RESEARCH ARTICLE

Biosynthesis of Titanium Dioxide Nanoparticles and its Efficacy against Tick *Rhipicephalus (Boophilus) microplus*

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Abstract

The purpose of the present study was to evaluate the acaricidal activity of synthesized titanium dioxide nanoparticles using aqueous flower extract of *Calotropis gigantea* and encapsulated *Azadirachta indica*, *Ricinus communis* on synthesized titanium dioxide nanoparticles (TiO_2 NPs) against ticks *Rhipicephalus (Boophilus) microplus*. The titanium dioxide nanoparticles were synthesized by addition of *C. gigantea* flower extract with titanium sulphate solution and thus synthesized nanoparticles were characterized using DLS (Dynamic Light Scattering) and zeta potential, FT-IR (Fourier transformed infrared spectroscopy) and SEM (Scanning electron microscope). The maximum efficacy was observed for A-TiO_2 (*A. indica* coated TiO_2 NPs) with LC_{50} and LC_{99} values of 8.264 and 47.097 ppm. Biofabricated TiO_2 NPs posses high acaricidal activity and can be used as alternative to chemical acaricides.

Key words: *Azadirachta indica, Calotropis gigantea, Rhipicephalus (Boophilus) microplus, Ricinus communis,* Titanium dioxide nanoparticles.

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Introduction

Ticks are second to mosquitoes as vectors of infectious pathogens to humans which transmit pathogens from one animal to another (de la Fuente *et al.,* 2008). Ticks and tick-borne diseases (TTBDs) affect 80 % of the world cattle population and are widely distributed throughout the world, particularly in tropical and subtropical countries (de Castro, 1997). Indiscriminate usage of acaricides leads to development of resistance in tick populations (Anand *et al.,* 2021).

The use of nanotechnology minimizes the problem of drug resistance and reduces the risk of presence of drug residues in milk and meat. Among nanomaterials, metal oxide NPs have many advantages and wide range of applications in therapeutic area, biotechnology, vehicles for gene and drug delivery (Kumar *et al.,* 2018). Titanium dioxide nanoparticles (TiO_2 NPs) is a metal oxide NP with good photocatalytic property, semiconductor, chemically stable (Zhao *et al.,* 2007), with strong oxidizing power and non-toxicity (Rajakumar *et al.,* 2014). Green synthesis of NPs is popularizing as one of the cost effective, eco-friendly and energy efficient synthesis of TiO_2 NPs which may be suitable alternatives for chemical and physical methods (Sagadevan *et al.,* 2022).

C. gigantean is a giant milk weed belongs to Asclepiadaceae family. The plant has medicinal properties like anti-bacterial, anti-cancer, anti-tumor, anti-oxidant, wound healing (Ningsih *et al*., 2022), and anti-migraine activity (Bhatia *et al.,* 2022). Acaricidal activity of *C. gigantean* has been identified (Marimuthu *et al.,* 2013). *Azadirachta indica* commonly called as neem is known for its medicinal (Hikaambo *et al*., 2022) and anti-cancer (Iman *et al.,* 2021) properties. *R. communis* belongs to Euphorbiaceae family popularly known as castor plant. It is fast growing perennial shrub grown commonly in India. Medicinal properties of the plant include anti-cancer, reversible anti-fertility, anti-diabetic, leishamicidal, hepatoprotective, antioxidant, insecticidal, and anti-microbial property (Kebede and Shibeshi, 2022; Khalid *et al*., 2022). Objective of the present study was to biosynthesise and characterize titanium dioxide (TiO_2) nanoparticles and assess the acaricidal activity of nanoscale TiO_2 NPs against *Rhipicephalus (Boophilus) microplus*.

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Materials and Methods

Synthesis and Characterization of Nanoparticles

Nanoparticles were synthesised as per Marimuthu *et al.* (2013) with slight modifications. Seventy millilitre 1 mM of freshly prepared titanium sulphate ($TiOSO_4$) solution was taken in conical flask, to it 30 mL of *C. gigantea* plant extract was added slowly and stirred the solution for 1 h and kept aside at room temperature over night. The formed nanoparticles of TiO_2 NPs were coated with 10 % w/v of *A. indica* and *R. communis* extract by encapsulation technique by mixing drop by drop to 50 mL of TiO_2 NPs solution and stirred continuously for 2 h and the mixture was left at room temperature for 24 h followed by centrifugation at 1750 g for 2 min. The sediments collected at bottom was washed twice with distilled water and dried overnight at 80°C. The obtained nanoparticle powder was further confirmed by following the characterization methods as described below.

Fourier transformed infrared spectroscopy (FT-IR): It is widely adopted technique compared to IR spectroscopy. Functional groups attached to the metallic nanoparticle surface show different FTIR pattern than those of free groups. FT-IR analysis was used to determine the functional groups of titanium dioxide nanoparticles. The surface adsorption of functional groups on nanoparticles at a spectral range of 500-3500 cm^{-1} was carried out as per Snigdha *et al.* (2020). The dried powder was mixed with KBr (potassium bromide) pellets as it acts as carrier for sample in infrared spectra (Bruker India Scientific Pvt, Ltd)

Dynamic light scattering (DLS) and Zeta potential: The nanoparticle solution was centrifuged and supernatant was collected to perform Dynamic light scattering (DLS) and Zeta potential as per Shigdha *et al.* (2020). The hydrodynamic diameter of nanoparticles was measured using DLS and the surface charge of nanoparticles by zeta analyzer (Nanopartica, HORIBA, SZ-100).

Scanning electron microscope (SEM): SEM was used to describe the surface morphology and shape as per Snigdha *et al.* (2020). The *C. gigantea* mediated nanoparticles, *A. indica* and *R. communis* coated TiO_2 nanoparticles were dried at 85°C for 24 h on copper grid for SEM analysis (TM3030 plus SEM).

Collection and Maintenance of Herbs and Parasites

Salubrious leaves of *A. indica*, flowers of *C. gigantea* and leaves of *R. communis* were collected from Tirupati and were certified by Department of Botany [GS02], Sri Venkateswara University, Tirupati.

Engorged *R. (Boophilus) microplus* ticks were collected from cattle in and around Tirupati, engorged female ticks collected from study area were identified and stored in a test tube and maintained at 80-90 % RH and 27±1°C. These ticks were kept under optimal conditions for the eggs to hatch. Thus obtained seed ticks were used for further studies. Seed ticks aged between 14-21 days were subjected to bioassays – Adult Immersion Test (AIT) and Larval Packet Test (LPT) as per FAO (2004) to asses acaricidal activity of the titanium dioxide nanoparticles.

Statistical Analysis

The mortality percentage of larval packet test and adult immersion test was subjected to probit analysis to evaluate lethal concentrations (LC_{50} and LC_{99}) and inhibitory concentrations (IC_{50} and IC_{99}) for various tested compounds using software Statistical Package for Social Sciences (IBM SPSS 22.V, Illionois, Chicago). The lethal concentrations, reproductive efficacy percent and oviposition inhibition were expressed at 95 % of fiducial limits. The mean and standard error values were calculated in Microsoft Excel 2007. *Post hoc* analysis was done using Duncan's test. The level of $p<0.05$ was considered as statistically significant.

Results and Discussion

Characterization of TiO_2 NPs by Fourier Transformed Infrared Spectroscopy (FT-IR)

The Fourier-transformed infrared (FT-IR) spectrum analysis of TiO_2 nanoparticles (NPs) revealed peaks corresponding to specific functional groups. The peak observed at 3417 cm^{-1} indicated the stretching of the alcohol [O-H] group, while the peak at 1623 cm^{-1} corresponded to bending of N-H group. The peak observed at 1081 cm^{-1} was associated with the stretching of C-O group (Fig. 1a).

Further analysis of A-TiO_2 NPs showed peaks at 3426 cm^{-1}, representing the stretching of O-H groups in alcohols, and at 1640 cm^{-1}, indicating the stretching of C=C groups in alkenes. Peaks at 1214, 1181, 1133, 1072 cm^{-1} corresponded to the stretching of C-N groups in aliphatic ethers. The peaks at 800, 693 and 621 cm^{-1} represented the bending of C=C groups in alkenes (Fig. 1b).

Similarly, R-TiO_2 NPs exhibited peaks at 3398 cm^{-1}, indicating the stretching of O-H groups in alcohols, and at 1652 cm^{-1}, corresponding to the stretching of C=C groups in conjugated alkenes. Additionally, a peak at 1400 cm^{-1} was observed, which was associated with the bending of O-H groups in carboxylic acids (Fig. 1c).

Rajakumar *et al*. (20120 and Gandhi *et al*. (2016) demonstrated similarities in peak positions for functional groups, albeit with slight variations in specific wave numbers. These findings provide valuable insights into the consistent presence of functional groups in TiO_2 NPs and contribute to a better understanding of their chemical composition. The FT-IR spectrum analysis of TiO_2 NPs helps in identifying and characterizing functional groups, which in turn suggest that the biosynthesized nanoparticles are surrounded by a thin layer of phytomolecules (Table 1, Fig. 1)

Table 1: FTIR tentative frequencies of TiO_2 NPs

Wave number (Cm^{-1})	Band assignment
3550-3200	Stretching of O-H
1600-1650	Stretching of C=C
1580-1650	Bending of N-H
1395-1440	Bending of O-H
1020-1250	Stretching of C-N
600-840	Bending of C=C

Characterization by Dynamic Light Scattering and Zeta Potential

Dynamic light scattering (DLS) was employed to determine the hydrodynamic diameter (HDD) and zeta potential of *C. gigantea*-mediated TiO_2 NPs, A-TiO_2 NPs, and R-TiO_2 NPs in a hydrosol solution. The recorded HDD values were 26 nm, 126 nm, and 47.6 nm, respectively. These measurements indicate the size distribution and average hydrodynamic size of the nanoparticles in the solution (Fig. 2). Furthermore, the Polydispersity Index (PI) values were determined as 0.232, 0.449, and 0.703 for *C. gigantea*-mediated TiO_2 NPs, A-TiO_2 NPs, and R-TiO_2 NPs, respectively. The PI values provide information about the particle sizes within the sample, with lower values indicating a more monodisperse distribution.

The mean zeta potential, a measure of the surface charge of nanoparticles, was found to be -59.2 mV for *C. gigantea*-mediated TiO_2 NPs, 51.1 mV for A-TiO_2 NPs, and 23.8 mV for R-TiO_2 NPs. These values indicate the stability and potential for nanoparticle aggregation in the solution, with higher absolute values indicating stronger repulsive or attractive forces between particles.

Albukhaty *et al*. (2020) reported a particle size of approximately 18 nm for TiO_2 NPs, which is consistent with the HDD values observed in our study. Raliya *et al.* (2014) demonstrated a hydrodynamic radius of nanoparticles of approximately 140.4 nm and a zeta potential of around -44.6 mV, supporting our results in terms of hydrodynamic size and zeta potential values.

Characterization by Scanning Electron Microscope (SEM)

Scanning electron microscope (SEM) analysis was conducted to examine the morphology and structure of TiO_2. The SEM micrographs (Fig. 3) displayed polydispersed nanoparticles, with the particle size indicated by a bar scale of 10 and 30 µm. The shape of the particles was predominantly spherical, exhibiting a characteristic round morphology. However, occasional agglomerates were observed, suggesting the tendency of nanoparticles to cluster together. The findings obtained align with previous studies conducted by Rajakumar *et al*. (2012, 2013) and Roopan *et al*. (2012). These studies also reported the spherical shape of TiO_2 NPs with agglomeration. The consistency between our results and prior research further validates the reproducibility and reliability of the observed particle morphology.

The spherical shape of TiO_2 NPs is attributed to the synthesis method and the inherent crystal structure of the material. The agglomerates, on the other hand, can arise due to the high surface energy of NPs, resulting in their tendency to aggregate. The presence of agglomerates may have implications for the dispersion and stability of the NPs in various applications

Acaricidal Activity of Nanoparticles

The acaricidal activity of TiO_2 NPs, A-TiO_2 NPs, and R-TiO_2 NPs was evaluated, resulting in significant larval mortality (100%), adult mortality (90-100%), and oviposition inhibition (94-100%). These findings demonstrate the effectiveness of these nanoparticles in controlling acarid populations. A-TiO_2 NPs displayed complete control over all life stages, while R-TiO_2 NPs showed notable adult mortality and oviposition inhibition (Table 2). The results are in accordance with Marimuthu *et al.* (2013), who observed complete larval and adult mortality at concentration of 20 ppm, and Rajakumar *et al.* (2014) reported 100 % mortality at concentration of 25 ppm. There are no earlier reports of *A. indica* and *R. communis* coated TiO_2 NPs on *R. (Boophilus) microplus.*

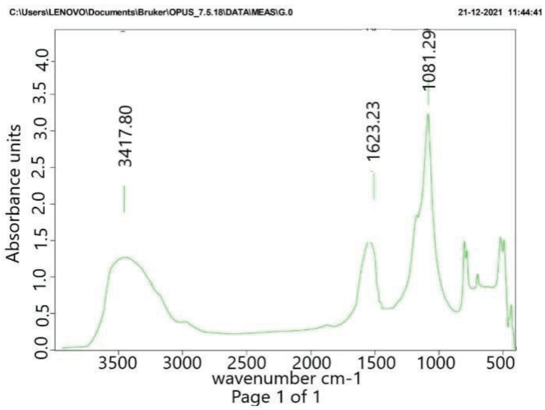
Conclusion

Nanoparticles are acting as good drug delivery agents and in preparation of nanoparticles, the eco-friendly and biological reducing agent and capping agent is recommended instead of chemical reducing agents. *Azadirachta indica* coated titanium dioxide nanoparticles have good acaricidal activity when compared to other synthesized compounds, and titanium dioxide nanoparticles have more oviposition inhibitory activity when compared with other compounds. Other plant extracts used in this study were having significant acaricidal property and can be used as alternative for chemical acaricides.

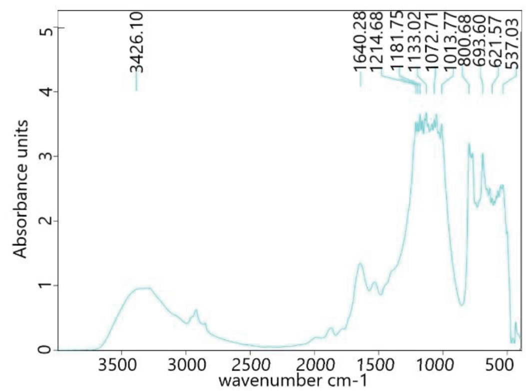
Table 2: Acaricidal activity of different test compounds against ticks *R. (Boophilus) microplus*

Test compound	Conc [ppm]	% AM	% OI	LC_{50} [ppm]	LC_{99} [ppm]	R^2	Intercept	% LM
TiO_2 NPs	5	20	50	11.345 [5.611-17.585]	57.131 [28.677-2798.356]	0.886	3.54	43.33
	10	30	59					62.00
	15	56.66	70					72.33
	20	83.33	86					87.66
	25	90	94					100
A-TiO_2 NPs	5	33.33	39	8.264 [2.805-12.302]	47.097 [24.111-1947.16]	0.918	2.39	48.33
	10	46.66	66					66.00
	15	76.66	80					78.33
	20	86.66	91					89.66
	25	100	100					100
R-TiO_2 NPs	5	20	41	9.596 [8.582-16.569]	54.219 [42.828-75.257]	0.976	3.12	46.00
	10	54	58.6					65.00
	15	66.66	76					75.00
	20	83.33	88					87.66
	25	93.33	97					100

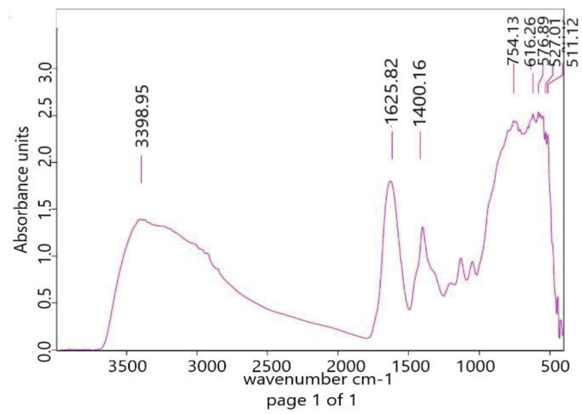
Conc- concentration, % AM- percent adult mortality, % OI percent oviposition inhibition, LC_{50}- lethal concentration 50, LC_{99}- lethal concentration 99, R^2- coefficient of determination, per cent, LM- percentage larval mortality, ppm- parts per million.



(1a)

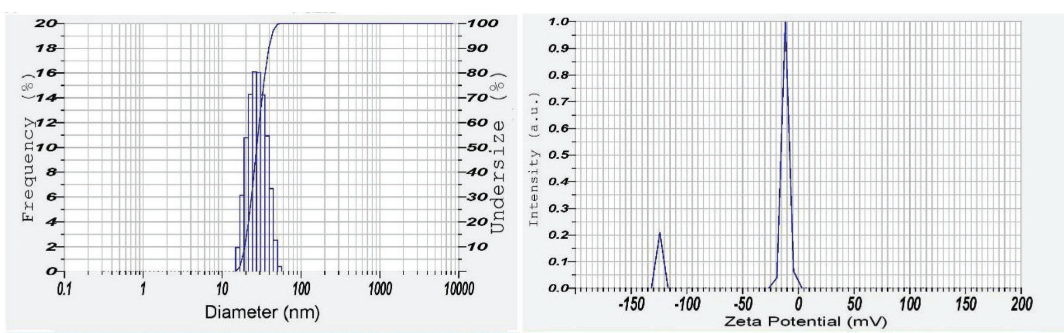


(1b)

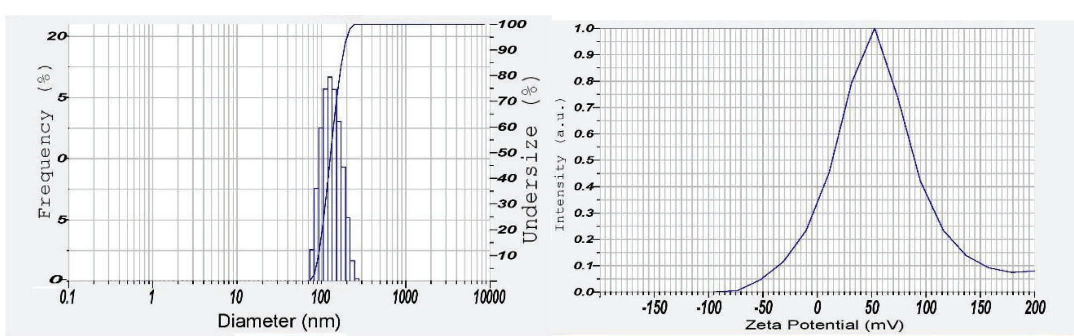


(1c)

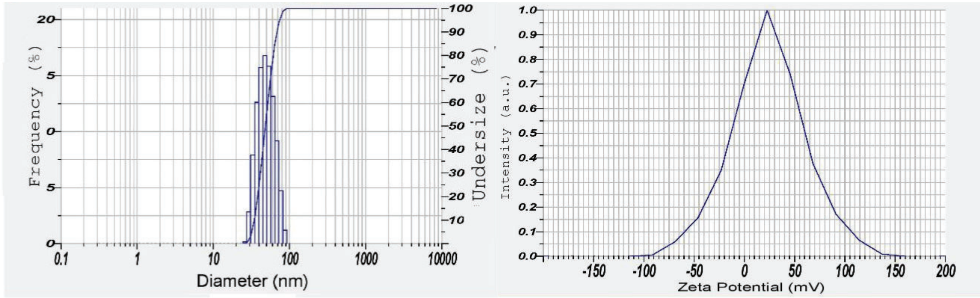
Fig. 1: FT-IR peaks of TiO_2 NPs (1a), A-TiO_2 NPs (1b), and R- TiO_2 NPs (1c).



(2a)

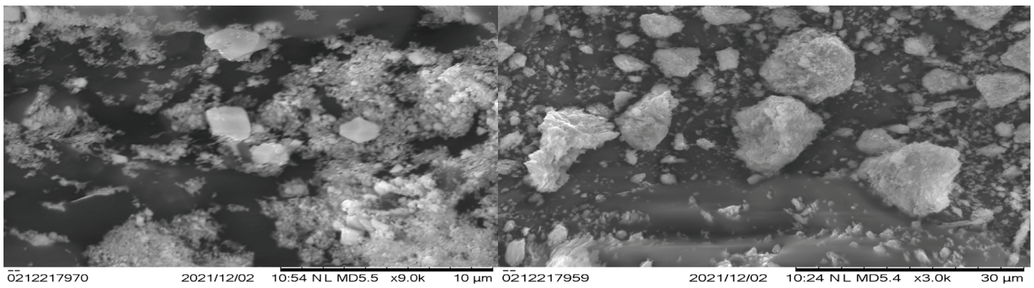


(2b)

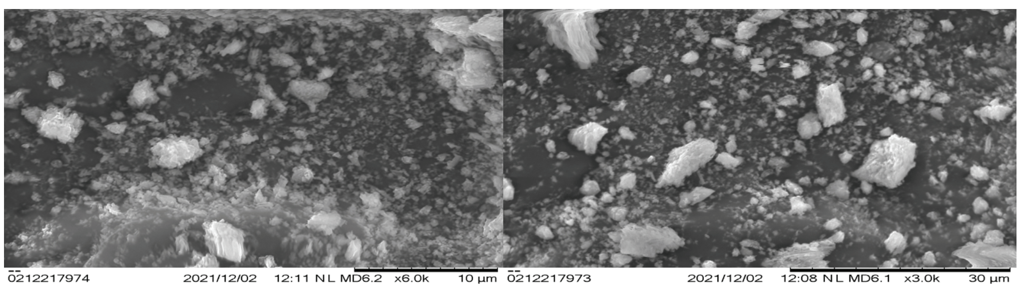


(2c)

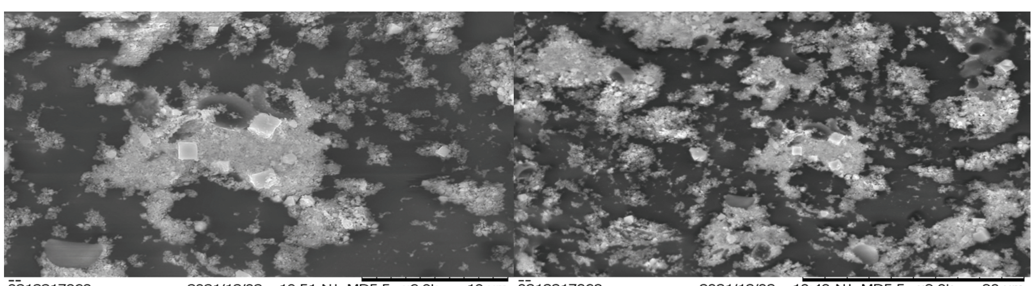
Fig. 2: 2a, 2b, 2c represents dynamic light scattering and zeta potential of TiO_2 NPs, A-TiO_2 NPs, R- TiO_2 NPs, respectively



(3a)



(3b)



(3c)

Fig. 3: 3a, 3b, 3c represents the SEM images in 10 and 30 µm of TiO_2 NPs, A-TiO_2 NPs, R- TiO_2 NPs, respectively

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References

Albukhaty, S., Al-Bayati, L., Al-Karagoly, H., & Al-Musawi, S. (2020). Preparation and characterization of titanium dioxide nanoparticles and *In vitro* investigation of their cytotoxicity and antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*. *Animal Biotechnology*, *30(1-4),* 1-7.

Anand, A., Lakshmanan, B., Kajal, T.A., Joseph, S., Aravindakshan, T.V., & Jose, J. (2021). Deltamethrin resistance in *Rhipicephalus* *sanguineus* and *Rhipicephalus [Boophilus] microplus* tick population in Kerala. *Journal of Veterinary and Animal Sciences*, *52(1),* 19-25.

Bhatia, S., Al-Harrasi, A., Kumar, A., Behl, T., Singh Sehgal, A., & Kabir, M. (2022). Anti-migraine activity of freeze-dried latex obtained from *Calotropis gigantea* Linn. *Environmental Science and Pollution Research*, *29(27460–27478),* 1-19.

de Castro, J.J. (1997). Sustainable tick and tick borne disease control in livestock improvement in developing countries. *Veterinary Parasitology*, *71*(2-3), 77-97.

de la Fuente, J., Estrada-Pena, A., Venzal, J.M., Kocan, K.M., & Sonenshine, D.E. (2008). Overview: ticks as vectors of pathogens that cause disease in humans and animals. *Frontiers in Bioscience*, *13*(13), 6938-6946

FAO (2004). Resistance management and integrated parasite control in ruminants – guidelines, module 1 – ticks: *Acaricide resistance: Diagnosis, management and prevention*. Animal Production and Health Division, Rome, pp. 25-77.

Gandhi, P.R., Jayaseelan, C., Vimalkumar, E., & Mary, R.R. (2016). Larvicidal and pediculicidal activity of synthesized TiO_2 nanoparticles using *Vitex negundo* leaf extract against blood feeding parasites. *Journal of Asia-Pacific Entomology*, *19*(4), 1089-1094.

Hikaambo, C.N.A., Kaacha, L., Mudenda, S., Nyambe, M.N., Chabalenge, B., Phiri, M., & Kampamba, M. (2022). Phytochemical Analysis and Antibacterial Activity of *Azadirachta indica* Leaf Extracts against Escherichia coli. *Pharmacology & Pharmacy*, *13*(1), 1-10.

Iman, M., Taheri, M., & Bahari, Z. (2021). The anti-cancer properties of neem [Azadirachta indica] through its antioxidant activity in the liver; its pharmaceutics and toxic dosage forms; a literature review. *Journal of Complementary and Integrative Medicine*, *19*(11), 203-211.

Kebede, B., & Shibeshi, W. (2022). *In vitro* antibacterial and antifungal activities of extracts and fractions of leaves of *Ricinus communis* Linn against selected pathogens. *Veterinary Medicine and Science, 8*, 1802-1815.

Khalid, A., Algarni, A.S., Homeida, H.E., Sultana, S., Javed, S.A., Abdalla, H., & Abdalla, A.N. (2022). Phytochemical, cytotoxic, and antimicrobial evaluation of *Tribulus terrestris* L., *Typha domingensis* Pers., and *Ricinus communis* L.: Scientific evidences for folkloric uses. *Evidence-Based Complementary and Alternative Medicine*, *2022*, 1-11.

Kumar, H., Venkatesh, N., Bhowmik, H., & Kuila, A. (2018). Metallic nanoparticle: a review. *Biomedical Journal of Scientific and Technical Research*, *4*(2), 3765-3775.

Marimuthu, S., Rahuman, A.A., Jayaseelan, C., Kirthi, A.V, Santhoshkumar, T., Velayutham, K., & Rao, K.V.B. (2013). Acaricidal activity of synthesized titanium dioxide nanoparticles using *Calotropis gigantea* against *Rhipicephalus microplus* and *Haemaphysalis bispinosa*. *Asian Pacific Journal of Tropical Medicine*, *6*(9), 682-688.

Ningsih, D.S., Idroes, R., Bachtiar, B.M., Khairan, K., Tallei, T.E., & Muslem, M. (2022). *In vitro* cytotoxicity of ethanolic extract of the leaf of *Calotropis gigantea* from Ie Jue Geothermal Area, Aceh-Indonesia, and its mouthwash formulation against dental pulp cells. *Journal of Applied Pharmaceutical Science*, *12*(2), 133-143.

Rajakumar, G., Rahuman, A.A., Jayaseelan, C., Santhoshkumar. T., Marimuthu, S., Kamaraj, C., &Jose, S. (2013). *Solanum trilobatum* extract-mediated synthesis of titanium dioxide nanoparticles to control *Pediculus humanus capitis, Hyalomma anatolicum anatolicum* and *Anopheles subpictus*. *Parasitology Research*, *113*(2), 469-479.

Rajakumar, G., Rahuman, A.A., Priyamvada, B., Khanna, V.G., Kumar, D.K., & Sujin, P.J. (2012). *Eclipta prostrata* leaf aqueous extract mediated synthesis of titanium dioxide nanoparticles. *Materials Letters*, *68,* 115-117.

Rajakumar, G., Rahuman, A.A, Roopan, S.M., Chung, I.M., Anbarasan, K., & Karthikeyan, V. (2014). Efficacy of larvicidal activity of green synthesized titanium dioxide nanoparticles using *Magnifera indica* extract against blood-feeding parasites. *Parasitology Research, 114*(2), 571-581.

Raliya, R., Biswas, P., & Tarafdar, J.C. (2014). TiO_2 nanoparticle biosynthesis and its physiological effect on mung bean [*Vigna radiata* L.]. *Biotechnology Reports*, *5***,** 22-26.

Roopan, S.M., Bharathi, A., Prabhakarn, A., Rahuman, A.A., Velayutham, K., Rajakumar, G., & Madhumitha, G. (2012). Efficient phyto-synthesis and structural characterization of rutile TiO_2 nanoparticles using *Annona squamosa* peel extract. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, *98*, 86-90

Sagadevan, S., Imteyaz, S., Murugan, B., Lett, J.A., Sridewi, N., Weldegebrieal, G.K., & Oh, W.C. (2022). A comprehensive review on green synthesis of titanium dioxide nanoparticles and their diverse biomedical applications. *Green Processing and Synthesis*, *11*(1), 44-63.

Snigdha K, Chengalva R V, Kothapalli S R, Yegireddy M, Bollini S., & Tollamadugu Naga Venkata Krishna Vara P. (2020). Assessment of acaricidal activity of nanoscale ZnO encapsulated piperine formulation against *Rhipicephalus microplus*. *IET Nanobiotechnology*, *14*(8), 722-731.

Zhao, Y., Li, C., Liu, X., Gu, F., Jiang, H., Shao, W., & He, Y. (2007). Synthesis and optical properties of TiO_2 nanoparticles. *Materials Letters*, *61*(1), 79-83.