

In-vitro* Anthelmintic and Acaricidal Activity of *Nicotiana tabacum* Leaf Extract Mediated AgNPs Against *Rhipicephalus (Boophilus) microplus

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ABSTRACT

A green synthesis of silver nanoparticle was carried out using *Nicotiana tabacum* leaf extract. Synthesized nanoparticles were characterized using UV-Vis absorption spectroscopy, DLS, TEM, FT-IR and XRD studies respectively. UV-Vis absorption spectroscopy of prepared silver colloidal solution showed absorption maxima at 385 nm. TEM analysis showed average particle size ranges from 5nm-100nm, while XRD confirms SAED pattern confirmed the crystalline nature of synthesized nanoparticles. FT-IR analysis indicated the involvement of carboxyl (C=O), hydroxyl (O-H) and amine (N-H) functional groups of *Nicotiana tabacum* leaf extract in preparation of silver nanoparticles. DLS shows the particle size of silver nanoparticles is 90.5nm and zeta potential is 22.9mv. Hence from this study we concluded that the nanoparticles synthesized from *Nicotiana tabacum* leaf extract shown very effective on in-vitro acaricidal activity (0.170 mg/ ml to 0.0027 mg/ml of AgNPs) were used and noted cent percent mortality was at 0.170 mg/ ml and anthelmintic activity (IC_{50} : 0.621 & IC_{90} : 3.476 mg/ml) AgNPs of *Nicotiana tabacum* was found to be more lethal.

Key words: Ag nanoparticle; Acaricidal Activity; Anthelmintic Activity; *Nicotiana tabacum*

INTRODUCTION

Nanobiotechnology is a fast-growing interdisciplinary area where nanotechnology extends the tools and technology platforms for the investigation and transformation of biological systems and, in turn, biology contributes inspiring models and bioassembled components to nanotechnology¹⁷. It is a field with great potential for countries rich in

biological diversity, such as India, whose biodiversity may be used as a key resource for biotechnological products and processes that are suitable for large-scale synthesis. There is immense interest in the biomedical applications of nanoparticles (NPs) owing to their size and structural similarity to biological molecules.

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Currently, the chemical methods widely used for NP synthesis have associated risks, such as chemical precursor contamination, solvent toxicity, and hazardous byproduct formation, which make alternative synthetic methods imperative. The promising alternative of “green chemistry” exploits the intricate biological pathways and biological resources of living systems, including bacteria, fungi, algae, viruses, plants, and plant extracts, for the bio production of NPs². It has been suggested that to achieve NP synthesis, techniques employing natural reagents such as biodegradable polymers, microorganisms, plant extracts, and sugars and vitamins as reductants and capping agents would be attractive. Though both plants and microbes are used for NP synthesis, plant extracts are preferable because of production advantages such as the lack of elaborate culture maintenance and the possibility of large-scale production³. Nanotechnology has achieved status as one of the critical research endeavors of the 21st century as scientists harness the unique properties of atomic and molecular assemblage built at the nanometer scale. These nanoparticles have been conventionally produced by physical and chemical means, which have inherent disadvantages like increased size, high energy requirements and capital intensiveness. Hence, biogenic synthesis, which involves the action of biological material produce metal nanoparticles, remains pertinent in present context. This method of synthesis is clean, less costly, employs ambient conditions and less energy intensive. The diverse application of nanocrystalline silver ranges from catalysis to photonics, biosensing and diagnostics. Recognizing the importance of developing eco-friendly methods for the synthesis of biologically active nanoparticles, scientists have additionally started looking into research relating to the synthesis of metallic nanoparticles embedded within bio moieties, possessing core-shell morphology. The core being the metal nanoparticle (MNP), while the shell comprises of biochemical-moieties either from a plant extract, or from a microorganism,

which can chemically interact with bio-organic molecules in a cellular environment. In both cases, MNP formation occurs when metal ions are reduced to its zero valent state. The reduction, employing microorganisms, is achieved through the reductase enzyme generated by the microorganism either by extracellular or intercellular route, while in case of plant extract the reduction process is through organic reducing agents present in the extract. The mechanism of reduction employing plant extract is complicated as there may be 25–30 individual reducing components acting individually or combined in groups that lead to obtaining MNP. A detailed mechanism to understand the formation of MNPs using plant extracts is still unknown. However, highly reproducible and chemically homogeneous stable dispersions of MNPs are achieved. Time consumption for synthesis of MNPs is the limitation of this process similar to the one employing microorganisms. However, some reports on the modified use of plant extracts have given insight into the possible organic moieties responsible for the metal ion reduction process along with greatly reducing the time duration for the synthesis. *Nicotiana tabacum*, or cultivated tobacco, is an annually-grown herbaceous plant. It is found only in cultivation, where it is the most commonly grown of all plants in the *Nicotiana* genus, and its leaves are commercially grown in many countries to be processed into tobacco. It grows to heights between 1 and 2 meters. Research is ongoing into its ancestry among wild *Nicotiana* species, but it is believed to be a hybrid of *Nicotiana sylvestris*, *Nicotiana tomentosiformis*, and possibly *Nicotiana otophora*. Tobacco contains the following phytochemicals: nicotine, anatabine, anabasine (an alkaloid similar to the nicotine but less active), glucosides (tabacine, tabacine,) 2, 3, 6 – trimethyl - 1, 4-naphthoquinone, 2 methylquinone, 2-naphthylamine, propionic acid, anathaline, anthalin, anethole, acrolein, anatabine, cembrene, choline, nicotelline, nicotianine, and pyrene. At present study the silver

nanoparticles was synthesized from *Nicotiana tabacum* leaf extract and it was characterized by using UV-visible spectroscopy (UV), Fourier Transformance Resonance Spectroscopy (FT-IR), X-Ray Diffraction (XRD), Dynamic Light Scattering (Particle size analyzer and Zeta Potential), Transmission Electron Microscopy (TEM), the nanoparticles showed very good *In-vitro* anthelmintic and acaricidal activity.

MATERIALS AND METHODS

Plant collection, extracts preparation and synthesis of silver nanoparticles

Nicotiana tabacum leaves were collected from Acharya N G Ranga Agricultural University, Tirupati. The leaves were washed thoroughly thrice in water to remove dust. Leaves were shade dried and ground into fine powder using blender.

The aqueous leaf extract was prepared as per¹³. Fifty grams of leaf powder was mixed with 500 mL distilled water and boiled for about 30 min. on a hot plate. After boiling filtration was done by using Whatman No.1 filter paper and clear aqueous extract was obtained. For future use it was stored at 4°C.

Silver nanoparticles (AgNPs) were synthesized as per the description of¹³. Silver nanoparticles were synthesized using 1:9 ratio mixing of *Nicotiana* leaf extract and 2mM silver nitrate solution and maintained at 80°C on a magnetic stirrer. After addition, the solution was kept at room temperature for 24 hours. Synthesis of nanoparticles was ascertained by a distinct change of the hydrosol. The reduction of silver ions (Ag⁺) was confirmed by UV-Vis spectrum of the hydrosol. Silver nanoparticles were collected by centrifuging at 10000 rpm for 4 min. of the hydrosol for further characterization.

Characterization of AgNPs

The Localized Surface Plasmon Resonance (LSPR) of AgNPs was recorded using UV-visible spectrophotometer (Cary 4000 UV-Vis spectrophotometer) with the scanning range 200–500 nm. Air-dried, platinum coated samples were performed using an analytical Transmission electron microscope (Hitachi s-

3500N) XRD (JEOL: JPX-8030 with CuK α radiation (Ni filtered = 13,418 Å) at the range of 40 kV, 20 A) and FT-IR (Bruker model, TENSOR 37). Particle sizing experiments were carried out by means of laser diffractometry, using Zeta sizer nano series (Malvern). Measurements were taken in the range of 0.1 and 1 \times 10⁸µm.

Adult motility test (In-vitro anthelmintic activity)

The adult motility test (AMT) was performed by slightly modifying to¹⁴. Adult *H. contortus* worms were collected from abomasums of freshly slaughtered infected sheep. It was conducted in Petri dishes at room temperature (27-30°C).

AgNPs at seven different concentrations (from 0.170 mg/ml to 0.0027mg/ml), Aqueous extract (6mg/ml; 3mg/ml; 1.5mg/ml; 0.75 mg/ml; 0.375 mg/ml; 0.1875mg/ml; 0.09375mg/ml and 0.046875 mg/ml). Piperazine at 12mg/ml used as positive control, PBS alone with worms served as negative control. Inhibition of motility was taken as indication of worm mortality/paralysis. The observations were taken at regular time intervals until worms in the negative control completely lost their motility. Each concentration of test compound was performed in triplicate.

Egg hatch assay

The egg hatch assay (EHA) was performed by slightly modifying ⁴ methods. Adult female worms of *H. contortus* has collected and washed thoroughly with phosphate buffer saline and crushed in the PBS for the liberation of eggs. Egg suspension was centrifuged for 3 min. at 2000 rpm and sediment is retained. Saturated solution of Sodium nitrate was used to re-suspend the sediment. The suspension was subjected for centrifugation as mentioned above and the top most fluid containing eggs was collected. Eggs were washed thrice with distilled water. By using the McMaster technique adjusted to a concentration of 200 eggs/ml.

Egg suspension of 1 ml containing approximately 200 eggs was taken in 24 well titration plates. Synthesized AgNPs and

aqueous extracts of *Nicotiana tabacum* were added to plates at different concentrations. Albendazole (2.5; 1.25; 0.62; 0.31mg/ml) were taken into wells as positive control, PBS along with egg solution taken as negative control. The plates were incubated at 28°C for 48 hrs. A drop of Lugol's iodine was added into each well to stop the reaction and number of eggs and L₁ larvae were counted. The experiment was performed in triplicate.

Evaluation of Acaricidal activity of leaf extracts against *Rhipicephalus (Boophilus) microplus*

Fully engorged adult ticks were collected from heavily infested cattle in and around Tirupati using forceps, with intact mouth parts, into a collection vial. These ticks were immediately brought to the laboratory for identification. Collected ticks were processed and examined microscopically identified as *Rhipicephalus (Boophilus) microplus* as per the descriptions¹.

Larval packet test (LPT)

The larval packet test was performed as per FAO⁵ to evaluate the *in-vitro* acaricidal activity of the compounds. Engorged female ticks were obtained from the cattle in the study area, identified, cleaned, stored in a petri dish and maintained RH (85-92%) and temperature

27.0 ± 1°C. Until oviposition female ticks were observed. Later eggs were separated and allowed to hatch in glass vials with cotton plug and kept in optimal conditions. The obtained seed ticks were maintained at 27.0 ± 1.0°C and 85-92 % RH for 14-21 days. 14 to 21 days seed ticks were used to larval packet test.

The dilutions of the phyto chemical compounds were made in distilled water and the synthetic compound deltamethrin was diluted in olive oil with trichloroethylene (1:2).

Packets made of Whatman filter paper No. 1 (12 cm x 18 cm) were impregnated with 3mL of respective compounds and dried at room temperature for two hours. About 100 larvae were placed in acaricide impregnated filter paper packet and the top of the packet was sealed with a clamp (Plate 6). These larval packets were incubated at 27.0 ± 1.0°C and 85-92 % RH for 24 hours. Mortality of larvae was assessed after 24 h of exposure. Lethal concentrations of the compounds were determined using the live and dead count. The percentage mortality in all of the experimental batches of larvae was corrected by applying Abbott's formula.

$$\text{Corrected percent mortality} = \frac{\% \text{ test mortality} - \% \text{ control mortality}}{100 - \% \text{ control mortality}} \times 100$$

Statistical Analysis

The oviposition inhibition percentage from egg hatch assay and mortality percentage from larval packet were subjected to probit analysis to calculate lethal concentration (IC₅₀ and IC₉₀), (LC₅₀ and LC₉₉) for respective compounds using Statistical Package for Social Sciences (SPSS 19.0 V IBM, Illinois, Chicago). The lethal concentrations were expressed by 95% fiducial limits. The 50 and 90 percent of the maximal oviposition inhibition index was calculated by Regression analysis using Microsoft Excel 2013. The level of significance was set at p<0.05. The results from AMA were analyzed with ANOVA using Statistical version.

RESULTS AND DISCUSSION

The *Nicotiana tabacum* leaf extract was used for the green-synthesis of AgNPs showing *in-vitro* acaricidal activity and anthelmintic activity. Visual observation of the color change of the AgNO₃ leaf extract indicated the bio reductive formation of AgNPs. Blending AgNO₃ with *Nicotiana tabacum* leaf extract at a room temperature of 37°C resulted in the formation of AgNO₃ NPs (AgNP), as indicated by a prominent color change to dark brown due to excitation of the AgNP surface plasmon vibration. The colorless AgNO₃ initially changed to light yellow, and finally to a brown, indicating the formation of the AgNPs. This color change took place more rapidly at 37°C at room temperature.

The color shift to dark brown took 30 minutes at room temperature, the control AgNO₃ solution without plant extract did not show any color changes under a similar set of conditions. In addition, the bio reductive formation of AgNPs was ascertained by UV–Vis, FTIR, XRD, DLS and TEM analysis.

The formation and stability of AgNPs synthesized via *Nicotiana tabacum* leaf extracts were initially examined by UV–Vis analysis; The UV–Vis absorption spectra obtained were typical and revealed the bio reductive formation of AgNPs. A high-intensity surface plasmon resonance band is seen at 385nm, along with the synthesized AgNPs characteristic wavelength range. The large number of weak absorption peaks at shorter wavelengths reveals the presence of many participating organic compounds that can interact to reduce the silver ions. It was also observed that the reduction of silver ions into silver nanoparticles started at the start of reaction and reduction was completed at almost 30 min at room temperature, indicating rapid biosynthesis of silver nanoparticles Figure. 1. The FT-IR analysis of the synthesized AgNPs divulged the two-fold function of the *Nicotiana tabacum* leaf extract as a bio reductant whose biomolecules participate in the reduction of the Ag⁺ ions, and as a capping agent that stabilizes the bio reduced AgNPs. The surface chemistry of the AgNPs synthesized by *Nicotiana tabacum* leaf is revealed by the appearance in the FT-IR spectra of IR bands at 3359, 2885, 2821, 2094, 1773, 1637, 1405, 1050, 617, and 572cm⁻¹. The *Nicotiana tabacum* AgNP FT-IR absorption spectra indicates the presence of the N–H stretching hydrogen-bonded primary amine (3359cm⁻¹), C–H stretching hydrogen (2885 and 2821cm⁻¹), N–H bonding (2094cm⁻¹), and C=O stretching (1773 and 1637cm⁻¹). The absorption peaks present at 1405cm⁻¹ indicates the presence of C=O stretching vibration of aldehyde/ketone and aromatic compounds. The peak at 1050cm⁻¹ indicates the presence of N=N stretching of the azo compound and the peak at 617cm⁻¹ indicates C–H group of alkynes, the peak

present at 572cm⁻¹ indicates C–Br stretching vibration of alkyl halides Figure. 2. The FT-IR spectroscopic study also confirmed that the protein present in *Nicotiana tabacum* leaf extract acts as a reducing agent and stabilizer for the silver nanoparticles and prevents agglomeration. The XRD pattern of the AgNPs synthesized by *Nicotiana tabacum* leaf extract was compared and interpreted using standard data. The major peaks at 35°, 52°, and 73° (2θ values) correspond to the reflections from the (111), (200) and (220) planes, respectively, and confirm the crystalline phase of the AgNPs. X-ray diffraction results clearly show that the silver nanoparticles formed by the reduction of Ag⁺ ions by the *Nicotiana tabacum* leaf extract are crystalline in nature Figure. 3. Additional as yet unassigned peaks are also observed suggesting that the crystallization of bio-organic phase occurs on the surface of the nanoparticles. The hydrodynamic diameter (size) of the *Nicotiana tabacum* leaf mediated synthesized AgNPs was found to be 90.5nm Figure. 4a and was measured as a function of scattering angle of the laser from the surface of the particle. Further, zeta potential of AgNPs was also measured and was recorded as -31.1mV Figure. 4b. The higher zeta potential (-31.1mV) clearly indicates the dispersity and stability of the prepared AgNPs. The TEM micrographs of AgNPs green-synthesized by *Nicotiana tabacum* leaf mediated synthesized AgNPs reveal their morphology and size, showing polydispersed AgNPs, most of which have a spherical and hexagonal morphology, with a size range of 5–100nm. It is evident that AgNPs were spherical in shape and were poly-dispersed. The measured average size of AgNPs was 20nm and occasional agglomeration of the AgNPs has been observed Figure. 5.

Egg hatch assay

In EHT, inhibitory concentration estimates of AgNPs and aqueous extracts of *Nicotiana tabacum* and albendazole are represented in Table 1. Based on IC₅₀ and IC₉₀ values most effective plants in the order of significance were; AgNPs (IC₅₀: 0.005 & IC₉₀: 0.045mg/ml)

aqueous extract, (IC₅₀: 0.621 & IC₉₀: 3.476 mg/ml) AgNPs of tobacco was found to be more lethal than aqueous extract. IC₅₀ and IC₉₀ values of for albendazole (1.120 & 2.212 mg/ml), though it was not statistically significant ($p \geq 0.05$) at IC₉₀. Over the centuries, plants were used as important source of medicines to treat different ailments of humans and animals. In India earliest records of curative properties of some herbs is documented in Rig-Veda. In this study, the wormicidal activity of the *Nicotiana tabacum* was validated by conducting AMT and EHA, results demonstrates the wormicidal activity of crude acetone extract of *N. tabacum* was found it as effective as albendazole. No published literature is available for the comparison of silver nanoparticle results. According to⁷ aqueous and methanol extracts of *N. tabacum* revealed dose dependent activity against gastrointestinal parasites of sheep.¹⁶ Tested the wormicidal activity of crude extract of *N. tabacum* and stated that crude extract as effective as levamisole. According to³ wormicidal activity of tobacco is mainly due to nicotine, a ganglionic stimulant that tends to activate the neuromuscular junctions and also causing the spastic paralysis finally leads to death of worms. Different parts of *N. tabacum* are widely used for their narcotic, anti-inflammatory, antirheumatic and antiparasitic properties¹¹. The ganglion stimulant would tend to activate these neuromuscular junctions causing a spastic paralysis in the worms leading to their death and expulsion from the host. The wormicidal activity could be due to its strong corrosive action on cuticle and tegument of helminthes.

Adult motility test

The silver nanoparticles and aqueous extracts of *Nicotiana tabacum* were showed dose dependent anthelmintic activity as the concentration of the compounds decreased the degree of immobilization got delayed in all the treatment groups. At higher dose AgNPs (0.170mg/ml) exhibited more anthelmintic activity, as worms were completely paralyzed within one minute of exposure and for aqueous extract higher dose (6mg/ml) worms were

completely paralyzed within five minute of exposure. At lowest dilution it took 17.33 min, 26.33 min for AgNPs and aqueous extracts respectively. Total time taken for mortality of worms for the above two extracts remained as 19.67 and 30.0 min Table 2 and 3. In PBS which acted as negative control, time taken for 100% mortality was 8 hrs. The total time taken for mortality of worms with reference drug Piperazine adepate (12mg/ml) was 8.67 min.

Acaricidal activity against *Rhipicephalus (Boophilus) microplus*

The larval packet test was used in the present study, to determine the acaricidal activity against *Rhipicephalus (Boophilus) microplus* with various concentrations of deltamethrin; *Nicotiana* aqueous leaf extract; AgNPs. A modified version of FAO⁵ larval packet test (LPT) was used to determine the compound activity to *R. (B). Microplus* larvae. The concentrations of deltamethrin (25 to 400 ppm) peak mortality (100%) were recorded at a concentration of 400 ppm. A total of seven concentrations of silver nanoparticles (0.170 mg/ml to 0.0027 mg/ml) were used and noted cent percent mortality was at 0.170 mg/ml. The effect of the crude *Nicotiana* aqueous leaf extract against *R. (B). Microplus* was found to be 100 percent at 6000 ppm concentration. With the increase in concentration level the percent mortality rate also increased. The lethal concentrations of various compounds against fresh larvae Table 4 Silver nanoparticles showed significantly ($P < 0.05$) lower LC₅₀ compared to respective plant extracts. The LC₉₉ values also in similar manner, aqueous extract showed least acaricidal activity with significantly ($P < 0.05$) higher LC₉₉ values. Deltamethrin is the most commonly used acaricide against ectoparasitic infestations like tick in India and entire world. By its wide applications, cattle tick *Rhipicephalus (Boophilus) microplus* is becoming resistant towards deltamethrin. In the present study high resistance was observed with deltamethrin at a concentration of 400 ppm. Previously some studies reported high resistance towards deltamethrin¹⁵ and with less resistance⁸. The reasons for the increased

resistance might be due to its widespread usage, increase α/β esterase activity and target site insensitivity⁹ (mutation in para-sodium channel gene).

To overcome these resistance problems, the leaf extracts of leaves of *Nicotiana tabacum*, were found to be alternative. It was selected based on their previous acaricidal activity reports, usage in traditional veterinary medicine and availability of plant. No published literature is available for the comparison of these results. In this study these results are similar to stated the acetone extracts more potent than aqueous extracts of *Nicotiana tabacum*. *N. tabacum* was used in the ethno-veterinary practice as an anthelmintic⁷, anti-inflammatory and anti-rheumatic agent¹¹. Zahir and Rahuman,¹⁴ evaluated the anti-parasitic activity of silver nanoparticles with different plant extracts from *Euphorbia prostrata* against *Haemaphysalis bispinosa* and *Hippobosca maculata*. It was

shown that the highest mortality was found in the hexane, chloroform, ethyl acetate, acetone, methanol and aqueous leaf extracts of *E. prostrata* and synthesized AgNPs against the adult of *H. bispinosa*. Several other plant extracts were also shown to possess acaricidal activity against *Rhipicephalus (Boophilus) microplus*, viz., ethanolic extract of *Sapindus saponaria*¹⁰ all these reports showed 80% efficacy against *Rhipicephalus (Boophilus) microplus* larvae. Ghosh *et al.* reported that, the leaf extracts of *Ricinus communis* had the highest mortality at 10 percent concentration. In a study by¹² demonstrated that the combination of aqueous extracts of *Azadirachta indica*, *Mangifera indica*, *Polyalthia longifolia*, *Annona squamosa* and *Ficus benghalensis* showed the 100 percent mortality as compared to a single plant extract against *Rhipicephalus (Boophilus) microplus* ticks.

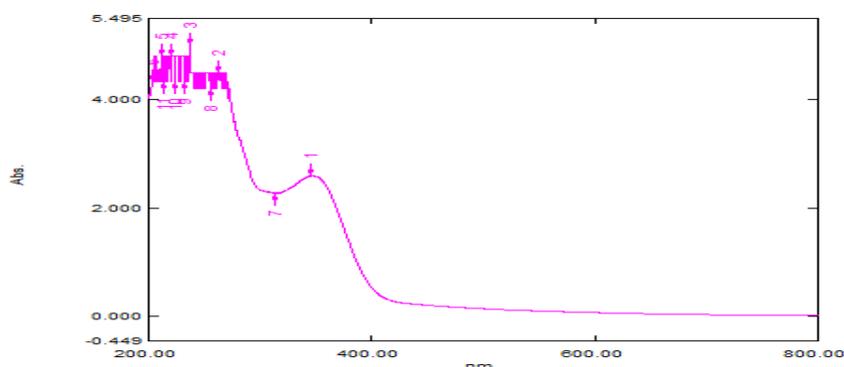


Fig. 1: Showing UV-visible spectroscopy recorded using Ag nanoparticles synthesized from *Nicotiana tabacum*

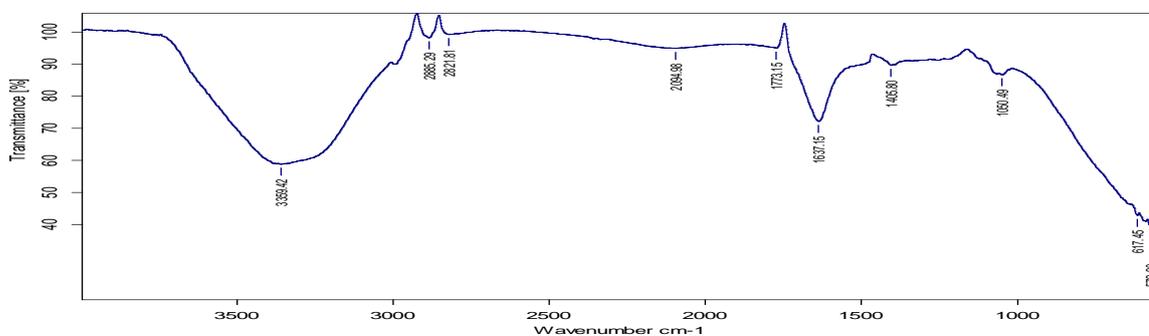


Fig. 2: FT-IR spectroscopy recorded using Ag nanoparticles synthesized from *Nicotiana tabacum*

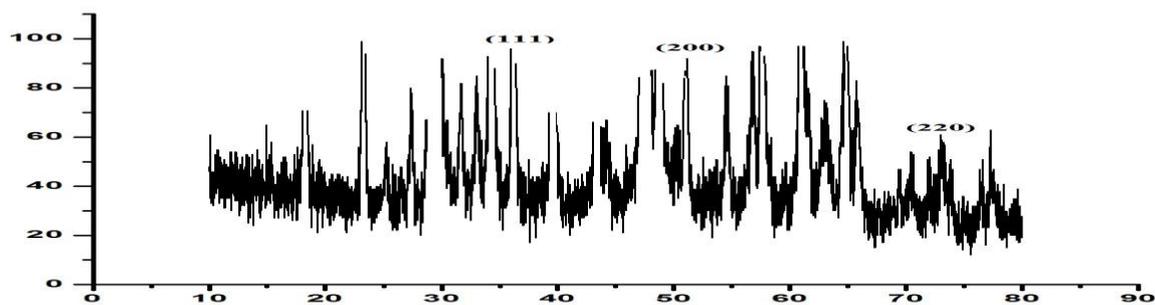


Fig. 3: XRD pattern of Ag nanoparticles synthesized from *Nicotiana tabacum*

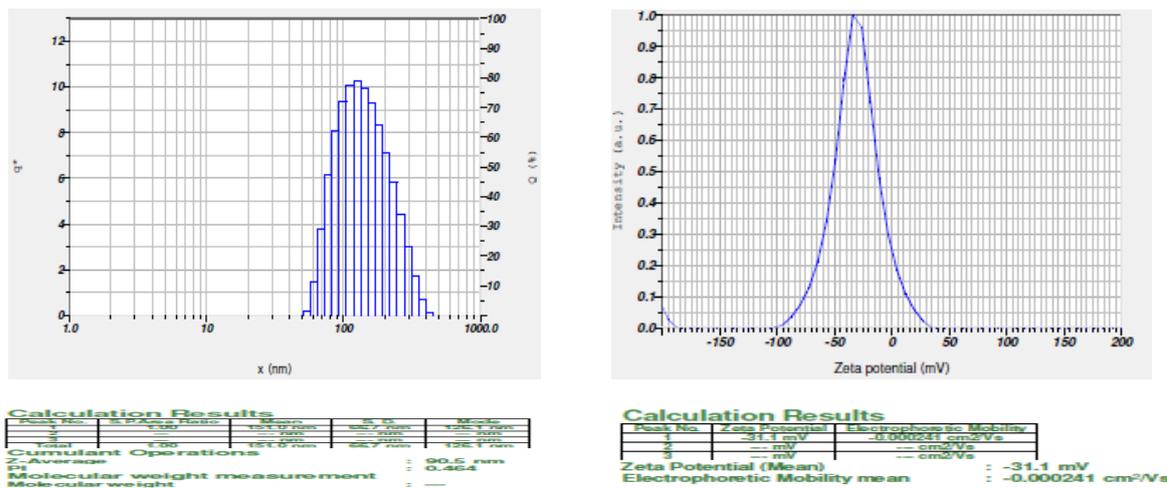


Fig. 4: (a) and 4 (b) Showing Dynamic light scattering results showing that particle size and zeta potential of Ag nanoparticles synthesized from *Nicotiana tabacum*

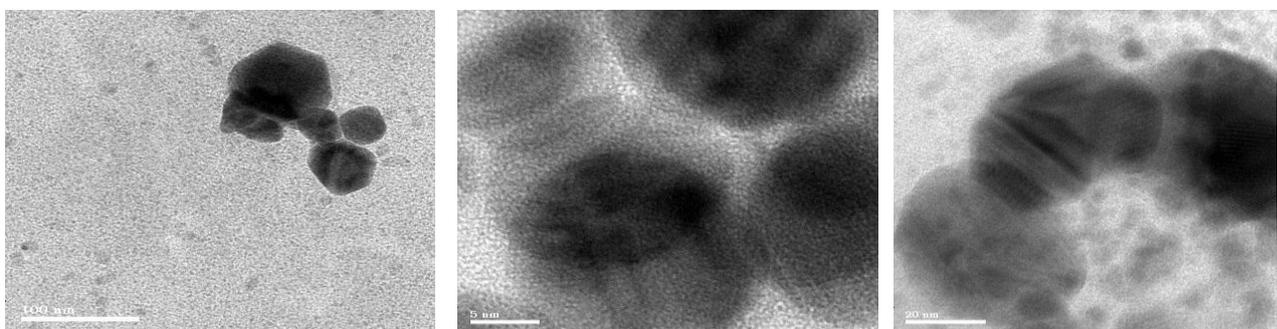


Fig. 5: TEM image of Ag nanoparticles synthesized from *Nicotiana tabacum* showing (20-100nm)

Table 1 Hatchability inhibition by various plant compounds

Compound	IC ₃₀	IC ₅₀	IC ₈₀	IC ₉₀	Slope (SE)	Intercept (SE)	X ² (df)
Albendazole	0.845 (0.612-0.942)	1.125 (0.825-1.274)	1.862 (1.435-2.581)	2.212 (1.791-3.684)	3.241 (0.111)	-0.078 (0.023)	171.312 (10)
AgNPS	0.002 (0.001-0.003)	0.005 (0.004-0.007)	0.022 (0.017-0.028)	0.045 (0.034-0.065)	1.312 (0.027)	3.112 (0.074)	111.603 (19)
Nicotiana Aq Ext	0.312 (0.264-0.342)	0.621 (0.542-0.703)	1.862 (1.705-2.418)	3.476 (2.934-4.283)	1.719 (0.032)	0.342 (0.026)	62.182 (19)

Values are inhibitory concentrations with 95% Fiducial Confidence Intervals in parenthesis
 IC : Inhibitory concentration; SE = Standard error; df = degrees of freedom
 Probit analysis using IBM SPSS 19.0 V

Table 2: Anthelmintic activity of AgNPs

Concentration (mg/mL)	Time (min) for paralysis			Time (min) for mortality		
		±			±	
0.17	1.00	±	0.00	1.00	±	0.00
0.085	2.00	±	0.00	3.67	±	0.33
0.043	5.33	±	0.33	6.67	±	0.33
0.021	7.67	±	0.33	9.67	±	0.33
0.011	11.67	±	0.33	13.67	±	0.33
0.0053	15.33	±	0.33	17.00	±	0.58
0.0027	17.33	±	0.67	19.67	±	0.33
Piperine 12mg/mL	6.33	±	0.33	8.67	±	0.33

Table 3: Anthelmintic activity of aqueous extract of *Nicotiana tabacum*

Concentration (mg/mL)	Time (min) for paralysis			Time (min) for mortality		
		±			±	
6.00	5.00	±	0.58	6.00	±	0.58
3.00	7.67	±	0.67	9.00	±	0.58
1.50	11.33	±	0.33	14.67	±	0.33
0.75	14.33	±	0.67	17.67	±	0.88
0.375	19.00	±	0.58	21.33	±	0.33
0.188	24.00	±	0.58	27.67	±	0.33
0.094	26.33	±	0.33	30.00	±	0.58
Piperine 12mg/mL	6.33	±	0.33	8.67	±	0.33

Table 4: Lethal concentrations of various compounds against fresh larvae

Compound	LC ₅₀	LC ₉₉	SD	SE	R ²	Slope	Intercept
Deltamethrin	67.18	1120.69 (710.48-1723.06)	0.618 (38.34-96.14)	0.097	0.9451	1.560	2.034
aqueous extract	762.58	6129.31 (386.28-1581.34)	0.915 (3128.31-NC)	0.165	0.9624	1.048	1.692
AgNPs	17.227	582.239 (7.96-37.27)	0.894 (269.11-1259.68)	0.171	0.9952	1.118	3.653

Values are inhibitory concentrations with 95% Fiducial Confidence Intervals in parenthesis

LC : Lethal concentration; SE = Standard error; SD = Standard deviation

Probit analysis using IBM SPSS 19.0 V

CONCLUSIONS

To conclude, in the present investigation we successfully developed an environmental friendly synthesis method for the production of biosynthesized AgNP by exploiting *Nicotiana tabacum* leaf extract as a potential bio-reductant. In particular, AgNPs were obtained by treating a solution AgNO₃ with the extract of *Nicotiana tabacum* leaves and subsequent microwave irradiation of the mixture. The prepared nanoparticles have been characterized by different techniques such as UV-vis, FT-IR, XRD, TEM and DLS. The rapid biosynthetic method developed in this study for producing silver nanoparticles has distinct advantages over chemical methods such as a high bio-safety, eco-friendliness, and non-toxicity to the environment. The green silver nanoparticles tend to have significant potential acaricidal activity when compared to the synthetic chemical compounds. Instead of using phyto-chemical compounds as acaricides

if used in the form of green synthesized metallic nanoparticles their potential acaricidal activity will be synergized. Applications of Ag *Nicotiana tabacum* nanoparticles based on these findings may lead to valuable discoveries in various fields such as medical applications.

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