B-5031
[1-8]

RESEARCH ARTICLE Indian Journal of Animal Research

Green Synthesized ZnO Nanoparticles (ZnO-NPs) and Its Acaricidal Action against *Rhipicephalus (B). microplus*

Raguvaran Raja[1], Debabrata Mondal[1], M.V. Jithin[1,2], Narayani Yadav[1], Dushyant Kumar Sharma[1,3], Sonam Bhatt[1,4], Karikalan Mathesh[5]

10.18805/IJAR.B-5031

ABSTRACT

Background: Tick population in India has developed resistance against all commonly available acaricides and there have been only limited research activities to develop novel acaricides due to the enormous costs involved. Therefore, alternative therapy is need of the hour to tackle the acaricidal resistance.

Methods: The present study envisages *Annona squamosa* seed based synthesis of ZnO nanoparticles (ZnO-NPs) for its acaricidal potential. Mean size of ZnO-NPs was 292.75 nm as per scanning electron microscope. Zeta potential of ZnO-NPs was (-) 38.9 mV. ZnO-NPs showed characteristic intense peaks in XRD analysis at 2θ of 31.89°and 45.64° due to its crystalline nature. In FTIR, the peak observed at 3754.73 cm^{-1} may be due to (-) OH stretching and deformation. ZnO NPs showed a strong absorbance peak at 300 nm confirmed the stability of nanoparticles.

Result: Critical analysis of reproductive index study with ZnO-NPs revealed consistent acaricidal property of 2% ZnO-NPs even in the next generation of tick. Cytotoxicity evaluation revealed absence of aggregation as well other morphological changes of RBC, which indicated safer use of synthesized ZnO NPs on the animal body.

Key words: Acaricide, *Annona squamosa*, Green synthesis, Nanoparticles, Zinc oxide.

INTRODUCTION

Parasitic diseases in particular ectoparasitic infestation is a global problem and considered as major obstacle in the health and production performance of animal industries. Among the ectoparasites, ticks and tick-borne diseases (TTBDs) continue to be the major constraint globally on profitable livestock production (Mondal *et al*., 2013). TTBDs were ranked high globally in terms of their impact on the livelihood of resource-poor farming communities in developing countries including India (Minjauw and McLeod, 2000). Calculated costs for control of TTBDs affecting Indian livestock was 498.7 million US $ per annum (Minjauw and McLeod, 2003). *Rhipicephalus (Boophilus) microplus*, is the most prevalent cattle tick in various agro climatic zones of India and infesting all age groups of cattle, horse, sheep, goat, deer (Ghosh *et al*., 2007), mithun (Rajkhowa *et al*., 2005), yak (Mondal, 2006) and wild herbivores (Singh *et al*., 1978).

At present, tick control is predominantly based on large-scale repeated use of synthetic acaricides, *viz.* cypermethrin, deltamethrin, fenvalerate, diazinon, amitraz, flumethrin and ivermectin. Application of chemical acaricides has had limited efficacy in reducing tick infestations and is often accompanied by serious drawbacks, including the development of acaricide resistant ticks, environmental contamination and residual contamination of acaricides in milk and meat (Kumar *et al*., 2020). As per the report published by FAO, the tick population in India has developed resistance against all commonly available acaricides and there have been only limited research activities to develop newer classes of acaricides due to the enormous costs involved (FAO, 2004). Nanotechnology has enormous potential in livestock sector various nanoparticles have been prepared for diagnostic and treatment purpose. Nowadays plant mediated nanoparticles synthesis has attracted attention of researchers globally.

Annona squamosa is a multipurpose tree with edible fruits and is of an enormous source of medicinal and industrial products (Zahid *et al*., 2018). Aqueous extract of *A. squamosa* seed extract with 8% concentration revealed lethal to 70.8% tick population by 24 h of treatment (Magadum *et al*., 2009). Metal nanoparticles such as Zinc Oxide, Copper, Silver, Nickel have been tested for their acaricidal

[1]Division of Medicine, ICAR-Indian Veterinary Research Institute, Bareilly-243 122, Uttar Pradesh, India.
[2]Department of Veterinary Clinical Complex, Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut-250 002, Uttar Pradesh, India.
[3]ICAR-Central Sheep and Wool Research Institute, Avikanagar-304 501, Rajasthan, India.
[4]Department of Medicine, Bihar Animal Sciences University, Patna-800 001, Bihar, India.
[5]Wildlife Section, Indian Veterinary Research Institute, Bareilly-243 001, Uttar Pradesh, India.

Corresponding Author: Raguvaran Raja, Division of Medicine, ICAR-Indian Veterinary Research Institute, Bareilly-243 122, Uttar Pradesh, India. Email: raguivri@gmail.com

How to cite this article: Raja, R., Mondal, D., Jithin, M.V., Yadav, N., Sharma, D.K., Bhatt, S. and Mathesh, K. (2024). Green Synthesized ZnO Nanoparticles (ZnO-NPs) and Its Acaricidal Action against *Rhipicephalus (B.). microplus*. Indian Journal of Animal Research. 1-8.doi:10.18805/IJAR.B-5031

Submitted: 03-10-2022 **Accepted:** 02-10-2024 **Online:**13-12-2024

efficacy against *R.* (B). *microplus* (Kirthi *et al.*, 2011; Ramyadevi *et al*., 2011; Santoshkumar *et al.*, 2012; Rajkumar *et al.*, 2013). ZnO nanoparticles showed potent acaricidal activity against *Hyalomma* spp (Norouzi *et al*., 2019). ZnO NPS could be used for pest management for stored product pest (Hamdy *et al*., 2023). There are limited studies available on the use of *A. squamosa* as an acaricide to control *R (B). microplus.* In addition to this, synthesis of ZnO-NPs using the seed extract of *A. squamosa* has not been reported till date. In the present study, an attempt was made for synthesis and characterization of *A. squamosa* seed based ZnO-NPs to develop potent acaricide against *R. (B) microplus* without hampering the normal body system of the host animal.

MATERIALS AND METHODS

Green synthesis of *A. squmosa* based ZnO-NPs

The work was conducted at medicine division of ICAR-IVRI, UP during 2018-2019. *A. squamosa* seed aqueous extract based ZnO-NPs were prepared by the method described earlier with some modifications (Banumathi *et al*. 2016). Briefly, zinc acetate dehydrate was added to 50 ml of distilled water under constant stirring using a magnetic stirrer. Eight per cent (8%) of aqueous extract of *A. squamosa* seed was introduced into the above solution after 10 minutes of stirring. The solution was kept under vigorous stirring for 6 hours thereafter; the synthesized nanoparticles were collected by centrifugation at 12000 rpm for 45 minutes. The pellet was subjected to lyophilization and the product was preserved in air tight container for further use.

Characterization of green synthesized ZnO-NPs

Scanning electron microscopy (SEM) Analysis

Scanning electron microscope was used to study the morphology of nanoparticles. Drop of sample was gold coated with JFC-1600 auto fine sputter gold coater. The coated sample was viewed under SEM (JEOL JSM-6610LV) and imaging was performed.

Zeta analysis

Zeta potential of the ZnO-NPs was obtained with the aid of zetasizer.

X-ray diffraction (XRD) analysis

XRD was used to analyze the crystalline pattern of ZnO-NPs. ZnO-NPs were coated on XRD grid and the measurements were recorded at an acceleration voltage of 40 kV and current of 30 mA.

Fourier transform infrared spectroscopy (FT-IR) analysis

The FTIR spectrum was recorded in the wave numbers ranging from 400 to 4000 cm^{-1} using FTIR spectroscopy.

U.V. visible spectroscopic analysis

The optical properties of ZnO-NPs were measured by UV-spectrophotometer. The study was carried out for 6 hours at room temperature and the absorption was measured at different wavelength starting from 300 nm to 800 nm using *A. squamosa* seed extract as reference.

Acaricidal activity of green synthesized ZnO-NPsby adult tick immersion test

Fully engorged adult female *R.* (*B). microplus* ticks were collected from infested cattle, washed with distilled water and dried using clean soft tissue paper. Adult tick immersion test (ATI) was performed as per the protocol described earlier with minor modification (Drummond, 1983). Different concentrations of ZnO -NPs were soaked on Whatman filter paper No. 1 in a 90 mm petridish. The control papers were soaked with distilled water. Petridishes were placed at a temperature of 28-30°C and 80% relative humidity (RH) in an incubator. Petridishes were opened at distinct time intervals (24, 48, 72 hours) and the number of live and dead ticks was recorded. Adult mortality was determined by using Abbott's formula.

$$\text{Corrected mortality (\%)} = \frac{\text{\% treated mortality - \% control mortality}}{\text{100 - \% control mortality}} \times 100$$

In vitro population limiting properties of green synthesized ZnO-NPs

The surviving ticks from the ATI test were weighed individually and kept in a tick rearing tube for oviposition. When oviposition ceased, the eggs laid in each tube were weighed separately and recorded to calculate the reproductive index. The reproductive index (RI), alteration in the egg mass production (DO %), alteration in the weight of adult tick (DR %) and the percent efficacy of the *A. squamosa* extract and ZnO NPs (E %) of ticks were calculated as follows:

$$\text{Reproductive index (RI)} = \frac{\text{Egg mass}}{\text{Engorged tick weight}}$$

DR % (% reduction of mean weight of adult female)

$$= 100 \times 1 - \frac{\text{PMTV}}{\text{PMTC}}$$

PMTV = Mean weight of adult female of treated group
PMTC = Mean weight of adult female of control group

DO % (% reduction of mean weight of eggs) =

$$100 \times 1 - \frac{\text{PATV}}{\text{PATC}}$$

PATV = Mean weight of eggs of treated group
PATC = Mean weight of eggs of control group
E % (Efficacy of drug) = 100 ×[1-(CRT-CRO)]
CRT = Reduction in weight of adult female (PMTV/PMTC)
CRO = Reduction in egg laying capacity (PATV/PATC)

Hemolytic activity of green synthesized ZnO NPs

Spectrophotometric method

Hemolytic potency of ZnO NPs was assessed by spectrophotometer method (Raghava *et al*., 1994). Drug samples were dissolved in 1 ml of PBS and after sonication, two fold serial dilutions were made. Twenty micro liter (µl)

of RBC suspension was added to 96 well plates to which 100 µl of each concentration of drug was added. For complete haemolysis, cells were suspended in 100 µl of distilled water (Positive control). For negative control cells were suspended in PBS with pH 7.4. Plate was incubated at 37°C for 90 minutes after that content was transferred to eppendorf tubes and centrifuged at 3000 rpm for 5 min. Supernatants were directly transferred to new 96 well plates by using multipipette**.** Optical density of each well was taken at 543 nm by using UV spectrophotometer.

$$H = \frac{OD_S - OD_0}{OD_{100} - OD_0} \times 100$$

The per cent hemolysis caused by ZnO NPs at a given concentration was calculated by the following formula

Where H, OD_S, OD_0, OD_{100} are per cent haemolysis, optical density in the presence of ZnO NPs, the presence of drug solubilising buffer and the presence of water, respectively.

Microscopic method

Haemolytic potency of ZnO NPs was assessed as described earlier with minor modifications (Simundic *et al.*, 2013). Two fold serial dilutions of the ZnO NPs were prepared in 1 ml of 1X PBS. Ten µl of ZnO NPs were mixed with 50 µl of final RBC suspension and kept at 37°C in incubator. After 24 hr of incubation, blood smears were prepared out of the sample and examined under microscope along with control.

RESULTS AND DISCUSSION

Green synthesis of ZnO-NPs

In our study, *A. squamosa* seed extract based ZnO-NPs were prepared and subjected to characterization and toxicity studies.

Characterization of green synthesized ZnO-NPs

Scanning electron microscopy (SEM) analysis

SEM (JOEL JSM-6610LV) was used to determine the size and shape of nanoparticles. SEM picture depicting the ovoid structure of nanoparticles is shown in Fig 1. Minimum size of ZnO NPs was 250 nm with a maximum of 303 nm and the average of 292.75 nm. The functional performance of nanoparticles depends on size, morphology and physical state (Galindo-Rodriguez *et al.*, 2004). *Plectranthus amboinicus* leaf extract-assisted biosynthesis of ZnO-NPs revealed rod shaped nanoparticles with a diameter range of 50-180 nm (Fu and Fu, 2015). Green synthesized ZnO-NPs using leaf extract of *Ixora coccinea* had spherical shaped nanoparticles with a diameter range of 80-130 nm (Yedurkar *et al*. 2016). These variations in the size and shape of nanoparticles are due to the nature of plant, amount of plant extract used, kind of parent zinc salt and application of particular centrifugal speed and time upon nano-structured component.

Zeta analysis

ZnO-NPs were dissolved in distilled water and after sonication (40 volts, 15 min), samples were subjected to zeta analysis (Malvern zeta sizer). Polydispersity index (PDI) and zeta potential of ZnO-NPs were 0.5 and -38.9, respectively (Fig 2). Zeta potential states the potential stability of synthesized nanoparticles. Zeta potential of ZnO NPs prepared using *Myristica fragrans* was (-) 22.1 mV (Faisal *et al*., 2021). If the sample has large negative or positive zeta potential values, particles will repel each other and there will be no aggregation (Hughes *et al*., 2015). On the other hand, if the sample has small zeta potential values there is more chance for aggregation of particles. Optimum zeta potential value is greater than (+) 30 mV or smaller than (-) 30 mV ((Yedurkar *et al*., 2016). Obtained zeta potential (-38.9 mV) in the present study indicated the stability of particles in colloids.

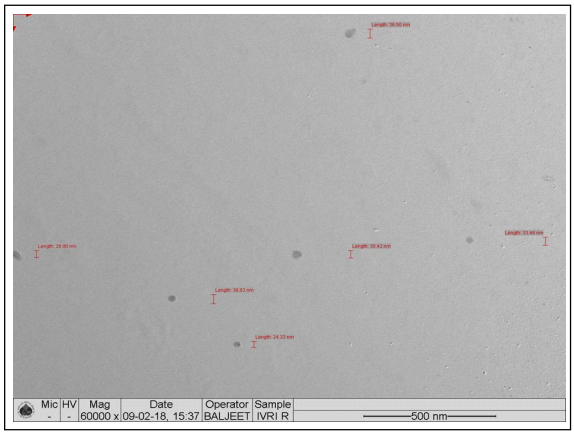

Fig 1: Transmission electron microscopic image of green synthesized ZnO NPs.

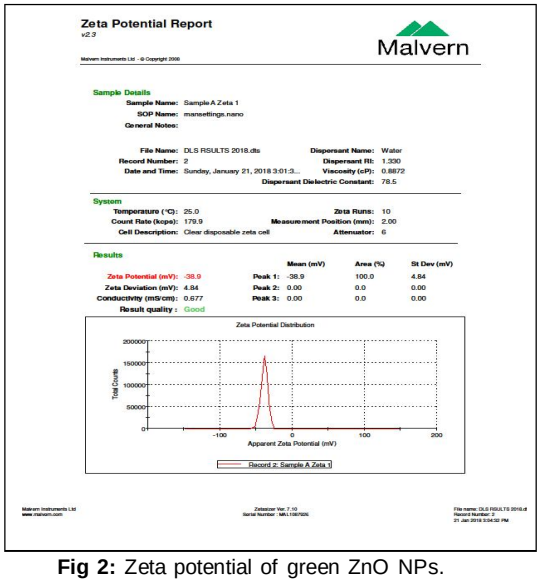

Fig 2: Zeta potential of green ZnO NPs.

X-ray diffraction (XRD) analysis

ZnO-NPs showed the characteristic intense peaks at 2θ of 31.89° and 45.64° due to its crystalline nature. In addition to this, moderately intense peaks at 2θ of 34.6° and 56.4° were also observed (Fig 3). These findings are consistent with previous studies (Fakhari *et al*., 2019; Unni *et al*., 2022). Almost similar values were reported (Thirunavukkarasu *et al*., 2016) for ZnO-NPs prepared by sol gel method with the characteristic intense peaks at 2θ of 31.85°, 34.32°, 36.47°, 47.61°, 56.62°. Likewise, another study also reported 2θ values at 31.84°, 56.7° for green synthesized ZnO-NPs (Yedurkar *et al*., 2016). This indicated crystalline nature of ZnO-NPs in the present study.

Fourier transform infrared spectroscopy (FT-IR) analysis

Possible functional groups present in the synthesized ZnO-NPs were confirmed by FTIR analysis. Peak observed at 3754.73 cm^{-1} may be due to-OH stretching and deformation. Similarly, peaks at 1643.05 cm^{-1} and 732.81 cm^{-1} correspond to zinc oxide stretching and vibration respectively (Fig 4). Similar results were reported by authors who reported absorption peak at 3398 cm^{-1} and OH stretching at (3517 cm^{-1}) for ZnO-NPs prepared by sol gel method, respectively (Yedurkar *et al*., 2016; Thirunavukkarasu *et al*., 2016). A previous study by Unni *et al*. (2022) reported FTIR peaks at 3640 cm^{-1} (hydroxyl group), 2100 cm^{-1} (C≡C terminal alkyne), 1739 cm^{-1} (C-O stretching) and 1490 cm^{-1} (C-H bending). Therefore, this inference confirmed the presence of ZnO-NPs of the present study.

U.V. visible spectroscopic analysis

Optical absorption property of ZnO-NPs was recorded by UV-spectroscopic analysis. ZnO-NPs showed a strong absorbance peak at 300 nm in the entire time span (1-6 hour) which confirmed the stability of synthesized nanoparticles (Fig 5). It was in accordance with previous study wherein author synthesized ZnO NPs from leaves of L. *leschenaultiana* and reported a strong absorbance peak

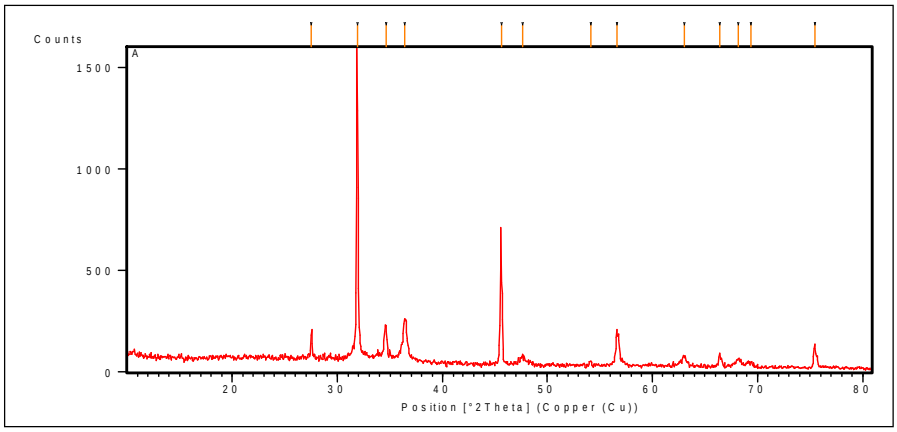

Fig 3: X-ray diffraction pattern analysis of green synthesized ZnO NPs.

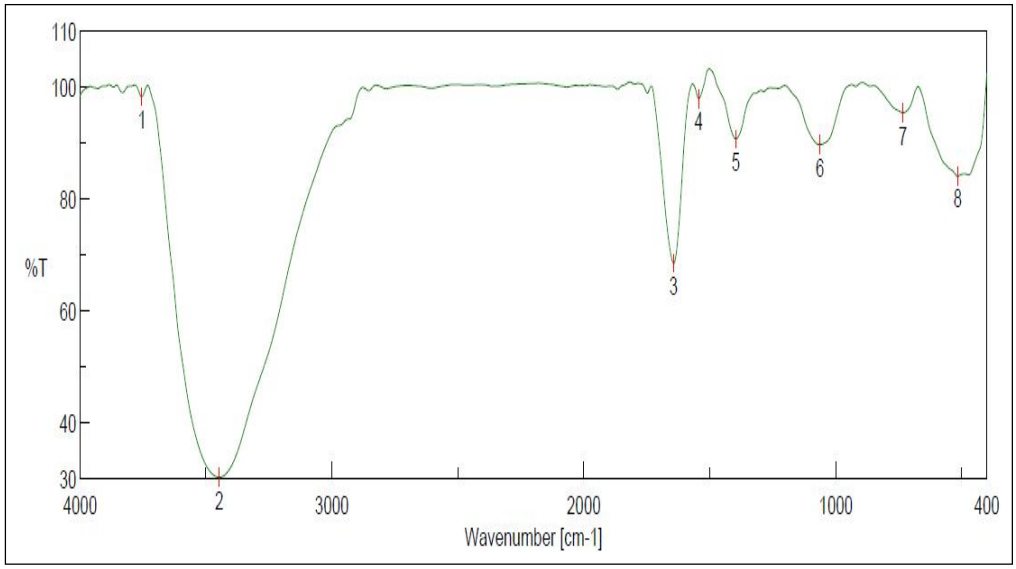

Fig 4: Fourier transform infrared spectroscopic analysis of green ZnO NPs.

at 383 nm (Banumathi *et al.*, 2016). So, the present study reconfirmed the stability of nanoparticles. ZnO NPs prepared from green alage showed absorbance peak at 370 nm confirmed successful formation of nanoparticles using *L.pruinosum* (Naiel *et al*., 2022).

Acaricidal activity of green synthesized ZnO-NPs by adult tick immersion test

Adult tick immersion test (ATI) (Fig 6) depicted no consistent tick mortality from 2 to 10% of aqueous extract (AE) at 24 hr, 48 hr and 72 hr. However, significantly ($p<0.05$) increased tick mortality was observed in both 48 hr and 72 hr as compared to 24 hr study in AE treated group. In contrast, significant reduction in tick mortality with 1% AE of *A. squamosa* was observed as compared to higher concentrations of 2 to 10% A.E (Table 1).

Contrary to AE of *A. squamosa*, green synthesized ZnO-NPs depicted inverse concentration dependent tick mortality at 24 hr, 48 hr and 72 hr with 2 to 10% concentrations. However, similar trend was not observed with 1% concentration of ZnO-NPs at any time intervals. Critical analysis of the study revealed consistent tick mortality in different time intervals with 2% concentration of ZnO-NPs (Table 1). Similarly, green synthesized ZnO NPs exhibited excellent acaricidal activity against all developmental stages of *Hyalomma dromedary* (Abdel-Ghany, 2022).

Evaluation of population limiting property of green synthesized ZnO-NPs

There was a significant reduction in DR% (reduction in adult tick weight) with higher concentration AE as compared to ZnO-NPs. Highest DO% (reduction in egg weight) was observed with 10% AE as compared to ZnO-NPs. There was consistently significant variation in the efficacy (E%) of AE as well as ZnO-NPs from 10% to 2% concentrations.

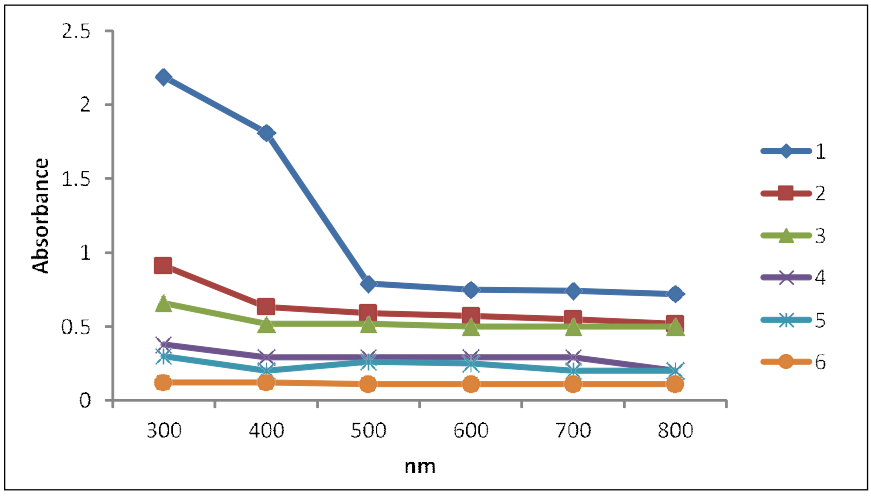

Fig 5: UV-Spectroscopic analysis of green synthesized ZnO NPs.

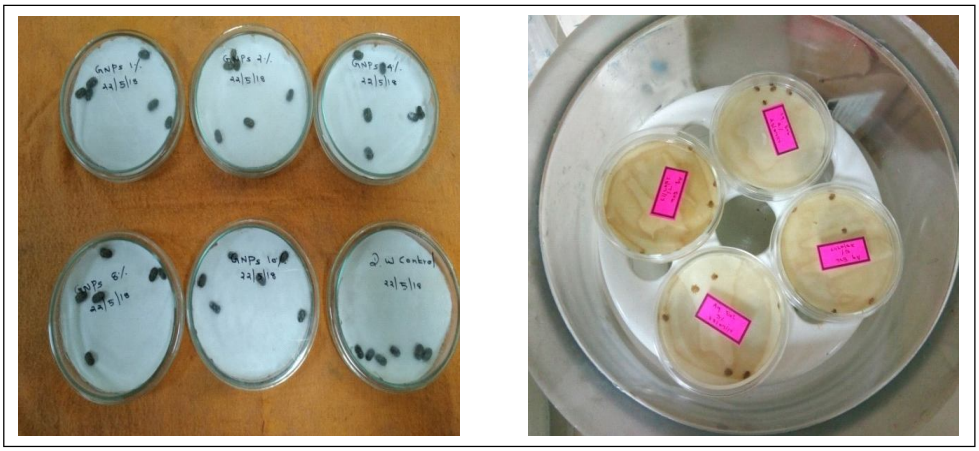

Fig 6: Adult tick immersion test.

Table 1: Per cent (%) Mortality of ticks in ZnO-NPs by adult tick immersion test.

Treatment group	Concentrations%	Tick mortality (%) at different hours		
		24	48	72
AE	10	83.33 ^{C}	100.00 ^{E}	100.00 ^{E}
	8	83.33 ^{C}	100.00 ^{E}	100.00 ^{E}
	4	83.33 ^{C}	100.00 ^{E}	100.00 ^{E}
	2	83.33 ^{C}	100.00 ^{E}	100.00 ^{E}
	1	16.66 ^{A}	16.66 ^{A}	16.66 ^{A}
ZnO-NPs	10	50.00 ^{B}	50.00 ^{BC}	50.00 ^{BC}
	8	50.00 ^{B}	50.00 ^{BC}	50.00 ^{BC}
	4	66.66 ^{BC}	66.66 ^{CD}	66.66 ^{CD}
	2	83.33 ^{C}	83.33 ^{DE}	83.33 ^{DE}
	1	16.66 ^{Aa}	33.33 ^{ABa}	66.66 ^{CDb}

(AE- Aqueous seed extract of *A. squamosa*, ZnO-NPs - Green synthesized ZnO-NPs).

Superscripts with different small letters varied significantly (pe"0.05) within different hours of each treatment concentrations of a group. Superscripts with different capital letters varied significantly (pe"0.05) between the different treatment concentrations at same time of treatment.

Table 2: Population limiting property of ZnO-NPs.

Parameters	Treatment	Groups treatment concentrations (%)				
		10	8	4	2	1
DR (%)	AE	64 ^{dB}	55 ^{cB}	53 ^{cB}	27 ^{bA}	8 ^{aA}
	ZnO-NPs	37 ^{dA}	33 ^{cA}	30 ^{bcA}	26 ^{bA}	22 ^{aB}
DO (%)	AE	34 ^{bB}	27 ^{aB}	27 ^{aB}	25 ^{aB}	25 ^{aB}
	ZnO-NPs	18 ^{dA}	15 ^{cA}	10 ^{bA}	9 ^{bA}	5 ^{aA}
E (%)	AE	83 ^{eB}	75 ^{dB}	71 ^{cB}	55 ^{bB}	24 ^{aA}
	ZnO-NPs	49 ^{dA}	44 ^{cA}	34 ^{bA}	33 ^{bA}	29 ^{aB}
R	AE	0.9 ^{dB}	0.82 ^{cB}	0.79 ^{cB}	0.52 ^{bA}	0.41 ^{aA}
	ZnO-NPs	0.6 ^{aA}	0.64 ^{abA}	0.66 ^{bA}	0.62 ^{abB}	0.62 ^{abB}

(AE- Aqueous seed extract of *A. squamosa*).

Superscripts with different small letters varied significantly (p≥0.05) within each treatment group of a parameter in study. Superscripts with different capital letters varied significantly (p≥0.05) between the two treatment groups of a parameter in study.

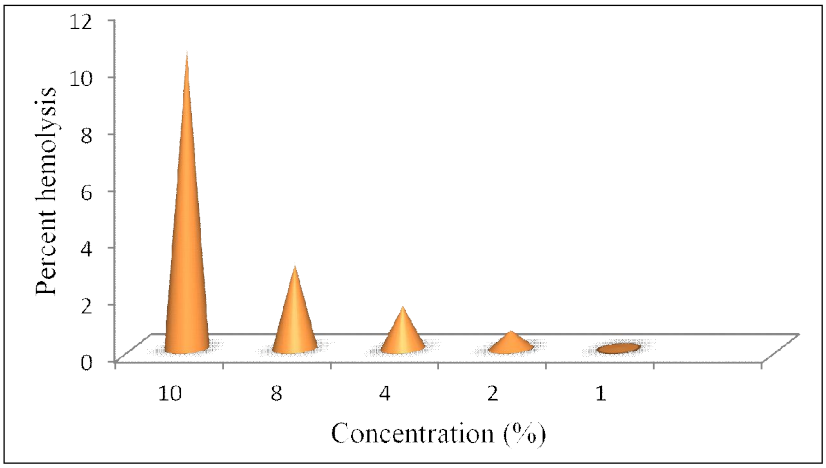

Fig 7: Hemolytic assessment of green synthesized ZnO NPs.

Regarding reproductive index (RI), ticks exposed with ZnO-NPs depicted consistent values at all concentrations as compared to AE. Critical analysis of reproductive index revealed consistent efficacy with the potent acaricidal property of 2% ZnO-NPs even in the next generation (Table 2) and may be a key component to reduce tick load from diverse pasture in the future.

Hemolytic assessment by spectrophotometer method

Suspension of RBC treated with two fold serial dilutions of ZnO NPs and % hemolysis estimated by spectrophotometric evaluation revealed positively correlated concentration dependent cytotoxicity at higher concentrations (4% and 8%) which continues to be toxic at further higher concentration (10%). Both 2% and 1% concentrations showed negligible per cent hemolysis. Increased concentration of nanoparticles depicted a positive correlation with cytotoxicity as was evident in Fig 7a. The study revealed concentration dependent cytotoxicity of ZnO-NPs but not affecting the host beyond the target species at lower concentrations but highly toxic at higher concentrations.

Hemolytic assessment by microscopic method

After 24 hr incubation of RBC suspension with two fold serial dilutions of ZnO-NPs revealed, an absence of aggregation as well as other morphological changes of RBC. Morphological changes in RBC of nanoparticles, positive control and negative control and treated groups are shown in Fig 7b. Interaction of nanoparticles with RBC can result in transformation of erythrocyte shape (Suwalsky *et al.*, 2005). Aggregation of RBC was observed at higher concentrations of ZnO-NPs which were synthesized chemically (Simundic *et al*., 2013; Raguvaran *et al*., 2015). Absence of aggregation as well as morphological changes in RBC was evident in the present study. Toxicity study of ZnO NPs on mice revealed non-significant changes in hematobiochemical parameters which was also eviden in histopathology of liver and kidney (Abdel-Ghany *et al*., 2022).

CONCLUSION

In the present study, potent acaricidal property with consistent efficacy was recorded with 2% ZnO-NPs without any cytotoxicity to the host beyond the target species *i.e.* tick, revealed present green synthesized ZnO NPs as a promising and potent acaricidal agent for further clinical use on livestock for tick control program.

ACKNOWLEDGEMENT

Authors sincerely thank Director, ICAR-IVRI Bareilly, UP for extending facilities.

Conflict of interest

There is no conflict of interest among the authors.

REFERENCES

Abdel-Ghany, H.S.M., Shafy, S.A., Abuowarda, M.M., El-Khateeb, R.M., Hoballah, E.M. and Fahmy, M.M. (2022). Acaricidal activity of biosynthesized zinc oxide nanoparticles against *Hyalomma dromedarii* (Acari: Ixodidae) and their toxic effects on swiss albino mice. Acta Parasitology. 67: 878-891.

Banumathi, B., Malaikozhundan, B. and Vaseeharan, B. (2016). *In vitro* acaricidal activity of ethnoveterinary plants and green synthesis of zinc oxide nanoparticles against *Rhipicephalus (Boophilus) microplus.* Veterinary Parasitology. 216: 93-100.

Drummond, R.O. (1983). Tick-borne livestock diseases and their vectors. Chemical control of ticks. Wimld An Rev, (FAO). 36: 28-33.

Faisal, S., Jan, H., Shah, A., Shah, S., Khan, A., Akbar, M.T., Rizwan, M., Jan, F.Wajidullah, Akhtar N., Khattak A., Syed, S. (2021). Green synthesis of zinc oxide nanoparticles using aqueous fruit extract of *Myristica fragrans:* Their Characterization and biological and environmental application. ACS Omega. 6: 9790-9722.

Fakhari, S., Jamzad, M. and Kabiri, F.H. (2019). Green synthesis of zinc oxide nanoparticles: A comparison. Green Chemistry Letters and Reviews. 12(1): 19-24.

FAO, (2004). Resistance Management and Integrated Parasite Control in Ruminants. Guidelines. Animal Production and Health Division, FAO, pp. 25-77.

Fu, L. and Fu, Z. (2015). Plectranthus amboinicus leaf extract-assisted biosynthesis of ZnO nanoparticles and their photocatalytic activity. Ceramics International. 41: 2492-2496.

Galindo-Rodriguez, S., Allemann, E., Fessi, H. and Doelker, E. (2004). Physicochemical parameters associated with nanoparticles formation in salting out, emulsification-diffusion and nanoprecipitation methods. Pharmaceutical Research. 21: 1428-1439.

Ghosh, S., Bansal, G.C., Gupta, SC., Ray, D., Khan, M.Q., Irshad, H., Shahiduzzaman, M.D., Seitzer, U., Ahmed, J.S. (2007b). Status of tick distribution in Bangladesh, India and Pakistan. Parasitology Research. 101: 207-216.

Hamdy, E., Al-Askar, A.A., EL-Gendi, H., Khamis, W.M., Behiry, S.I., Valentini, F., Abd-Elsalam, K.A., Abdelkhalek, A. (2023). Zinc oxide nanoparticles biosynthesized by Eribotrya japonica leaf extract: Characterization, Insesticidal and Antibacterial properties. Plants. 12: 28-26.

Hughes, J.M., Budd P.M., Grieve, A., Dutta, P., Tiede, K.and Lewis, J. (2015). Highly monodisperse, lanthanide containing polystyrene nanoparticles as potential standard reference materials for environmental “nano” fate analysis. Journal of Applied Polymer Science. 4206: 1-9.

Kirthi, A.V., Rahuman, AA., Rajakumar, G., Marimuthu, G., Santhoshkumar, T., Jayaseelan, C. and Velayutham, K. (2011). Acaricidal, pediculocidal and larvicidal activity of synthesized ZnO nanoparticles using wet chemical route against blood feeding parasites. Parasitology Research. 100: 461-472.

Kumar R, Sharma A.K, Ghosh S. (2020). Menace of acaricide resistance in cattle tick, Rhipicephalus microplus in India: Status and possible mitigation strategies. Veterinary Parasitology. doi: 10.1016/j.vetpar.2019.108993.

Kumar, S., Rahuman, T., Bagavan, AA., Marimuthu, A., Jeyaseelan, S., Kirthi, C., Kamraj, A.V., Kamaraj, C., Rajakumar, C., Zahir, G., Elango, A.A., Velayutham, G., Iyappan, K., Siva, M., Karthik, C., L. and Rao, K.V.B. (2012). Evaluation of stem aqueous extract and synthesized silver nanoparticles using *Cissus quadrangularis* against *Hippobosca maculata* and *Rhipicephalus (Boophilus) microplus*. Experimental Parasitology. 132: 156-165.

Magadum, S., Mondal, D.B. and Ghosh, S. (2009). Comparative efficacy of *Annona squamosa* and *Azadirachta indica* extracts against *B. microplus Izatnagar* isolates. Parasitology Research. 105: 1085-1091.

Minjauw, B. and Mc Leod, A. (2003). Research Report, DFID Animal Health Programme. Centre for Tropical Veterinary Medicine, University of Edinburgh, UK.

Minjauw, B., McLeod, A. (2000). Epidemiology and economics of tick-borne diseases, their effects on the livelihood of poor in east and southern Africa and in India. Consultancy report to the Animal Health Programme (AHP) of the Department for International Development (DOID), Nairobo, Kenya, pp. 94.

Mondal, D.B., Sarma, K., Saravanan, M. (2013). Upcoming of the integrated tick control program of ruminants with special emphasis on livestock farming system in India. Ticks and Tick-Borne Diseases. 4: 1-10.

Mondal, D.B. (2006). Health Issues in High Altitude Wildlife and Their Management. Compendium of Lectures: ICAR Summer School on Recent Trends in Wildlifehealth Management and Forensics w.e.f. 8th to 28th September, 2006. Organized by Centre for Wildlife Conservation Management and Disease Surveillance, IVRI, Izatnagar and sponsored by ICAR, pp. 248-257.

Naiel, B., Fawzy, M., Halmy, M.W.A., *et al.* (2022). Green synthesis of zinc oxide nanoparticles using sea lavender [*Limonium pruinosum* (L.) Chaz.] extract: Characterization, evaluation of antiskin cancer, antimicrobial and antioxidant potential. Science Report. 12: 20370.

Norouzi, A., Ataei. A., Hejazy, M., Shahbazi, P. (2019). Acaricidal activity of zinc oxide nanoparticles against Hyalomma spp *in vitro.* Nanomedicine Research Journal. 4: 234-238.

Raghava, G.P.S., Goel, A., Singh, A.M. and Varshney, G.C. (1994). Technical Report: A Simple Microassay for Computing Hemolytic Potency of Drugs. Biocomputing. 17: 1148-1152.

Raguvaran, R., Manuja, A., Singh, S., Chopra, M., Manuja, B.K. and Dimri, U. (2015). Zinc Oxide Nanoparticles Induced Haemolytic Cytotoxicity in Horse Red Blood Cells. International Journal of Pharmaceutical Science and Research. 6: 1166-69.

Rajakumar, G., Rahuman, A.A., Velayutham, K., Ramyadevi, J., Jeyasubramanian, K., Marikani, A., Elango, G., Kamraj, C., Santhoshkumar, T., Marimuthu, S., Zahir, A.A., Bagavan, A., Jeyaseelan, C., Kirthi, A.V., Iyappan, M. and Siva, C. (2013). Novel and simple approach using synthesized nickel nanoparticles to control blood sucking parasites. Veterinary Parasitology. 191: 332-339

Rajkhowa, S., Rajkhowa, C., Bujarbaruah, K.M., Hazarika, G.C., (2005). Seasonal dynamics of Boophilus microplus infesting mithum Bos frontalis of Nagaland. Indian Journal of Animal Science. 75: 25-26.

Ramyadevi, J., Jeyasubramanian, K., Marikani, A., Rajakumar, G., Rahuman, A.A., Santhoshkumar, T., Kirthi, A.V., Jayaseelan, C., Marimuthu, S. (2011). Copper nanoparticles synthesized by polyol process used to control hematophagous parasites. Parasitology Research. 109: 1403-1415.

Simundic, M., Drasler, B., Sustar, V., Zupanc, J., Stukelj, R., Makovec, D., Erdogmus, D., Hagerstrand, H., Drobne, D. and Kralj-Iglic, V. (2013). Effect of engineered TiO_2 and ZnO nanoparticles on erythrocytes, platelet-rich plasma and giant unilamelar phospholipid vesicles. BMC Veterinary Research. 7: 1-13.

Singh, J., Borkakoty, M.R., Miranpuri, G.S., Lahkar, B.C., (1978). Ixodid ticks infestingdomestic and wild animals of Assam, India. Indian Journal of Parasitology. 2: 49-50.

Suwalsky, M., Villena, F., Norris, B., Soto, M.A., Sotomayor, C.P., Messori, L. and Zatta, P. (2005). Structural effects of titanium citrate on the human erythrocyte membrane. Journal of Inorganic Biochemistry. 99: 764-770.

Thirunavukkarasu, C., Archana, R., Sharmila, S., Janarthanan, B. and Chandrasekaran, J. (2016). Preparation and characterization of ZnO nanoparticles using *moringa oleifera* extract by green synthesis method. Asian Journal of Phytomedicine and Clinical Research. 4: 121-132.

Unni, V., Abishad, P., Ram, V.R., Niveditha, P., Yasur, J., John, L., Prejit, N., Juliet, S., Latha, C., Vergis. J., Kurkure, N.V., Barbuddhe, S.B. and Rawool, D.B. (2022). Green synthesis and characterization of zinc oxide nanoparticles using piper longum catkin extract and its *in vitro* antimicrobial activity against multi-drug-resistant non-typhoidal *Salmonella spp.*, Inorganic and Nano-Metal Chemistry. doi: 10.1080/24701556. 2022.

Yedurkar, S., Maurya, C. and Mahanwar, P. (2016). Biosynthesis of Zinc Oxide Nanoparticles Using *Ixora coccinea* Leaf Extract-A Green Approach. Open Journal Synthesis Theory and Applications. 5: 1-14.

Zahid, M., Mujahid, M., Singh, P.K., Farooqui, S., Singh, K., Parveen, S. and Arif, M. (2018). *Annona squamosa* Linn. (custard apple): An aromatic medicinal plant fruit with immense nutraceutical and therapeutic potentials. International Journal of Pharmaceutical Science and Research. 9: 1745-59.