



IN VITRO ACARICIDAL ACTIVITY OF SELENIUM NANOPARTICLES AGAINST *RHIPICEPHALUS* SPP. TICKS AND EVALUATION OF THEIR TOXICITY ON MAMMALIAN CELLS

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Summary

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The *Rhipicephalus* species of ticks is responsible for transmission of pathogenic microorganisms in vertebrate animals and humans. Recently, the use of nano-biotechnology has been proposed as a useful approach to fight against ticks. The aim of this study was to evaluate the acaricidal activity of selenium nanoparticles (Se NPs) against the *Rhipicephalus* spp. *in vitro* and to determine their toxicity by MTT assay. The acaricidal activity of Se NPs in concentrations of 50, 100, 150 and 200 µg/mL was investigated after 15, 30 and 60 minutes, and distilled water and cypermethrin were used as positive and negative controls, respectively. Then the toxicity of each concentration was evaluated by MTT assay. Mortality percentages and LC₅₀ values were calculated. Data were analysed by GraphPad Prism 6.0 software. The results of this study showed that the concentration of 200 µg/mL of Se NPs had the highest acaricidal effect (95%) in the exposure time of 60 minutes and that the spray method was more effective than the contact method. The results of the MTT toxicity test showed that Se NPs had very little toxicity in all concentrations. The median lethal concentration (LC₅₀) values were obtained at 125 µg/mL in 60 min. It was concluded that the Se NPs contained potent acaricidal compounds and may be used as an acaricide compound against *Rhipicephalus* spp.

Key words: antitick, *in vitro*, *Rhipicephalus* spp., selenium nanoparticles, toxicity

INTRODUCTION

Ticks are blood-sucking ectoparasites of vertebrates and humans that transmit various pathogens such as protozoa, bacteria, rickettsials, and viruses during blood feeding (Ravindran *et al.*, 2023). Ticks are the cause of weight loss, anorexia, anaemia, poisoning and general stress of ani-

mals and decrease livestock production such as meat and milk (Abdel-Ghany *et al.*, 2021; Hussain *et al.*, 2024). *Rhipicephalus* spp. are one of the most important species of ticks in Iran, which cause great economic damage to livestock and at the same time transmit protozoan

diseases such as theileriosis, babesiosis, anaplasmosis, as well as Crimean-Congo haemorrhagic fever virus (CCHF) (Norouzi *et al.*, 2020). The use of acaricides causes resistance in ticks, and on the other hand, their persistence in the environment cause environmental hazards and adverse effects on human health (Norouzi *et al.*, 2021). The transmission of a pathogen by a tick, including *Rhipicephalus* spp., depends on ecological, physiological and behavioural characteristics, the probability of finding a host, as well as their ability to survive. They typically spend less energy to find a host and can use their energy reserves to survive for relatively long periods. They also use more energy to absorb a typically large blood meal than other arthropod vectors, which increases the tick's potential as a vector (Pfäffle *et al.*, 2013).

Recently, the use of nanoparticles has been proposed as an alternative to old chemical pesticides. The use of these compounds is increasing day by day because environmental pollution, side effects and toxicity are low ((Kumar *et al.*, 2022; Norouzi *et al.*, 2024). Selenium (Se) plays vital roles in selenoproteins and enzymes, acts as the redox centre of all these enzymes and is essential for their biochemical activity (Fairweather-Tait *et al.*, 2011). There are 25 selenoprotein genes in the human genome. Se is incorporated as selenocysteine in various antioxidant enzymes such as glutathione peroxidase (GPX), thioredoxin reductase and selenoprotein P. Some other important compounds containing Se are sodium selenite, selenomethionine and selenocysteine, which can act as anti-cancer agents (mainly chemopreventive) by different mechanisms (Seale, 2019). The pharmacological effect and toxicity of Se depend on the concentration, redox

state and the type of Se compound. Studies show that anticancer effects are observed at doses close to toxic levels (Yücel *et al.*, 2020). A large number of reports indicate that Se causes G2/M cell cycle arrest and induces apoptosis in cancer cells through the mitochondrial pathway (Sun *et al.*, 2020). Therefore, Se reduces the incidence of many cancers, including lung, prostate, and colorectal cancer, with at least side effects on normal cells (Pietrzak *et al.*, 2024; Barnhart *et al.*, 2024; Shelton *et al.*, 2024). The therapeutic applications of Se NPs include anti-cancer, antioxidant, anti-inflammatory and anti-diabetes, and its anti-cancer activity is due to their pro-oxidant properties in these cells, which induces the synthesis of reactive oxygen species (ROS) that leads to mitochondrial and endoplasmic reticulum damage, which in turn leads to DNA damage (Khurana *et al.*, 2019). So far, the acaricidal effect of Se NPs has not been studied in the world and in Iran, so this study was conducted to evaluate the acaricidal activity of Se NPs against the *Rhipicephalus* spp. *in vitro* and to determine their toxicity by MTT assay.

MATERIALS AND METHODS

Ethical approval

This study was approved by the Ethics Committee of University of Tabriz, Tabriz, Iran with reference number IR.TBZMED.VCR.REC.1402.062.

Ticks collection

In this experimental and *in vitro* study, the cluster random sampling of villages around East Azarbaijan province was done with the consent of livestock farmers. In this study, female ticks were collected from three domestic animals (goat,

sheep, and cow) by anaesthesia and forceps sampling method and sent to the laboratory with information containing the name of the animal, the date of collection, and the place of capture. Finally, ticks were identified under a stereomicroscope (Olympus, Okayama-shi Okayama, Japan), based on the identification key. *Rhipicephalus* spp. gnathosoma is distinct with the lateral margins of the basis capituli forming angular protrusions; causing a hexagonal shape of the basis. Other features seen in *Rhipicephalus* include lack of ornamentation, eyes present, pale rings present on the legs, festoons present, and ventral plates present (Dantas-Torres *et al.*, 2019).

Characterisation of Se NPs

The selenium nanoparticles amorphous nanopowder was a white powder with average particle size 20–30 nm and true density 2.4 g/cm³. Se NPs were purchased from the Pishgaman Iranian Nanomaterial Company (Iran). They were characterised by transmission electron microscopy (TEM, Leo 906, Zeiss 100 KV, Germany) and scanning electron microscopy (SEM, EM3200, KYKY Technology Development Ltd, Beijing, China). The Se NPs used in this experiment possessed analytical grade with the highest purity. Different concentrations of selenium nanoparticles were prepared by dissolving them in sterile distilled water at 45 °C on a rotary device (Heidolph Instruments GmbH & Co. KG, Schwabach, Germany). In the end, 0.45 and 0.20 µm filters were used to homogenise the constituent particles.

Evaluation of the acaricidal effect of Se NPs by spraying and contact methods

Under *in vitro* conditions, the acaricidal activities of the Se NPs were evaluated at concentrations of 50, 100, 150 and 200

µg/mL at exposure times of 15, 30 and 60 min, performed through spraying and contact methods. For the spraying method, ten adult female ticks were transferred to Petri dishes, and then different concentrations of Se NPs were sprayed directly on the ticks. With the same method, cypermethrin 10% (Hekar, Iran) with the same concentration was administered as a positive control. All the Petri dishes were left for 10, 30 and 60 minutes to monitor the acaricidal activity of the Se NPs preparation. After the end of the time, the legs of the ticks were touched with an entomological pin under the stereo microscope (Olympus, Okayama-shi Okayama, Japan), if the legs did not move, the tick was considered dead. Two repetitions were considered for each dilution for the two methods (Kim *et al.*, 2015).

For contact method, under optimal conditions, circular filter papers 4.8 cm in diameter were treated with the provided concentrations of Se NPs (50, 100, 150 and 200 µg/mL). After drying at room temperature for 2–3 minutes, ten live adult ticks were transferred to the dried filter paper, water-soaked cotton was placed in Petri dishes to provide moisture, and finally the Petri dishes were covered with their lids and the parafilms were sealed. After the end of the time, the legs of the ticks were touched with an entomological pin under the stereo microscope (Olympus, Okayama-shi Okayama, Japan) (Baran *et al.*, 2020).

Toxicological effects of Se NPs on Rhipicephalus spp.

The cytotoxicity of the Se NPs was determined by the MTT assay. To measure cytotoxicity, 1×10⁴ healthy Vero cells were cultured separately at a 96-well plate for 24 h. The cells were cultured in four replicates at 50, 100, 150 and 200 µg/mL,

respectively, and incubated again for 48 hours. Then, 20 μ L of MTT solution (thiazolyl blue tetrazolium bromide 98%, Sigma-Aldrich Co., USA) at a concentration of 5 mg/mL were added to each well and incubated for 3h. The supernatant was then removed and 150 μ L of DMSO was added to dissolve the formazan crystals. At each step, centrifugation was performed to remove the liquid. The absorbance was recorded at 570 nm using a plate reading spectrophotometer (Aqua-Labo, UVILINE 9600, France). Cell viability percentage was calculated by the following formula: Percentage of cell viability = (OD negative control / OD of tested sample) $\times$ 100%.

Statistical analysis

The data were expressed as a mean $\pm$ SD and analysed using GraphPad Prism software version 6.0 by a two-way ANOVA test for comparison between the test and controls.

RESULTS

The results of this study revealed that different concentrations of Se NPs with the exception of the concentration of 50 μ g/mL during 15-minute exposure had an acaricidal effect in the contact evaluation method and the concentration of 200 μ g/mL with the exposure time of 60 minutes had the highest acaricidal effect (95%). On the other hand, the results of this study showed that the spraying method was more effective than the contact method. The lethal concentration (LC_{50}) values were obtained at 100 μ g/mL in 30 min.

Fig. 1, 2 and 3 illustrate the TEM, SEM and X-ray diffraction images of Se NPs, respectively. The mortality rate of ticks after exposure to different concentra-

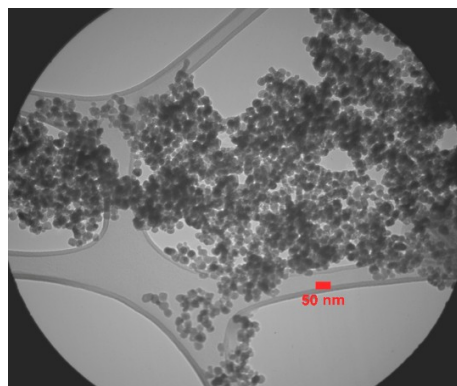


Fig. 1. TEM image of 50 nm Se NPs.

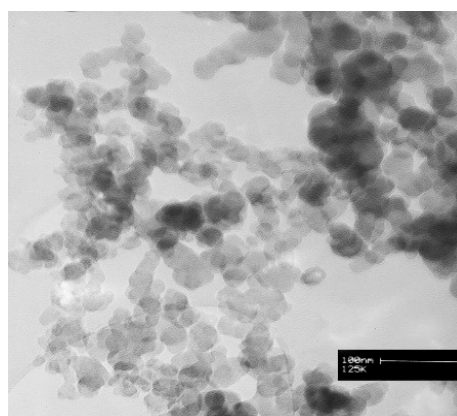


Fig. 2. SEM image of 30 nm Se NPs.

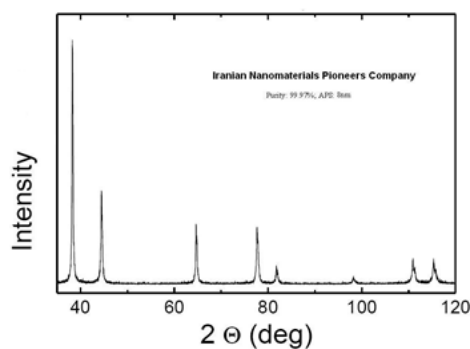
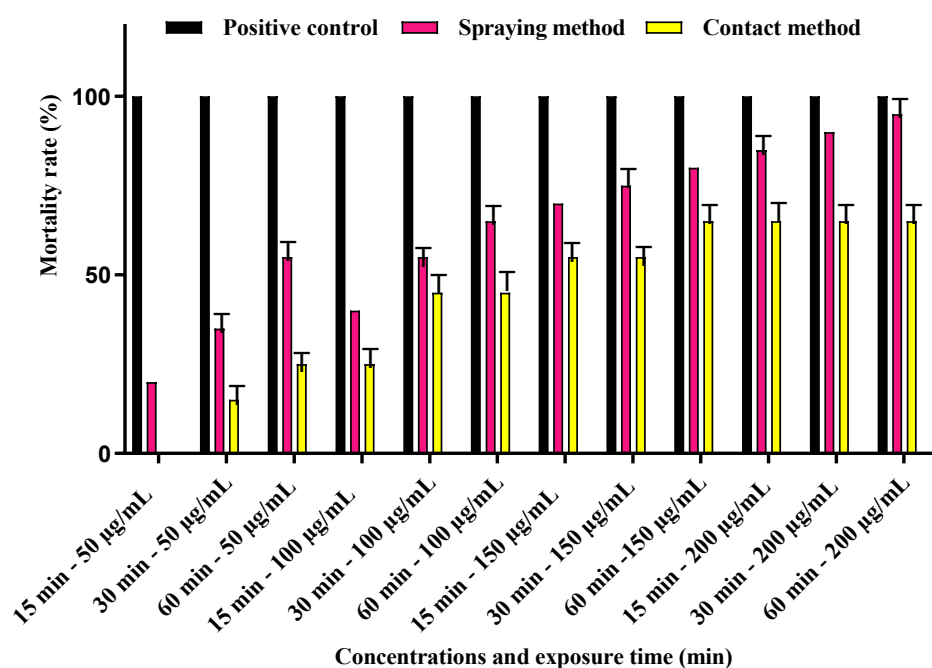


Fig. 3. X-ray diffraction (XRD) of Se NPs.

Table 1. The *in vitro* acaricidal activity (%) of Se NPs against *Rhipicephalus* spp.

Concentrations	Exposure times	Positive control	Spraying method	Contact method	Negative control
50 µg/mL	15 min	100 ± 0.0	20 ± 0.0	0 ± 0.00	0.0 ± 0.0
	30 min	100 ± 0.0	35 ± 4.76	15 ± 4.89	0.0 ± 0.0
	60 min	100 ± 0.0	55 ± 7.07	25 ± 7.07	0.0 ± 0.0
100 µg/mL	15 min	100 ± 0.0	40 ± 0.0	25 ± 4.89	0.0 ± 0.0
	30 min	100 ± 0.0	55 ± 4.62	45 ± 4.76	0.0 ± 0.0
	60 min	100 ± 0.0	65 ± 7.07	45 ± 7.76	0.0 ± 0.0
150 µg/mL	15 min	100 ± 0.0	70 ± 0.0	55 ± 4.89	0.0 ± 0.0
	30 min	100 ± 0.0	75 ± 4.89	55 ± 3.57	0.0 ± 0.0
	60 min	100 ± 0.0	80 ± 7.07	65 ± 4.04	0.0 ± 0.0
200 µg/mL	15 min	100 ± 0.0	85 ± 7.07	65 ± 4.89	0.0 ± 0.0
	30 min	100 ± 0.0	90 ± 4.89	65 ± 4.89	0.0 ± 0.0
	60 min	100 ± 0.0	95 ± 7.07	65 ± 4.89	0.0 ± 0.0

**Fig. 4.** Acaricidal effects of the Se NPs against *Rhipicephalus* spp. by spraying and contact methods.

tions of the Se NPs in various exposure times is presented in Table 1 and Fig. 4. The results of MTT toxicity test showed that Se NPs had very little toxicity in all

concentrations (Fig. 5). Therefore, in case of possible use of this nanoparticle as an acaricidal agent, skin cells will not suffer from tissue toxicity.

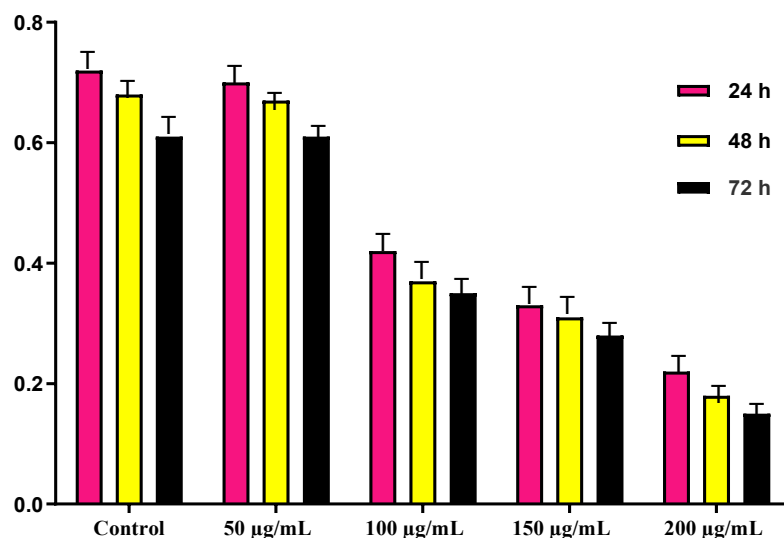


Fig. 5. Results of MTT assay on *Vero* cells.

DISCUSSION

In recent years, pesticide residues in agricultural land, pastures, groundwater, and food have caused concern. Therefore, it is necessary to search for new insect repellents, among which nanoparticles have attracted a lot of attention in the control of arthropods and ticks (Marimuthu *et al.*, 2011). The prevalence of *Rhipicephalus* spp. in domestic animals causes adverse effects on the growth performance and reduces livestock production. On the other hand, they transmit various diseases in humans and animals. No study has evaluated the acaricidal activity of Se NPs on *Rhipicephalus* spp. ticks, so this one was conducted to determine the acaricidal activity of Se NPs against the *Rhipicephalus* spp. *in vitro* and to determine their toxicity by MTT assay. The results revealed that the concentration of 200 µg/mL with exposure time of 60 minutes

attained the highest acaricidal effect (95%) and the results of MTT toxicity test showed that nano-Se had very little toxicity in all concentrations. The study conducted by Abdel Ghany *et al.* (2021) showed that nickel oxide (NiO) nanoparticles had a good acaricidal activity on eggs, larvae, engorged nymphs and fully fed females of *Hyalomma dromedarii* with LC₅₀ values for on embryonated eggs, larvae and engorged nymphs of 5.00, 7.15 and 1.90 mg/mL, respectively.

Another study showed that the concentration of 250 µg/mL of iron nanoparticles had the highest acaricidal effect (85.7%) on *Hyalomma* spp. ticks after 10 minutes of exposure (Norouzi *et al.*, 2020). An earlier study by Nourozi *et al.* (2019) found that zinc nanoparticles at a concentration of 250 mg/mL led to 100% mortality of *Hyalomma* spp. Marimuthu *et al.* (2011) surveyed the acaricidal activity of green synthesised silver nanoparticles

(Ag NPs) prepared by aqueous extract of *Mimosa podica* leaves against *Rhipicephalus (Boophilus) microplus* and obtained an LC₅₀ of 8.98 mg/L. The findings of Marimuthu *et al.* (2013) showed that titanium dioxide nanoparticles prepared by *Calotropis gigantean* extract possessed significant acaricidal activity on *Rhipicephalus microplus* larvae. Jayaseelan & Rahuman (2012) studied the effect of Ag NPs on *Hyalomma anatolicum* and *H. marginatum isaaci* and obtained LC₅₀ of 0.78 mg/L.

The green synthesis of Ag NPs from *Euphorbia prostrata* leaves had a good potential as an ideal eco-friendly approach for the control of *Haemaphysalis bispinosa* (Zahir & Rahuman, 2012). Santhoshkumar *et al.* (2012) studied the effect of *Cissus quadrangularis* stem aqueous extract and synthesised Ag NPs on *Rhipicephalus microplus* and obtained LC₅₀ of 50.00 mg/L. Copper nanoparticles synthesised by polyol process showed an LC₅₀ of 1.06 mg/L against *R. (B.) microplus* larvae (Ramya Devi *et al.*, 2011). Avinash *et al.* (2017) investigated for the first time, *in vitro* acaricidal activity of green Ag NPs on deltamethrin resistant *Rhipicephalus (Boophilus) microplus* and demonstrated that the DN-Ag NPs had significant acaricidal activity against *R. (B.) microplus*.

The reason for the different acaricide properties of Se NPs in the present study was the difference in nanoparticle type, tick genus and species, different concentrations and exposure times. The results indicated that Se NPs had potent acaricidal activity and might provide new acaricidal compounds for the effective control of *Rhipicephalus* spp. However, further investigations are needed to determine the efficacy of Se NPs *in vivo*. The limitation of this study was that other

characteristics of selenium nanoparticles were not provided to us by the manufacturing company, and that because of lack of satisfaction of the livestock farmers and their opposition, it was not possible for us to conduct experiments *in vivo*.

CONCLUSION

The nanoparticles are considered as one of the useful tools to control ticks, and the continuous search for environmentally friendly pesticides is necessary. Our results proved that Se NPs showed anti-tick effects against *Rhipicephalus* species and the concentration of 200 µg/mL with exposure time of 60 minutes resulted in the highest percentage of acaricidal effect (95%). According to results from the MTT toxicity test, the Se NPs had very little toxicity in all concentrations. It is suggested that this study be conducted at higher concentrations to achieve 100% mortality.

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