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AGRO WASTE OF *ZIZYPHUS JUJUBA* EXTRACT-MEDIATED SYNTHESIS OF SILVER NANOPARTICLES AND ITS ANTITERMITE ACTIVITY

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ABSTRACT

The present study deals with a cost effective and eco-friendly approach for green synthesis of silver nanoparticles (Ag NPs) using seed aqueous extract of *Zizyphus jujuba* as capping and stabilizing agent. Observations of nanoparticles under UV-visible spectrophotometer were promising and rapid production of surface plasmon resonance was observed at 426 nm. XRD pattern suggests the formation of crystalline particles with face centered cubic (fcc) structure. FTIR spectroscopy peak at 1384.1 cm^{-1} corresponds to C-N stretching of aromatic amine group. SEM showed spherical, aggregated shaped NPs with an average size of 42.1nm. EDAX analysis confirmed the presence of elemental metal signal. AFM analysis showed the irregular surface morphology and the presence of both individual and agglomerated nanoparticles. Anti-termitic activity was investigated using varying concentrations of aqueous seed extract of *Z. jujuba*, $AgNO_3$ solution and synthesized Ag NPs by no-choice bioassay test for 24 h. In the present study, the efficacy of the aqueous seed extract of *Z. jujuba* and green synthesis of Ag NPs is a simple, cost-effective and eco-friendly approach to control the *C. formosanus*.

KEYWORDS: Silver nanoparticles, *Zizyphus jujuba*, XRD, *Centrocestus formosanus*, AFM analysis.

INTRODUCTION

Silver nanoparticles (Ag NPs) were synthesized by several physical, chemical and biological methods. Such nanoparticles possess unique electrical, optical as well as biological properties and are thus applied in catalysis, biosensing, imaging, drug delivery, nano device fabrication and in medicine.[1] The use of plant extract for the synthesis of nanoparticles is advantageous over other environmentally benign biological processes as it eliminated the elaborate process of maintaining cell cultures. The green synthesis of various plants have been reported, the potential of plants as biological materials for the synthesis of nanoparticles are yet to be fully explored.[2]

The most common method of synthesizing silver nanoparticles is chemical reduction, where the silver nanoparticles are synthesized as colloidal dispersions in water or organic solvents.[3] The chemicals that are widely used as reductants for chemical synthesis of silver nanoparticles are ethylene glycol[4], sodium citrate[5] and sodium borohydride.[6] The synthesis of silver nanoparticles by alternate methods using biological systems like bacteria, fungi and plants has increased owing to the environmental concerns.[7] The green synthesis method should concern the utilization of non-toxic chemicals, environmentally benign solvents and renewable materials.[8]

Bio-inspired approaches were explored in the Ag NPs synthesis using aqueous seed extract of *Macrotyloma uniflorum*[9], *Syzygium cumini*[10], *Medicago sativa*[11] and *Jatropha curcas*[12] have been reported by many authors (Table 1). Termites are highly destructive polyphagous insect pests, which largely damage house hold materials, finished goods, plants and agricultural crops such as sugarcane, millet, barley and paddy.

Table. 1. The comparative study of different seed extracts based synthesized nanoparticles.

Trivial name	Botanical name	Nanoparticle	Morphology	Size (nm)	Reference
Horse Gram	*Macrotyloma uniflorum*	Ag NPs	Anisotropic	1-12	[9]
Alfalfa	*Medicago sativa*	Ag NPs	Spherical, flower-like, triangular	5-108	[11]
Jatropha	*J. curcas*	Ag NPs	Spherical	15-50	[12]
Pomegranate	*Punica granatum*	Ag NPs	Spherical	15-30	[29]
Jack fruit	*Artocarpus heterophyllus*	Ag NPs	Irregular and clusters	3-25	[43]
Jambul	*Syzygium cumini*	Ag NPs	Spherical	73-92	[44]
Ladys fingers	*Abelmoschus esculentus*	Au NPs	Spherical	45-75	[45]
Velvet bean	*Mucuna pruriens*	Au NPs	Spherical	6-17.7	[46]
Black seed	*Nigella sativa*	Au NPs	Crystalline and poly shaped	24-30	[47]

Termite colonies also heavily attack fodder crops and make tunnels in subtropical and tropical soil.[13] It is also known that termites damage a variety of materials ranging from paper fabrics to even non-cellulosic materials such as asbestos, asphalt bitumen, lead and metal foils.[14] Termites are the most troublesome pest of plants, trees and wooden structures. They severely damage agricultural crops and borough infrastructure. There are about 2,500 species of termites in the world and only 10% have pest status. Out of 300 species in India, about 35 have been reported as damaging agricultural crops and timbers in buildings. They cause over 3 billion dollars in damage to wooden structures annually throughout the United States.[15,16]

For controlling termites, synthetic termiticides have been used for a long time. At the advent of termite control, persistency of the chemical was regarded as a boon, as it provided protection for longer periods. But soon it was realized that, chlorinated, persistent type of insecticides posed a great hazard to environment, due to their residual effects. Therefore, some of the termiticides like DDT, aldrin, dieldrin, heptachlor and BHC were banned and a search for other less persistent insecticides were started. Recently, chloropyriphos has been successfully employed as soil treatments against subterranean termite. Continuous use of synthetic termiticides for soil as well as crop treatment has been allowed for the present time because of the lack of any effective substitute. World over, research is going on for an effective formulation, which can reduce the damage by termites, at the same time being environmentally acceptable.[17]

Zizyphus jujuba (Rhamnaceae) extract possessed antifungal, cytotoxic, antitermite, insecticidal[18] antifungal[19] antioxidant and antimicrobial activities.[20] The compounds jujuboside **A**, jujuboside **B**, palmitic acid, palmitoleic acid, stearic acid, oleic acid, linoleic acid, arachidic acid and docosanoic acid were isolated from *Z. jujube*.[21] Ag NPs modified with alkyl carboxylate and alkylamine were used for nanoparticle-assisted laser desorption/ionization imaging mass spectrometry to identify fatty acids, such as stearic, oleic, linoleic, arachidonic, and eicosapentaenoic acids, as well as palmitic acid, in mouse liver sections.[22] Ag NPs were produced by hydrazine hydrate and oleylamine/oleic acid systems in order to investigate the effects of reducing agents with different strengths on the reduction mechanism.[23] The current study aimed to explore the synthesized Ag NPs using *Z. jujuba* seed extract by reduction of Ag^+ to Ag^- from $AgNO_3$ solution was investigated for its antitermite activity against *C. formosanus*.

MATERIALS AND METHOD

Materials: Silver nitrate ($AgNO_3$) was procured from Hi-media Lab Pvt. Ltd. Seeds of *Z. jujuba* were collected from our Guindy campus, Chennai, Tamil Nadu, India. Seeds were surface sterilized with 0.2% mercuric (II) chloride ($HgCl_2$) solution for 5 min and thoroughly washed with deionised water to remove adsorbed $HgCl_2$ present on the outer coat of the seeds.[24]

Synthesis of Ag NPs: 10 g of *Z. jujuba* dried seed powder was added to 100 ml of deionized water at 60 °C for 5 min. In the typical synthesis of Ag NPs, 13 ml of the aqueous seed extract of *Z. jujuba* was added to 87 ml of 1 mM (10^{-3}M) solution of $AgNO_3$ in 250 ml Erlenmeyer flask at room temperature for 30 min. The reaction mixture was kept in a dark room until the color change was observed. Color changes in the reaction solution have been observed for the characterization of Ag NPs.

Characterization of Ag NPs: UV–vis spectra of the aliquots were monitored as a function of time of reaction on a Schimadzu 1601 spectrophotometer in 300 - 700 nm range operated at a resolution of 1 nm. For XRD studies, dried nanoparticles were coated on XRD grid and the spectra was recorded using Philips PW 1830 X-ray generator operated at a voltage of 40 kV and at a current of 30 mA with Cu Kα1 radiation. FTIR spectra of the samples were measured using a Perkin elmer spectrum instrument in the diffuse reflectance mode at a resolution of 4 cm^{-1} in KBr pellets. For electron microscopic study, 25 µL of sample was sputter-coated on copper stub and the images of nanoparticles were analyzed (SEM; JEOL, Model JFC-1600). Topography was studied using an Atomic Force Microscope (AFM) (Veeco Pico Force) working in the contact mode. In particular, AFM as used to characterize the uniformity and grain size of silver films deposited on different substrates. AFM images have been processed using WSxM software ver. 4.0.

Collection of termite: The test termite species, *C. formosanus* were collected from in and around the Guindy campus of University of Madras, Chennai and was identified at Zonal Entomological Research Centre, Nandanam, Tamil Nadu, India. Termites were kept in plastic and enamel trays. They were maintained and reared in the laboratory.[13] Water and newspaper were used as the food source. Termites were maintained in glass jars (height-24”, diameter 10”) in complete dark conditions at 28±20 C, 75±5 RH.

Anti-termite activity: The no-choice bioassay method[25, 26] was employed to evaluate the anti-termitic activity of the aqueous extract, $AgNO_3$ and synthesized Ag NPs *Z. jujuba seed* extracts. During preliminary screening one gram of material was dissolved in 100 mL of double distilled water, completely dissolved fresh seed aqueous extract of *Z. jujuba* and $AgNO_3$ solution were prepared at a concentration of 100, 80, 60, 40, 20 mg/L and synthesized Ag NPs solution was prepared at a concentration of 10, 8, 6, 4 and 2 mg/L. A pieces of filter paper samples (Whatman no. 3 and 8.5 cm in diameter), treated with acetone was used as the control. After the solvent (acetone) was removed from the treated filter papers by air-drying at 26±2º C. 25 active termites (22 workers and 3 soldiers) above the third instar were put on each piece of filter paper kept in a Petridish (90 mm dia). The dishes with covers were then placed in an incubator at 20±28º C. A few drops of water were periodically dripped onto the bottom edge of each Petridish. Three replicates were maintained for each test sample. The envelopes were opened after 24h of exposure and the number of alive and dead termites was recorded.

Data analysis: The average termite mortality data were subjected to probit analysis for calculating LD_{50}, LD_{90} and other statistics at 95% fiducial limits of upper confidence limit and lower confidence limit were calculated by using the software developed by Reddy et al.[27] Results with P<0.05 were considered to be statistically significant.

RESULTS AND DISCUSSION

UV-spectra analysis: Ag NPs were synthesized within 30 min of incubation using aqueous seed extract of *Z. jujuba*. It was observed that the addition of the *Z. jujuba* extract into the flask containing the aqueous $AgNO_3$ solution, the color of the medium changed to dark brown within 30 min which was due to the excitation of surface plasmon vibrations within the synthesized Ag NPs which indicated the formation of Ag NPs.[28] The effect of the reaction time on Ag NPs synthesis was also evaluated with UV–visible spectra and with time the peak becomes sharper. The surface plasmon resonance band of Ag NPs appears at 426 nm and even after 30 min of incubation only slight variation was observed (Fig. 1A and B). UV–visible spectra of the Ag NPs obtained on varying the mixing ratios of aqueous seed extract of *Punica granatum* and the maximum peak was observed at 420 nm.[29]

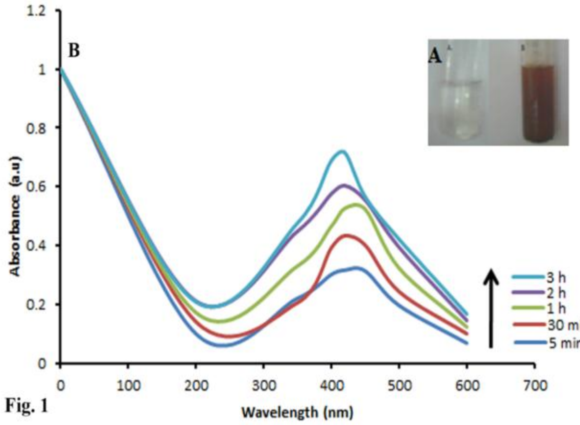

Fig. 1. (a) Aqueous solution of 10^{-3} mM $AgNO_3$ with *Z. jujuba* seed extract (left) before adding the seed extract and (right) after addition of seed extract at 30 min. (b) UV–vis spectra of aqueous $AgNO_3$ with *Z. jujuba* seed extract at different time intervals.

X-ray diffraction (XRD) studies

Seed extract of *Z. jujuba* (vaccum dried synthesized AgNps) subjected to XRD analysis. The number of Bragg reflections with 2θ values of 38.28º, 46.66º, 63.26º and 77.33º correspond to the (111), (200), (220) and (311) sets of lattice planes were observed which may be indexed as the band for face centered cubic structures of silver, respectively (Fig. 2). Similar study was conducted by Lukman et al.[11] who reported that the XRD patterns of Ag NPs synthesized from seed extract of *Medicago sativa* showed four distinct diffraction peaks at 38.32˚, 44.48˚, 64.68˚, and 77.64˚ corresponding to the respective (111), (200), (220) and (311) crystalline planes.

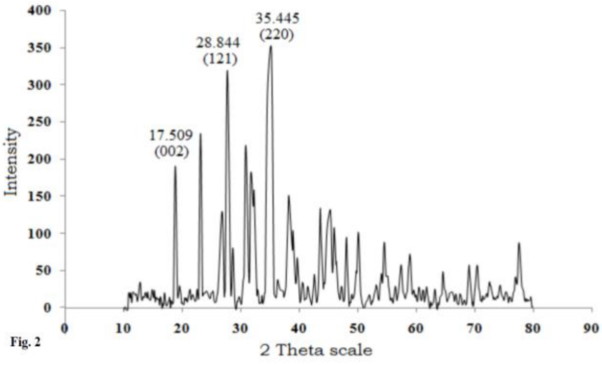

Fig. 2. XRD pattern of synthesized Ag NPs from aqueous seed extracts of *Z. jujube.*

Fourier transformed infrared (FTIR) spectroscopy analysis: FTIR band intensities in different regions of the spectrum for the bulk $AgNO_3$, *Z. jujuba* seed powder and synthesized Ag NPs were analyzed. There was a shift in the following peak spectra showed sharp and strong absorption band at 1758, 1620 and 1642 cm^{-1}

assigned to the stretching vibration of (NH) C=O group (Fig. 3). The strong absorption at 1744 cm^{-1} might be carbonyl stretching vibration of the acid groups of different fatty acids present in the extract. The bands at 1650 and 1550 cm^{-1} are characteristic of amide I and II band.[30] In the present study, the bands 3420, 3422 and 3454 C–H bend alkanes are commonly found in the proteins. These amide groups may also be in the aromatic rings. It is well known that proteins can bind to silver nanoparticle through either free amine groups or cysteine residues in the proteins[31] and therefore stabilization of silver nanoparticles by the surface bound proteins is possible in the present green synthesis.

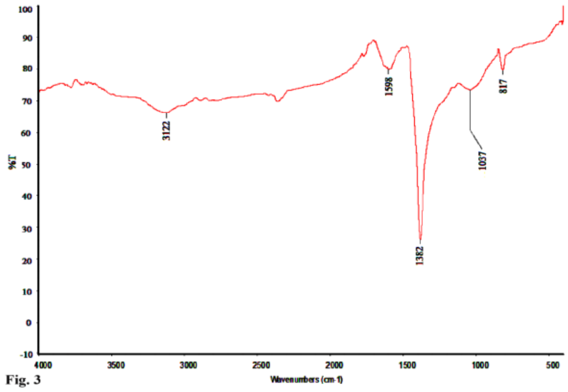

Fig. 3. FTIR spectra of (A) 1 mM $AgNO_3$ (B) dried *Z. jujuba* seed powder (C) Ag NPs synthesized from aqueous seed extracts of *Z. jujube.*

Scanning electron microscope (SEM) analysis

SEM analyses of synthesized Ag NPs were spherical and aggregated shape with an average size of 42.1 nm. Ag NPs were synthesized using seed extract of *Elettaria cardamomom* and the SEM image showed the spherical in shape with the size range of 40-70 nm.[32] EDAX analysis of the Ag NPs showed the presence of elemental metal signal which was also confirmed (Fig. 4A and B) in this study.

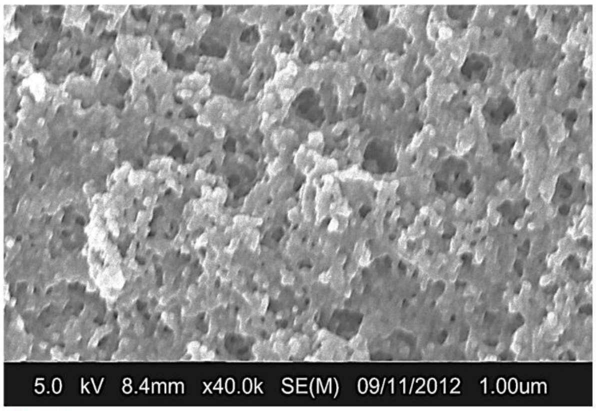

Fig.4A

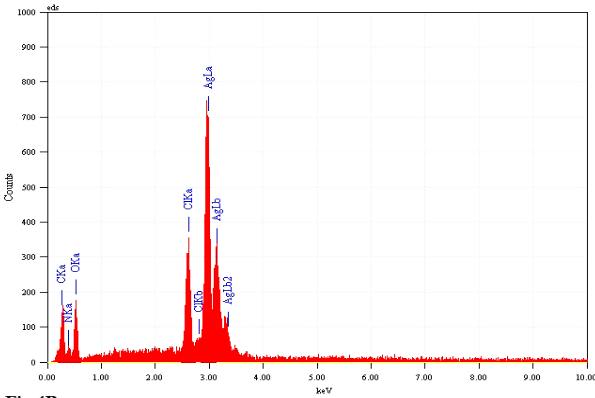

Fig.4B

Fig. 4. (A) SEM micrograph showing the Ag NPs synthesized using *Z. jujuba* (B) EDX spectrum of the biomass showing presence of silver.

Atomic Force Microscopy (AFM): The synthesized Ag NPs were characterized by AFM for its size, morphology, agglomeration analysis and three dimensional views. The irregular surface morphology was explained by the presence of both individual and agglomerated nanoparticles. The strong crystalline nature could be seen in the form of diagonal formations with ridges. The AFM provides suitable data regarding the green synthesized Ag NPs using seed extract of *Z. jujuba* (Fig. 5A and B). The AFM analysis showed that the synthesized Ag NPs using *Syzygium cumini* seed extract were spherical in shape.[10]

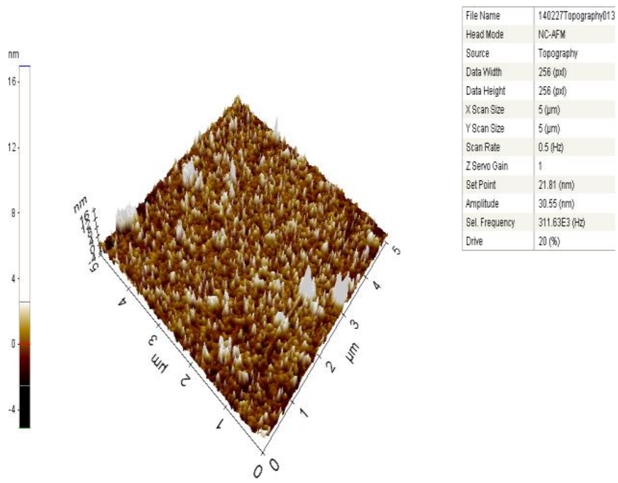

Fig. 5. AFM images of the synthesized Ag NPs showing (A) increased surface view and (B) 3D view

Anti-termite activity of Ag NPs: In the present study, aqueous extract, $AgNO_3$ solution and synthesized Ag NPs were evaluated for their anti-termitic potencies for the first time against *C. formosanus.* The maximum mortality was observed in the aqueous extract, $AgNO_3$ solution and synthesized Ag NPs against *C. formosanus* with LD_{50} values of 41.03, 37.68 and 3.77 mg/l; the LD_{90} values being 121.09, 110.03 and 8.42 mg/l and r^2 values of 0.989, 0.987 and 0.916, respectively (Table 2).

Table. 2: LD_{50} and LD_{90} values of aqueous, $AgNO_3$ and Synthesized silver nanoparticles using seed extract of *Zizyphus jujuba* against *Coptotermes formosanus*.

Extract /Material	Concentration (µg/ml)	SD % aMortality	LD50	LCL-UCL	LD90	LCL-UCL	*r2*
Aqueous	100	87±2.31	41.03	34.54-48.74	121.09	103.42-136.31	0.989
extract	80	72±3.87					
	60	62±1.30					
	40	48±0.34					
	20	29±4.19					
AgNO3	100	100±0.00	37.68	31.12-45.51	110.43	97.23-124.01	0.997
(1Mm solution)	80	82±0.54					
	60	64±1.67					
	40	51±3.92					
	20	32±4.91					
Synthesized	10	100±0.00	3.77	3.0-4.7	8.42	6.12-13.45	0.916
AgNPs	8	84±3.29					
	6	69±1.94					
	4	48±2.67					
	2	36±4.83					

Significant at $P<0.05$ level

LD_{50} lethal concentration that kills 50% of the exposed adult, LD_{90} lethal concentration that kills 90% of the exposed adult, UCL upper confidence limit, LCL lower confidence limit and regression a Mean value of three replicates

Meepagala et al.[33] have reported the effect of vulgarone B isolated from *Artemisia douglasiana*, apiol isolated from *Ligusticum hultenii* and cnicin isolated from *Centaurea maculosa* exhibited significant mortalities than in untreated controls in laboratory bioassay against *C. formosanus*. The methanolic leaf extract of *Detarium microcarpum* possessed strong antifeedant activity against termites.[34] Grace and Yates[35] reported that neem insecticide formulation, margosan-O, containing 0.3% azadirachtin and 14% neem oil, were toxic against the *C. formosanus.* The fermented extract from leaves of *Musa paradisiaca* at 100% concentration prevented termite attack for 50 days.[36]

Verma and Verma[37] have reported that the 5% chloroform leaf extract of *Lantana camara* possessed most effective termiticidal effects. The essential oil from the leaf of *Tagetes erecta* rich in (Z)-ocimene showed termiticidal activity and complete mortality of *Odontotermes obesus* was observed at a dose of 6 ml/Petriplate of leaf essential oil after 24 h of exposure.[38] The dichloromethane root extracts from *Echinops albicaulis*, *E*. transiliensis, *E*. *ritro* and *E*. *spinosissimus* 100% mortality by day 14 and the seed extracts of *Withania somnifera* and *Hygrophila articulate* were highly toxic in a 6-day period against the Formosan subterranean termite, *C. formosanus*.[39] Arihara et al.[40] reported the three new sesquiterpenes,(4S)-2,6,10-bisaboratrien-4-ol-1- one (**1**),1,8-epoxy-1 (**6**),2,4,7,10-bisabora pentaen-4-ol (**2**), and 1-methoxy-4-cadinene (**3**), have been isolated from the black heartwood of *Odontotermes japonica* and the isolated compounds were examined for termiticidal activity against *C. formosanus*.

The organic and inorganic nano-sized particles are increasing by gaining attention in medical applications due to their amenability to biological functionalization.[41] The chemical synthesis of nanoparticles might lead to the presence of some toxic chemical species adsorbed on the surface that may have adverse effects in its application. Plant-mediated biological synthesis of nanoparticles provides advancement over chemical methods as it is eco-friendly, cost effective, and easily scaled-up for large scale synthesis. Therefore, there is an urgent need to develop a green process of nanoparticle synthesis.[42] The synthesis of nanoparticles by green method can potentially eliminate this problem.

CONCLUSION

This is an eco-friendly and inexpensive approach for green synthesis of aqueous seed extract of *Z. jujuba* against *C. formosanus*. The present green synthesis showed that the renewable source of *Z. jujuba* could be used as an effective reducing agent for the synthesis of Ag NPs. These results suggested that the seed extract of *Z. jujuba* and synthesized NPs have the potential to be effectively control the population of C. *formosanus*. The results reported in this paper open the possibility of further investigations of efficacy on the termite control properties of natural product extracts.

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