Efficacy of plant-mediated synthesized silver nanoparticles against *Sitophilus oryzae*

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ABSTRACT

Silver nanoparticles (Ag NPs) were synthesized by using aqueous leaves extracts of *Euphorbia prostrata* as a simple, non-toxic and ecofriendly green material. The present study was based on assessments of the pesticidal activity to determine the efficacies of aqueous leaves extracts of *E. prostrata*, silver nitrate ($AgNO_3$) solution (1mM) and synthesized Ag NPs against the adult of *Sitophilus oryzae* L. The synthesized nanoparticles were characterized with UV- visible spectroscopy, X- ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR) and Scanning electron microscope (SEM) analysis. The nanoparticles were rod in shape and size of 25-80nm with an average size of 52.4 nm. Pesticidal bioassay tests were conducted at varying concentrations for 14 days. The LD_{50} values of aqueous extract, $AgNO_3$ solution and synthesized Ag NPs were 213.32, 247.90, 44.69 mg/kg $^{-1}$; LD_{90}=1648.08, 2675.13, 168.28 mg/kg $^{-1}$, respectively. These results suggest that the leaves aqueous extracts of *E. prostrata*, and synthesized Ag NPs have the potential to be used as an ideal eco-friendly approach for the control of the *S. oryzae*. This is the first report on the pesticidal activity of the plant extracts and synthesized nanoparticles.

Keywords *Euphorbia prostrata*, *Sitophilus oryzae*, silver nanoparticles, XRD, FTIR, SEM

INTRODUCTION

Rice is the most important food crop for more than half of the world's population. Losses in rice storage due to insect pests affect food availability for a large number of people. Milled rice is attacked by various insect pests during storage (Cogburn, 1980). Storage and upkeep of agricultural products are very important post harvest activities. Considerable amount of food grains is being spoiled after harvest due to lack of sufficient storage and processing facilities (Singh and Satapathy, 2003). The rice weevil, *Sitophilus oryzae* L. (Coleoptera: Curculionidae) is a major pest of stored rice in India, and has been spread worldwide by commerce. Both, the adults and larvae feed on whole grains. They attack wheat, corn, oats, rye, barley, sorghum, dried beans and cereal. It causes extensive losses in the quality and quantity of commercial products as well as deterioration of seed viability worldwide (Madrid *et al.*,1990).

Currently, chemical control is the most commonly used strategy against the pests. There are many chemicals that are toxic to stored-grain pests, including insecticides such as organophosphates, pyrethroids and fumigants such as methyl bromide and phosphine (Park *et al.*, 2003; Kljajic and Peric, 2006). These chemicals are effective for pest control but have several problems to users (Subramanyam and Hagstrum, 1995; Okonkwo and Okoye, 1996). Thus, repellents, fumigants, feeding deterrents and insecticides of natural origin are all rational alternatives to synthetic insecticides. Botanical insecticides composed of essential oils may prove to be a reasonable alternative to the more persistent synthetic pesticides (Chiasson *et al.*, 2004). It has also provoked undesirable effects, including toxicity to nontarget organisms and fostered environmental and human health concerns (Lee *et al.*, 2001).

Nanotechnology is now poised to enter commercialization era. NPs are showing promise in different fields of agricultural biotechnology (Rahman *et al.*, 2009). Stadler *et al.* (2010) successfully applied nano alumina against two stored grain pests like *S. oryzae* and *Rhyzopertha dominica*. Green Ag NPs have been synthesized using various natural products like *Azadirachta indica* (Tripathi *et al.*, 2009); *Glycine max* (Vivekanandhan *et al.*,2009) and *Camellia sinensis* (Begum *et al.*,2009). Present study was carried out on *Euphorbia prostrata* Ait. (Euphorbiaceae) is a small, prostrate, hispidly pubescent annual herb found all over India. They are occurring in two forms, red and green, recognized as two ecotypes; the former being tolerant to high calcium levels in the soil (Bhatnagar and Prakash, 1973). The active principles in *E. prostrata* are chiefly flavonoids, phenolic acid and tannins. The flavonoids possess a catecholic B-ring that seems to be responsible for the toxicant activity to insects (Onyilagha *et al.*, 2004), and this activity vary in agreement with the chemical structure of these compounds (Larsson *et al.*,1992). Kumar *et al.*,(2010) reported that the ethanolic leaves extract of *Annona squamosa* was tested against *S. oryzae*, the phytochemical investigation of this plant is mainly contains tannins and phenolic compounds. Elumalai *et al.* (2010) have reported that the aqueous extract of shade dried leaves of *Euphorbia hirta* was used for the synthesis of Ag NPs and their antibacterial activities. The antimicrobial activity of aqueous extracts of *E. prostrata* is highly effective against *Shigella dysenteriae* type 1 induced diarrhea in rats (Kamgang *et al.*, 2007). Nanoencapsulation is currently the most promising technology for protection of host plants against insect pests. Thus nanotechnology will revolutionize agriculture including pest management in the near future. Over the next two decades, the green revolution would be accelerated by means of nanotechnology (Bhattacharyya *et al.*, 2010). In this study, the pesticidal activities of synthesized Ag NPs using the leaves of *E. prostrata* were assessed. We report the synthesis of Ag NPs, reducing the silver ions present in the solution of silver nitrate by the cell free aqueous leaves extract of *E. prostrata*. Furthermore, these biologically synthesized nanoparticles and aqueous extracts of *E. prostrata* were found to produce a high parasitic activity.

MATERIALS AND METHODS

Insects rearing

S. oryzae was collected from infested rice obtained from a local market and reared in glass jars under laboratory conditions of 30°C ± 1°C, 75 ± 5% relative humidity (RH) in continuous darkness. The RH was maintained by using saturated solution of sodium chloride (Winston and Bates, 1960). After the pupal stage the adults less than 24 hrs old were used for the experiments.

Materials

Fresh leaves of *E. prostrata* were collected in and around Melvisharam, Vellore district, Tamil Nadu, India and the taxonomic identification was made by Dr. C. Hema, Department of Botany, Arignar Anna Government Arts College for Women, Walajapet, Vellore, India. The voucher number (CAH/EP/114-06) was given and kept in our research laboratory for further reference. Silver nitrate (99.9% pure) was obtained from Qualigens Fine Chemicals, Mumbai, India.

Preparation of plant extracts

The leaves was finely cut into small pieces and the aqueous extract was prepared by mixing 10 g of dried leaves powder with 500 mL of water (boiled and cooled distilled water) with constant stirring on a magnetic stirrer (Minjas and Sarda 1986). The suspension of dried leaves powder in water was left for 3 h, filtered through Whatman no. 1 filter paper, and the filtrate was stored in amber colored air tight bottle at 10°C and used within a week.

Synthesis of Ag NPs

The fresh leaves of *E. prostrata* broth solution was prepared by taking 2 g of thoroughly washed and finely cut leaves in a 300 mL Erlenmeyer flask along with 100 mL of sterilized double distilled water and then boiling the mixture of 60^{o} C for 15 min before finally decanting it. After boiling, the solution was cooled, decanted, and 12 mL of this broth was added to 88 mL of 1mM aqueous $AgNO_3$ solution and the resulting solution became brown in color. This extract was filtered through nylon mesh (spectrum) followed by Millipore hydrophilic filter (0.22µm) and used for further experiments (Parashar *et al.*, 2009). A control was setup to maintained without *E. prostrata* extract and color intensity of the extracts was measured at 420 nm for different intervals (15, 30, 60, 120, 240 and 300 min respectively).

Pesticidal Bioassay

The bioassay on *S. oryzae* was performed in small plastic screw capped jars. Each jar had a radius of 6 cm and height of 6.5 cm. The caps were perforated to allow aeration. 20 g of rice (IR64) was placed in each jar. Rice in each jar was mixed individually with aqueous leaves extracts of *E. prostrata*, 1mM $AgNO_3$ solution and synthesized Ag NPs. Different concentrations of 1000, 800, 600, 400, 200 mg/kg^{-1} of aqueous extract, $AgNO_3$ and synthesized Ag NPs in the concentration ranging from 250, 200, 150, 100, 50 mg/kg^{-1} rice were prepared. Then, the jars were shaken manually for approximately 1 min to achieve equal distribution on rice (Subramanyam and Roesli, 2000). For each dose, there were five replicates. The control papers were impregnated with aqueous plant extract, $AgNO_3$ and synthesized Ag NPs with rice. The jars were kept for 24 hrs before 20 unsexed adults of *S. oryzae* were introduced into each jar. All bioassays were performed at 30°C ± 1°C, 75 ± 5% r.h. Insect mortality was checked after 1, 3, 5, 7, and 14 days as per the method of Debnath *et al.*(2010). After the bioassay was complete, all the live insects were removed and the treated boxes were retained for two months beyond the experiment to check if there were any fresh *S. oryzae* progeny in the rice.

Characterization of the synthesized nanoparticles

Synthesis of Ag NPs solution with leaves extract may be easily observed by UV–vis spectroscopy. The bioreduction of the Ag+ ions in solutions was monitored by periodic sampling of aliquots (1 mL) of the aqueous component after

20 times dilution and measuring the UV–vis spectra of the solution. UV–vis spectra of these aliquots were monitored as a function of time of reaction on a Schimadzu 1601 spectrophotometer in 300–700nm range operated at a resolution of 1 nm. Further, the reaction mixture was subjected to centrifugation at 60,000×g for 40 min; resulting pellet was dissolved in deionized water and filtered through Millipore filter (0.45 µm). An aliquot of this filtrate containing Ag NPs was used for X-ray diffraction (XRD), and Fourier transform infrared (FTIR) analysis. For electron microscopic studies, 25 ìL of sample was sputter-coated on copper stub, and the images of nanoparticles were studied using scanning electron microscopy (SEM; JEOL, Model JFC-1600) and the measurements were operated at an accelerating voltage of 120 kV and later with an XDL 3000 powder. FTIR spectra of the samples were measured using Perkin-Elmer Spectrum One instrument in the diffuse reflectance mode at a resolution of 4 cm”1 in KBr pellets. Powder samples for the FTIR was prepared similarly as for powder diffraction measurements. The FTIR spectra of leaves extracts taken before and after synthesis of Ag NPs were analyzed which discussed for the possible functional groups for the formation of Ag NPs. For XRD studies, dried nanoparticles were coated on XRD grid, and the spectra were recorded by using Phillips PW 1830 instrument operating at a voltage of 40 kV and a current of 30 mA with CuKá1 radiation.

Data analysis

Lethal concentration (LD_{50}, LD_{90}) and their associated confidence intervals were estimated from 24 h concentration mortality data using probit analysis (Finney, 1971). LD_{50} at the 50% and slope levels were considered significantly different if their associated confidence intervals did not overlap. All differences were considered significant at 5% level.

RESULTS

Results from the pesticidal activity showed that the synthesized Ag NPs using *E. prostrata* were more effective than the aqueous extract and $AgNO_3$ solution (Table 1). Complete mortality (100%) was observed on 7th days for the synthesized Ag NPs but in aqueous extract and the $AgNO_3$ solution the same mortality was observed after 14 days (Table 1). The LD_{50} value of aqueous extract, $AgNO_3$ and synthesized Ag NPs were 213.32, 247.90, 44.69 mg/kg^{-1}, respectively (Table

Table 1. Insecticidal activity of aqueous extract of *E. prostrata*, silver nitrate solution and synthesized Ag NPs against the adults of *Sitophilus oryzae*

Test samples	Concentrations mg/kg^{-1}	Exposure hours (Mean±SD)				
		1st day	3rd day	5th day	7th day	14th day
Aqueous extract	1000	15±0.707	40±1.000	57±1.342	68±2.302	89±1.483
	800	09±0.447	25±0.708	44±1.780	61±2.489	76±1.304
	600	06±0.836	19±0.834	33±1.303	39±1.782	62±2.881
	400	02±0.574	11±0.447	24±0.830	33±1.517	46±1.484
	200	-	3±0.500	16±0.836	22±0.547	35±1.581
Silver nitrate	1000	17±0.894	37±0.894	49±1.789	60±2.236	80±1.000
	800	11±0.832	30±0.707	41±1.484	55±2.237	72±1.517
	600	08±0.000	19±0.834	36±0.837	43±1.304	58±1.792
	400	05±0.702	14±0.836	20±1.225	28±0.894	46±1.484
	200	-	07±0.547	13±0.894	21±1.781	32±1.140
Synthesized Ag NPs	250	55±2.237	70±1.224	88±1.674	100±0.000	100±0.000
	200	47±2.881	58±2.190	79±1.924	92±1.140	97±0.836
	150	40±1.582	45±1.581	66±2.000	85±2.000	91±1.225
	100	24±0.445	31±1.141	56±1.484	71±1.644	80±1.584
	50	18±0.895	27±0.540	37±1.517	60±1.122	71±1.484

Mean value of five replicates ± SD; Control—nil mortality

Table 2 LD_{50}, LD_{90}, and other statistical analysis of aqueous extract of *Euphorbia prostrata*, silver nitrate solution and synthesized Ag NPs against the adults of *Sitophilus oryzae*

Test samples	LD_{50} (mg/kg^{-1})	UCL–LCL (mg/kg^{-1})	LD_{90} (mg/kg^{-1})	UCL–LCL (mg/kg^{-1})	?2 (df=4)
Aqueous extract	213.32±23.22	258.84-167.78	1648.08±339.97	2314.43-981.71	4.19
Silver nitrate	247.90±29.18	305.10-190.70	2675.13±76.34	4171.49-1178.72	7.82
Synthesized Ag NPs	44.69±5.80	56.06-33.32	168.28±15.38	198.44-138.12	9.86

Control—nil mortality. Significant at $p < 0.05$ level.

LD_{50} =lethal concentration that kills 50% of the exposed larvae, LD_{90}= lethal concentration that kills 90% of the exposed parasites, UCL: upper confidence limit; LCL: lower confidence limit; x^2=Chi-square; df: degree of freedom.

2). This goes prove to that concentration plays an important role in insecticidal activity.

E. prostrata leaves extract without $AgNO_3$ did not show any change in color. Reduction of silver ion into Ag particles during exposure to the plant extracts could be followed by color change. Ag NPs exhibit dark yellowish brown color in aqueous solution due to the surface plasmon resonance phenomenon. The result obtained in this investigation is very interesting in terms of identification of potential weeds for synthesized Ag NPs.

UV-vis spectrograph of the colloidal solution of Ag NPs has been recorded as a function of time. Absorption spectrum of *E. prostrata* leaves extracts at different wavelengths ranging from 300 - 600 nm revealed a peak at 420 nm (Fig. 1).The synthesis solutions of Ag NPs in each case contained many molecules and some of these become attached or adsorbed on the surface of Ag NPs. FTIR analysis of these nanoparticles can characterize such molecules associated with Ag NPs. To identify Ag NPs associated molecules, FTIR spectra were recorded as shown in Fig 2. The FTIR spectra of Ag NPs exhibited prominent peaks at 3,431, 1,616, 1,381, 1,045, 818, 509 and 420 cm^{-1} (Fig. 2). The X-ray diffraction pattern of Ag NPs produced by leaves extract is shown in Fig. 3. The control thin films of the leaves extract as well as the $AgNO_3$ did not show the characteristic peaks. The XRD spectrum compared with the standard confirmed spectrum of silver particles formed in the present experiments were in the form of nanocrystals, as evidenced by the peaks at 2è values of 23.22°, 27.85°, 30.91°, 31.80°, 32.00°, 34.62°, 35.34°, 38.26° , 44.45° ,45.38° ,64.58°, and 77.49° the pure silver lattice constant has been estimated to be Alpha=4.081. The value of that is consistent with Alpha=4.0862 Å reported by the JCPDS file no. 4-0783. This estimation confirmed the hypothesis of particle monocrystallinity. The sharpening of the peaks clearly indicates that the particles were in the nanoregime. SEM analyses of the synthesized Ag NPs were clearly

Fig. 1. UV–vis absorption spectra of Ag NPs synthesized by *Euphorbia prostrata* leaf extract after 6 hrs

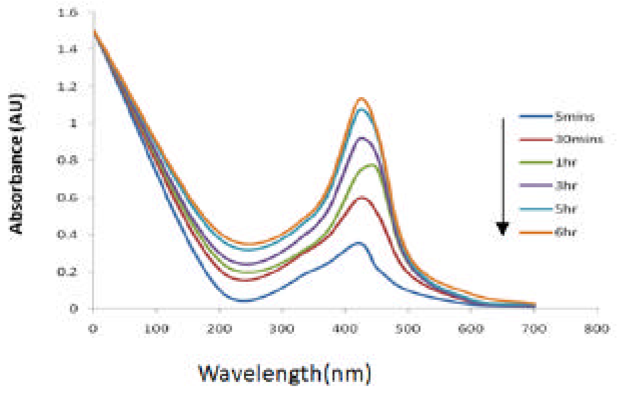


Fig. 2. FTIR spectra of vacuum dried powder of synthesized Ag NPs using *Euphorbia prostrata* leaf extract

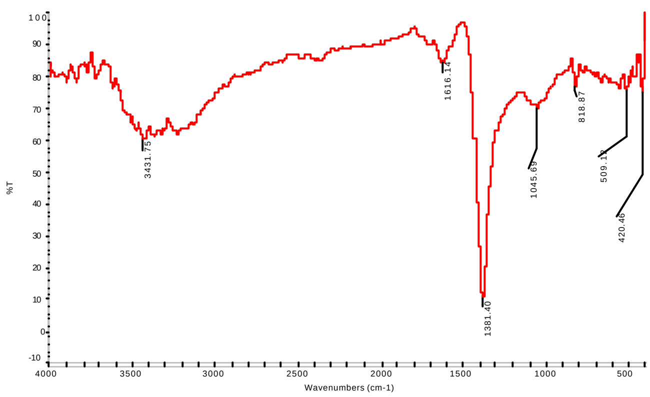


Figure 3. XRD pattern of Ag NPs synthesized by *Euphorbia prostrata* leaf aqueous extract

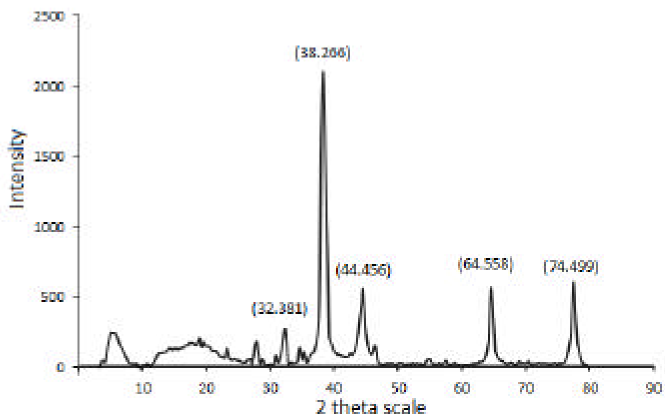
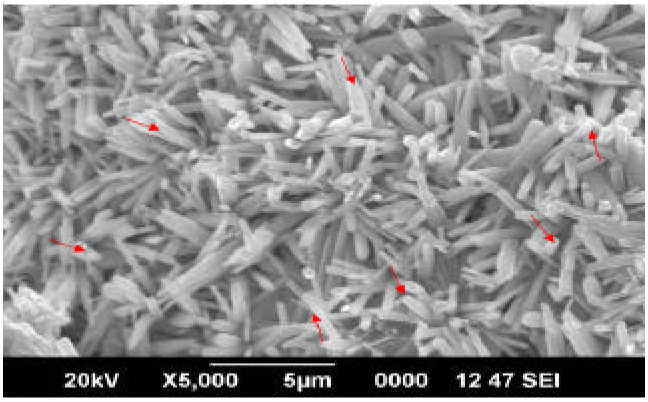


Fig. 4 Scanning electron micrographs of Ag NPs synthesized with *E. prostrata* leaf extract with 1.0 mM AgNO3 solution and incubated at 60°C for 6 h at pH 7.0 (magnified ×5000 inset bar represents 5 µm)



distinguishable measured ranged in size of 25-80 nm with an average size of 52.4 nm. Representative SEM micrographs of the reaction mixtures containing 10 mg of *E. prostrata* leaves extract powder and 1.0 mM of silver nitrate incubated for 6 hrs magnified at 5000× and 10,000× are shown in Fig. 4 a and 4b.

DISCUSSION

Plants or their extracts can be efficiently used in the synthesis of gold and Ag NPs as a greener route. Control over the shape and size of nanoparticles seems to be very easy with the use of plants. Such nanoparticles produced using plants have been used in various applications for human benefit. Elucidation of the mechanism of plant-mediated synthesis of nanoparticles is a very promising area of research (Kumar and Yadav, 2009).The potential uses and benefits of nanotechnology are enormous. These include agricultural productivity enhancement involving nanoporous zeolites for slow release and efficient dosage of water and fertilizer, nanocapsules for herbicide delivery, vector and pest management and nanosensors for pest detection (Scrinis and Lyons, 2007a). Nanoparticles help to produce new pesticides, insecticides and insect repellants (Owolade *et al.*, 2008). Nanoencapsulation is a process through which a chemical such as an insecticide is slowly but efficiently released to a particular host plant for insect pest control. Nanoencapsulation with nanoparticles in form of pesticides allows for proper absorption of the chemical into the plants unlike the case of larger particles (Scrinis and Lyon, 2007b). It has been observed that nanoparticles loaded with garlic essential oil are efficacious against *Tribolium castaneum* (Yang *et al.*, 2009).

The results of the present study show that synthesized Ag NPs from *E. prostrata* extract in relation to silver nitrate in exhibited various levels of reduction of adult *S. oryzae* and offered various degrees of protection to plants compared with *E. prostrata* based aqueous extract formulations. The present study shows that mixtures of plant extracts at 1g/kg^{-1} exhibited insecticidal activity against the target pests and thus indicating their potentials for development as botanical based nanoparticles. Various studies have been taken in use for the detection of the usage of botanicals as control in the stored product pest. Debnath *et al.* (2010) reported that the diatomaceous earth was used to design amorphous nano sized hydrophilic, hydrophobic, and lipophilic, surface-functionalized silica nanoparticle (SNP) was tested against rice weevil *S. oryzae* and its efficacy was compared with bulk-sized silica, application of hydrophilic SNP at 1 g kg^{-1} could kill more than 80% of the insects, after 7 days of exposure, 95 and 86% mortality were obtained with hydrophilic and hydrophobic SNPs at 1 g kg^{-1}, respectively. Biosythesis of gold and Ag NPs using aqueous solution of *C. sinensis* extract contains mainly polyphenols compounds and flavonoids, which are the natural source for the synthesis of Ag NPs (Begum *et al.*, 2009).

Goswami *et al.* (2010) reported that the applications of different surface functionalized hydrophilic nanoparticles, silica (SNP), aluminium oxide (ANP), zinc oxide (ZNP) and titanium dioxide (TNP) nanoparticle were tested against rice weevil *S. oryzae*. Insect mortality due to silica nanoparticles treatment was obtained at dose rates almost comparable with those of commercially available DE formulations ranging from 500 to 5000 mg kg^{-1} (Subramanyam and Roesli, 2000; Vardeman *et al.*, 2007). The above highlights putative effects of nanoparticles on insects, as these small particles are present in their entire body parts.

In UV spectral analysis the generation of color is due to excitation of surface plasmon in metal nanoparticles (Mulvaney, 1996). The reduction of silver ions and formation of stable nanoparticles occurring within 4 hrs of reaction was also achieved by Vilchis-Nestor *et al.* (2008) with *C. sinensis* extract. In basil plant the plasmon intensity at the reaction time of 11 hrs is near to that at 15 hrs, meaning completion of the reaction. Ag NPs were observed to be stable in solution and show very little aggregation (Ahmad *et al.*,2010). Chandran *et al*.(2006) reported that the synthesized Ag NPs using *Aloe vera* only after 24 hrs of reaction and in presence of ammonia, which in the study mentioned enhances the formation of a soluble silver complex. Sakulku *et al*.(2009) have reported the low release rate of nanoemulsion with large droplet size that resulted in prolonged mosquito repellant activity compared to the nanoemulsion with small droplet size. Although an attempt to develop essential oil for pesticides and insecticides has been made in a variety of water-soluble formulations such as nanoemulsion incorporated with â-cypermethrin (Wang *et al.*,2007) and essential oil-loaded microcapsules for pest control (Moretti *et al.*, 2002). XRD pattern clearly illustrates that the Ag NPs formed in this present synthesis were crystalline in nature. The purified nanoparticles showed the presence of bands due to O–H stretching (around 3431 cm^{-1}), C = O group (1616 cm^{-1}), C–C and C–N stretching (1381 cm^{-1}), O–H stretch (1045 cm^{-1}), and C–H alkenes (818). The spectra showed sharp and strong absorption band at 1616 cm^{-1} assigned to the stretching vibration of (NH) C=O group. The band 1381 developed for C–C and C–N stretching, respectively, and was commonly found in the proteins.

For the SEM studies, reaction mixtures were air-dried on silicon wafers. As a result, a coffee ring phenomenon was observed. It is well-known that when liquids that contain fine particles were evaporated on a flat surface, the particles accumulate along the outer edge and form typical structures (Chen and Evans, 2009). Ag NPs were synthesized using leaves extract of *Acalypha indica* and the SEM image showed the size of the control silver nitrate obtained was greater than 1000 nm size, where as synthesized Ag NPs measured 20–30 nm in size (Krishnaraj *et al.*, 2010). The Ag NPs synthesized by treating silver nitrate solution with *Eucalyptus hybrida* leaves extract, the Ag NPs formed were predominantly cubical with uniform shape; considerably change their optical and electronic properties (Xu and Käll, 2002).

The position of the peak changed with increasing incubation time. It was reported earlier that peak shift is an indicator of size change. Nanoparticle size also determines the colour of the solution, the smaller the size of Ag NPs and the greater the colours shift towards red (Mock, 2002). The difference in this regard with the present study may be due to difference in pest species, dose of the treatment, methodology of research, varietal and commodity variations, and laboratory and environmental differences. Some other interesting findings were reported by different scientists on plants but with different insect species.

One exciting finding from these experiments is that no fresh insect infestation is found in the Ag NPs treated stored rice even after 2 months of treatment. The nanocides can be removed by conventional milling process unlike sprayable formulations of conventional pesticides, leaving residues on the stored grain. Therefore, Ag NPs has an excellent potential as stored grain as well as seed protecting agent if applied with proper safety measures. This study could lead to open up newer pathways of using nanomaterial based technology in pesticide industry.

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