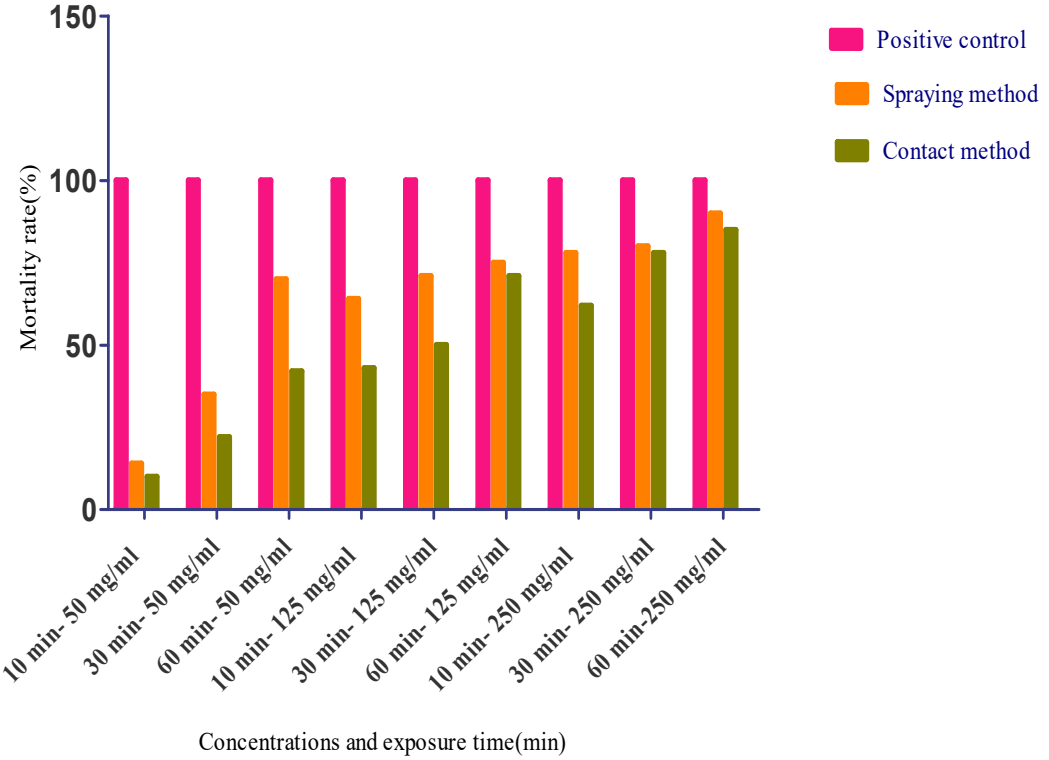

Fig. 3. Acaricidal effects of various concentrations of the SiO2-NPs against *Hyalomma* spp. by spraying and contact method

NPs have very little toxicity in all concentrations Fig. 4. Therefore, in case of possible use of this nanoparticle as an acaricidal agent, skin cells will not suffer from tissue toxicity.

In recent years, resistance to existing anti-arthropod agents has caused much public concern. These concerns have also been exacerbated by the problem of drugs remaining on farmland, pastures, groundwater and food. Therefore, the search for new insect repellents is an urgent and necessary policy. Hence, the existence of nanoparticles as emerging materials has attracted a lot of interest in the control of arthropods and ticks [14]. The prevalence of *Hyalomma* spp. in domestic animals has many destructive effects. Heavy contaminants have adverse effects on animal growth rate, quality and livestock production. On the other hand, it causes the transmission of various diseases in humans and animals. There has been no study evaluating the acaricidal activity of SiO2-NPs on tick *Hyalomma* spp., so this experiment was conducted to determine acaricidal activity of SiO2-NPs against the *Hyalomma* spp. *in vitro* and to determine its toxicity by MTT assay. The results revealed all concentrations of SiO2 nanoparticles had acaricidal effect and concentration of 250 mg/

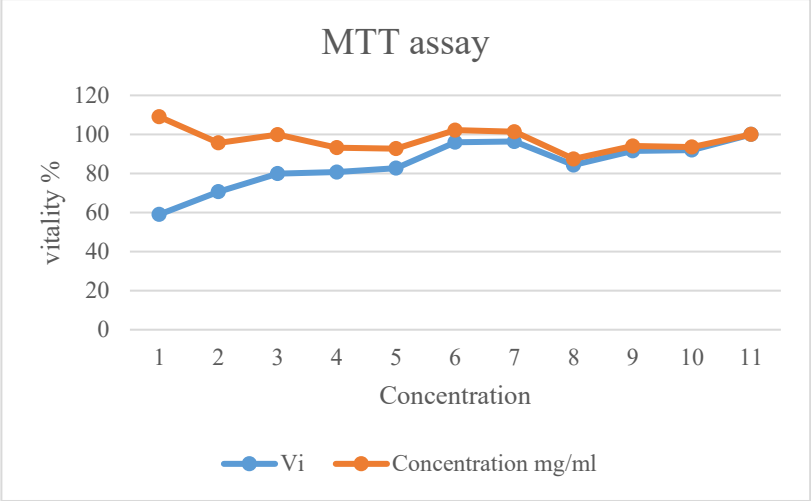

Fig. 4. MTT assay results on *Vero* cell

ml of SiO2-NPs had the highest acaricidal effect (90%) at 60 min exposure time and the results of MTT toxicity test showed that nano-SiO2 have very little toxicity in all concentrations.

Abdel-Ghany et al. (2021) investigated acaricidal activity of nickel oxide nanoparticles against *Hyalomma dromedarii*. The results of their study showed that nickel oxide nanoparticles have a good acaricidal activity in all stage of *Hyalomma dromedarii* life [2]. Norouzi et al. 2019 showed acaracidal activity of Iron nanoparticles on *Hyalomma* spp. They showed acaricidal activityof Iron nanoparticles in all concentrations and the most acaricdal effects at 250 µg/ml at 10 minutes (85.7%). The LC50 of Iron nanoparticles was 50 µg/ml in 60 min for *Hyalomma* spp. Oue results showed that the spray application method was more potent and most effective than the contact method [3]. Zn NPs have shown 100% mortality on Hyalomma spp. by spraying methods and 87.7 % by contact method at concentration of 250 mg/ml [6].

Marimuthu et al. (2011) studied the acaricidal activity of silver nanoparticles prepared by aqueous extract of *Mimosa pudica* leaves against *Rhipicephalus (Boophilus) microplus* and obtain LC_{50} of 8.98 mg/L [14]. Marimuthu et al. (2011) used 50 mg of Titanium dioxide nanoparticles on *Rhipicephalus microplus larvae*. They indicated that TiO_2 NPs had significant acaricidal activity against the larvae of *R. (B.) microplus* [15]. Jayaseelan et al. (2012) studied the effect of silver nanoparticles on *Hyalomma anatolicum* and *H. marginatum isaaci* and obtained LC50 of 0.78 mg/L [16]. The efficacy of silver nanoparticles against the adult cattle tick *Haemaphysalis bispinosa* was reported [17].

Santhoshkumar et al. (2012) studied the effect of silver nanoparticles on the *Rhipicephalus microplus* and obtained LC50 of 50.00 mg/L [18]. ZnO NPs showed good acaricidal activity against *R. (B.) microplus* larvae with LC_{50} of 13.41 mg/L [19]. Copper nanoparticles showed an LC_{50} of 1.06 mg/L against the larvae of *R. (B.) microplus* [20]. Avinash et al. (2017) showed silver nanoparticles efficacy on Deltamethrin resistance *Rhipicephalus (Boophilus) microplus* [21]. Gandhi et al. (2017) a study on the *Rhipicephalus microplus* tick and obtained a LC50 of 6.87 mg/L [22].

The reason for the different acaricide properties of SiO2-NPs in the present study is the difference in nanoparticle type, genus and species of tick, different concentrations and times exposure. The results indicated that SiO2-NPs had potent acaricidal activity and might provide new acaricidal compounds for the effective control of *Hyalomma* spp. Further investigations are needed to determine the efficacy of SiO2-NPs *in vivo.*

CONCLUSION

The nanoparticles can be applied as an effective approach for controlling of ticks. It is important to search for ecofriendly pesticides. Our results suggest that SiO2-NPs have the anti-parasitic effects against to *Hyalomma* spp. ticks at all

concentrations and times of exposure. 250 mg/ml of SiO2-NPs had the highest acaricidal effect (90%) at 60 min exposure time.

ACKNOWLEDGMENTS

We thank the expert of the parasitology department of the Faculty of Veterinary Medicine of Tabriz University.

CONFLICT OF INTEREST

The authors of this have declared there is no conflict of interest.

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