

Original Research Article

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Synthesis of Green Silver Nanoparticles from Soybean Seed and its Bioefficacy on *Spodoptera litura* (F.)

Goutam B. Hosamani^{1*}, R.R. Patil¹, V.I. Benagi²,
S.S. Chandrashekhar³ and B.S. Nandihali¹

¹Department of Agricultural Entomology, College of Agriculture, University of Agricultural sciences, Dharwad Karnataka -580005, India

²Department of Plant pathology, College of Agriculture, University of Agricultural sciences, Dharwad Karnataka -580005, India

³Department of Seed Science and Technology, College of Agriculture, University of Agricultural sciences, Dharwad Karnataka -580005, India

*Corresponding author

ABSTRACT

Keywords

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Now a day's nanotechnology has become one of the most promising approaches in pest management. Nanoparticles represent a new generation of environmental remediation technologies that provide cost-effective solution to some of the most challenging insect pests. *S.litura* (F.) (Lepidoptera: Noctuidae) is a major polyphagous pest of field crops and vegetables, causing severe damage to the plants in all phenological crop stages. The overall objective of this investigation was to look for new control strategy through evaluating the effect of Soya based Ag Nano -particles on the 2nd and 3rd instar larvae of *S.litura* under laboratory conditions. In the present study, we have reported the synthesis of silver green nanoparticles from soybean seed extract by Sunlight exposure method and its influence on *S.litura*. The synthesized soybean based silver nanoparticles were characterized by UV-Vis spectroscopy and particle size analyser (Nicom). The result revealed that soybean based AgNO₃ nanoparticles had a mean diameter of 87 nm (PSA). Nano Ag particles were the most effective compounds possessing insecticidal activity. Increased concentration and exposure period enhanced larval mortality. AgNPs caused characteristic symptoms of sluggishness, inactiveness, the larvae refusing to feed resulting in larval mortality.

Introduction

Nanotechnology is defined as the application of science, engineering and technology to develop novel materials and devices in different fields in the nanorange. For the

synthesis of silver nanoparticles, there are multiple processes that one could use. Some of these are the electrochemical method, sonochemical method, microwave-assisted processes and lately green chemistry approach. The synthetic nanoparticles though

available and effective are toxic and hazardous to the environment and to the user. Green nanoparticles are very important in developing sustainable technologies for the future. Synthesis of nanoparticles by plants is a green chemistry approach that interconnects nanotechnology and biotechnology. Plant extracts are used for bio reduction of metal ions to form nanoparticles. It has been demonstrated that plant metabolites like sugars, terpenoids, polyphenols, alkaloids, phenolic acids and proteins play an important role in reduction of metals ions into nanoparticles and support their subsequent stability. Synthesis of nanoparticles from plant extracts or microorganisms are safe to the environment and the user. Further, they are easily available and possess a broad range of metabolites. The integration of the principles of green chemistry to nanotechnology toward the synthesis of green nanoparticles is a current requirement in pest management (Srikanth *et al.*, 2017).

Spodoptera litura (Lepidoptera: Noctuidae) is a polyphagous pest, invading more than 180 plant species. Nowadays, manmade pesticides have been extensively used for controlling this pest on diverse crops, but have adverse side effects especially the frequency of resistance, mutilation to the environment, pest resurgence and fatal effects on non-target organisms have imposed a shift to more ecofriendly approach for managing this pest. Indiscriminate exploitation of insecticides, multiple generations of insects per annum, year-round availability of host crops contributed to the insecticide resistance of *S.litura* against almost all the insecticide groups (Kranthi *et al.*, 2002) including the new insecticides like lufenuron (Sudhakaran, 2002).

The adverse effects due to synthetic pesticides on pests and their subsequent impacts to ecological imbalance demands sustainable alternatives.

Synthetic Nano-Ag was effective against *S. litura* larvae (Chakravarthy *et al.*, 2012a). More than half of them relied to nanomaterials prepared through the so-called green synthesis method and nanomaterials synthesized from extracts from plants Beneli (2018) bacteria (Ayano *et al.*, 2014) and even dead insects (Jha and Prasad 2012) have been successfully employed to reduce and stabilize nanoparticles in aqueous suspensions. It is quite difficult and challenging to identify such insecticidal activity in the area of new chemistry. The present investigation was aimed to synthesize and evaluate the insecticidal effect of Soyabean seed based green silver nanoparticles.

Materials and Methods

soybean seeds (DSb 21) were collected from Main Agricultural Research Station, UAS, Dharwad. Synthesis and characterization of green silver nanoparticles was done in the Green Nanotechnology laboratory, while the bioassay was done in the Post graduate laboratory of the Department of Agricultural Entomology, University of Agricultural Sciences, Dharwad. 10 grams of partially grounded seeds were incubated in 100 ml of millipore water for 2 hours in hot water bath. Finally extract was centrifuged at 9000 rpm for removal of heavy seed debris followed by filtration with Whatman No. 1 filter paper. This resulted in production of milky colour seed extract that was stored at 4° C for further use.

Biosynthesis of silver green nanoparticles from soybean seeds

Protocol of Indrakumar (2016) was followed for synthesis of AgNp from soybean seed extract. 1mM aqueous silver nitrate (AgNO₃) was obtained from Hi Media Laboratory, Mumbai and used as a precursor, soybean seed extract was used as reducing and capping agent for synthesis of silver nanoparticles. 1.0

ml of soybean seed extract was added to 10 ml of 1 mM aqueous silver nitrate and taken into 30 ml screw test tube mixed well and exposed to bright sunlight for 3 h. Colour change was observed for the confirming the synthesis of silver nanoparticles (sunlight exposure method).

Further the synthesised soya seed based AgNps were characterized to know the size and other parameters using UV-Visible Spectrophotometer (UV-Vis), Particles Size Analyzer (PSA) NICOMP make.

Maintenance of insect culture

The egg masses were collected from the field of UAS Dharwad, brought to the laboratory and incubated. On hatching the first instars were released on host plant castor *Ricinus communis* in the laboratory and reared at 25°C - 28°C temperature and 70 per cent relative humidity.

Bioassay studies

The castor leaves dipped (leaf dip method) in green nanoparticles of different concentrations were provided as first feed early in the morning from the first day of both 2nd and third instar and the other two were normal feeds. Complete randomized bock design (CRD) design was used three replications were maintained. Ten larvae were used per replication. Based on instar duration observations were recorded on larval mortality at 24, 48, 72 and 96 Hours after treatment (HAT). Per cent mortality was calculated by using the formula.

Per cent mortality =

$$\frac{\text{Number of dead insects}}{\text{Total number of insects}} \times 100$$

Statistical analysis

The results obtained were subjected for statistical analysis (ANOVA) using a completely randomized block design. The mean values of treatments were then subjected to Duncan's Multiple Range Test (DMRT).

Results and Discussion

Biosynthesis of AgNps from soyabean seed extract

Synthesized soybean based silver nanoparticles were confirmed by colour changes commencing from colourless to different colours. Within 10 minutes of exposure to sunshine the colour changed from colourless to faint yellow and by 3 hours yellow coloured solution changed to dark orange completely. Further, the same colour was retained. The UV-Vis absorption peak was recorded between 425-435 nm with average particle size of 87 nm (Fig. 1 and 2). The present study with respect to synthesis is in full conformity with the description of Prasad (2014) who used soybean seed extract for synthesis of silver nanoparticles through magnetic stirring method. He characterized nanoparticles by means of UV-Vis and sharp band scattered at 434 nm clearly notifying the formation of silver nanoparticles TEM image obtained from green synthesized AgNPs were nearly monodisperse spherical shape and size ranged from 25 nm to 50 nm. In the present study the size of the particle was 87 nm, 425 nm UV-Vis and agrees with the report of Prasad (2014).

Similarly Indrakumar (2016) used soybean seeds for the synthesis of nanoparticles. Among the three methods he followed Sunlight exposure method was effective in synthesis of stable AgNPs. The UV-Vis absorption peak was recorded between 425-435 nm. The particle size of synthesized

nanoparticles was below 100 nm and the shape was spherical as being reported in the present study.

Efficacy of soybean seed based Green AgNp's on second and third instar of *S.litura*

The chemical, Emamectin benzoate @ 0.2 gm/L was significantly superior to all other treatments and showed cent per cent mortality of 2nd and 3rd instar at 24 hrs and 48 hrs after treatment. However, Precursors, the seed extract and water control did not effect any mortality.

The effect of different concentrations of AgNPs on second instar is presented in Table 1. At 24 hours 10000 ppm was superior to all other treatments (26.67 %) but on par with 8000 ppm, 6000 ppm that recorded 26.67 and 23.33 per cent mortality respectively. This was followed by 4000 ppm that recorded 20 per cent. Next best were 2000 ppm and 1000 ppm but were on par to each other with 16.67 per cent mortality. This was followed by 750 and 500 ppm which differed significantly from one another effecting 13.33 and 10.00 per cent mortality. Least percent mortality was noticed in 250 ppm. At 48 hours 10000 ppm was superior to all other AgNps but for 8000 and 6000 ppm recording 60 per cent, 53.33 per cent mortality. However, least per cent mortality (20 %) was recorded in 250 ppm. At 72 hours 10000 and 8000 ppm gave cent percent mortality but were on par to each other as well with chemical treatment. This was followed by 6000 ppm and 4000 ppm with 96.67 and 86.67 per cent mortality but did not differ among themselves. This was followed by 2000, 1000 and 750 ppm with mortality of 80.00, 80.00, 63.33 and 53.33 per cent respectively exercising similar effects. Least mortality of 46.67 % was recorded in 250 ppm.

In third instar at 24 HAT 10000 ppm, 8000 ppm, 6000 ppm and 4000 ppm were superior to all other treatments with 20.00, 20.00, 16.67 and 16.67 per cent mortality and were on par with each other. Next best was 2000 ppm and 1000 ppm both recording 13.33 per cent mortality and were on par to each other. Lowest mortality was noticed in 750 ppm. At 48 hours 10000 ppm and 8000 ppm recorded 46.67 and 43.33 per cent mortality and were superior to all other AgNps but on par to each other. Next best was 6000, 4000, 2000 ppm recording 36.67, 36.67 and 33.33 per cent mortality respectively without differing among themselves. This was followed by 1000, 750 and 500 ppm and all behaved similarly. However, least per cent mortality (10 %) was recorded in 250 ppm. At 72 hours 10000 ppm was significantly superior to all other treatments with 76.67 per cent mortality. This was followed by 8000 ppm, 6000 ppm and 4000 ppm but they differed significantly from one another. Next best were 2000, 1000, 750, 500 ppm and were on par to each other. Minimum mortality of 23.33 per cent was produced in 250 ppm. At 96 hours 10000 ppm gave cent percent mortality but was on par with chemical treatment but superior to all AgNp treatments. This was followed by 8000 ppm, 6000 ppm and 4000 ppm and differed significantly from one another. This was followed by 2000 and 1000 ppm both recording 82.50 per cent mortality & but were on par to each other. This was followed by 750 ppm (75.45 %). Minimum mortality (56.67 %) was recorded in 250 ppm. The use of green soya based AgNPs caused inactiveness, the larvae became sluggish ultimately refusing feed and resulting in larval mortality (Table 2).

At 48 hrs the AgNp extract on 2nd instar recorded (20.00 %-63.33 %) mortality and 46.67- 100 per cent mortality at 72 h while the 3rd instar larvae recorded mortality rate of 23.33 -76.67 per cent at 72 h and 56.67 %-100

% mortality at 96 hours of exposure. The mortality rate increased as the toxic concentration increased. The present study on use of green AgNPs is strengthened by the report of Kasmara *et al.*, (2017) who reported that *Lantana camara* nano extracts proved to

be more effective in managing *S. litura* 3rd instar larvae since the observed mortality rate was 3-10 % higher in 24 h and 13-26 % higher in 48 h as being reported in the present study both for 2nd and 3rd instar.

Fig.1 UV-Vis spectrum of Silver nanoparticle synthesized by Soybean seed extract

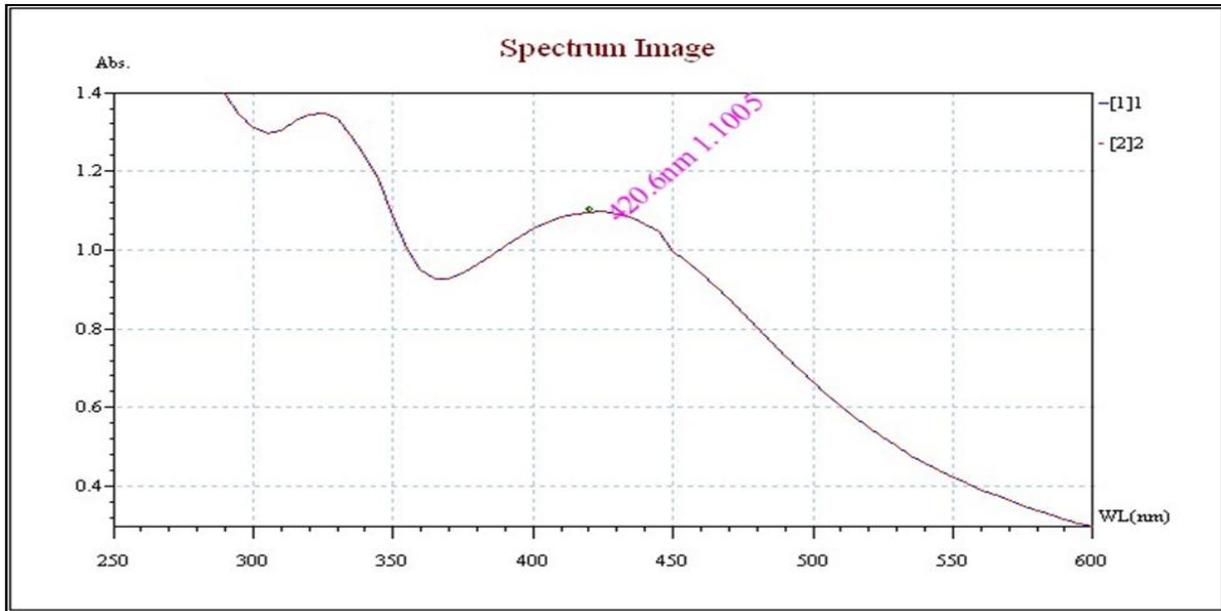


Fig.2 Particle size of Silver nanoparticle synthesized from soybean seed extract of Average Size 87 nm

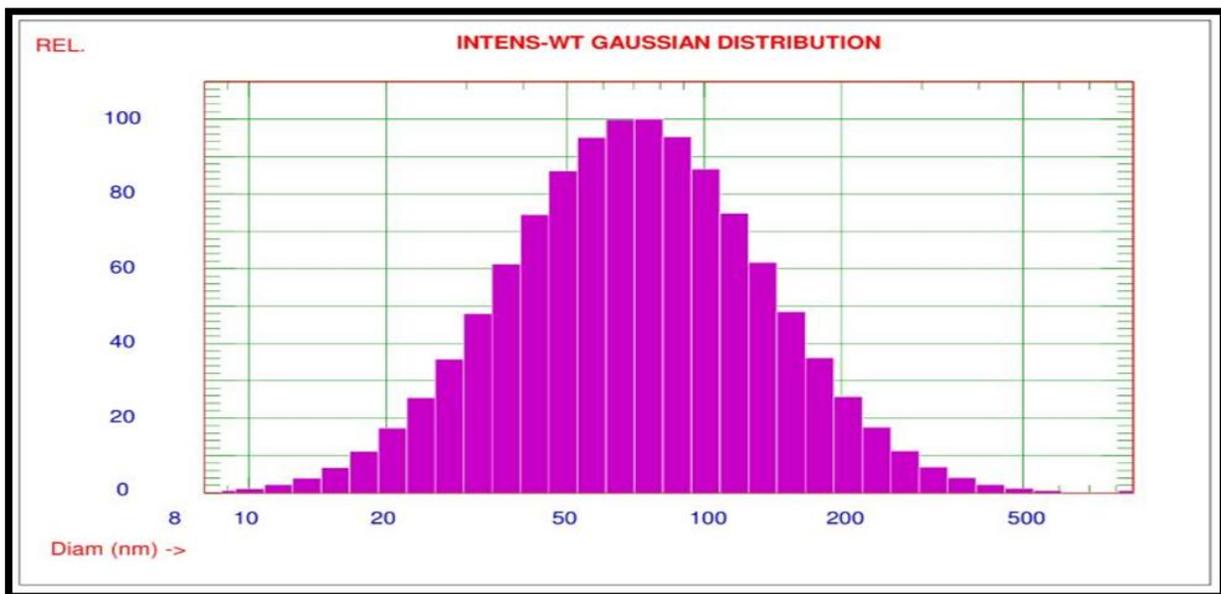


Table.1 Effect of Soybean seed based Green Silver nanoparticles on percent larval mortality of *S. litura* (second instar)

Treatments / Concentrations in ppm	Hours After Treatment (HAT)		
	24 hours	48 hours	72 hours
250	3.33 (1.91) ^g	20.00 (7.33) ^f	46.67 (7.55) ^e
500	10.00 (4.95) ^f	30.00 (7.95) ^e	53.33 (8.35) ^d
750	13.33 (6.10) ^e	36.67 (8.74) ^e	63.33 (9.75) ^c
1000	16.67 (7.33) ^d	43.33 (9.27) ^{cd}	80.00 (10.00) ^c
2000	16.67 (7.33) ^d	43.33 (9.27) ^{cd}	80.00 (10.00) ^c
4000	20.00 (8.05) ^c	46.67 (9.36) ^c	86.67 (11.02) ^b
6000	23.33 (8.74) ^b	53.33 (9.88) ^{bc}	96.67 (11.54) ^b
8000	26.67 (9.27) ^b	60.00 (10.34) ^b	100.00 (18.43) ^a
10000	26.67 (9.27) ^b	63.33 (10.96) ^b	100.00 (18.43) ^a
AgNO₃ alone	0.00 (0.00) ^h	0.00 (0.00) ^g	0.00 (0.00) ^f
Soya bean seed extract	0.00 (0.00) ^h	0.00 (0.00) ^g	0.00 (0.00) ^f
Untreated control	0.00 (0.00) ^h	0.00 (0.00) ^g	0.00 (0.00) ^f
Emmamectin benzoate @ 0.2g/l	80.00 (16.43) ^a	100.00 (18.43) ^a	100.00 (18.43) ^a
S.Em±	0.89	0.81	0.58
CD @ 1%	1.20	1.10	1.04

Figures in the parentheses are angular transformed values.
In vertical columns, means followed by same letter do not differ significantly by DMRT (P=0.05)

Table.2 Effect of Soybean based seed Green silver nanoparticles on percent larval mortality of *S. litura* (third instar)

Treatments / Concentrations in ppm	Hours After Treatment (HAT)			
	24 hours	48 hours	72 hours	96 hours
250	0.00 (0.00) ^e	10.00 (4.74) ^e	23.33 (5.35) ^h	56.67 (5.95) ^g
500	0.00 (0.00) ^e	23.33 (6.15) ^d	40.00 (6.35) ^f	66.80 (6.05) ^g
750	10.00 (4.62) ^d	26.67 (6.74) ^d	50.00 (6.50) ^f	75.45 (7.15) ^f
1000	13.33 (6.54) ^c	30.00 (7.15) ^d	53.33 (7.45) ^f	82.50 (8.35) ^e
2000	13.33 (6.54) ^c	33.33 (7.95) ^c	53.33 (7.45) ^f	82.50 (8.35) ^e
4000	16.67 (7.33) ^b	36.67 (8.74) ^c	56.67 (8.60) ^e	85.00 (9.45) ^d
6000	16.67 (7.33) ^b	36.67 (8.74) ^c	60.00 (9.30) ^d	87.35 (10.50) ^c
8000	20.00 (8.13) ^b	43.33 (9.36) ^b	70.00 (10.00) ^c	90.00 (14.75) ^b
10000	20.00 (8.13) ^b	46.67 (9.88) ^b	76.67 (11.30) ^b	100 (18.43) ^a
AgNO₃ alone	0.00 (0.00) ^e	0.00 (0.00) ^f	0.00 (0.00) ⁱ	0.00 (0.00) ^h
Soya bean seed extract	0.00 (0.00) ^e	0.00 (0.00) ^f	0.00 (0.00) ⁱ	0.00 (0.00) ^h
Untreated control	0.00 (0.00) ^e	0.00 (0.00) ^f	0.00 (0.00) ⁱ	0.00 (0.00) ^h
Emmamectin benzoate @ 0.2g/l	70.00 (15.32) ^a	100 (18.43) ^a	100 (18.43) ^a	100 (18.43) ^a
S. Em ±	0.32	0.25	0.60	0.21
CD @ 1%	1.29	1.02	1.20	0.95

Figures in the parentheses are angular transformed values.

In vertical columns, means followed by same letter do not differ significantly by DMRT (P=0.05)

Indra kumar (2016) for the first time revealed the larvicidal activity of green nanoparticles synthesized from *Carrissa carandas* AgNPs, *A. indica* AgNPs, *L. acidissima* CuNps, *T. peruviana* AgNPs, K.K AgNPs and *G. max* AgNPs extracts on 5th and 6th instar *S. litura* larva. *G. max* AgNPs recorded 73.24 per cent mortality and was significantly superior to all other treatments including precursors and the plant extracts except insecticidal control. According to the Giovanni Benelli (2018) silver nanoparticles have a significant impact on insect antioxidant and detoxifying enzymes, leading to oxidative stress and cell death. Ag nanoparticles also reduced acetylcholine esterase activity, while polystyrene nanoparticles inhibited CYP450 isoenzymes resulting in larval death.

The present study is also in line with Jyothsna and Usha Rani (2015) who observed that Silver nanoparticle treatments decreased body weights of *S.litura* third instar larvae. Treatments affected the activity of detoxifying enzymes. Oxidative stress induced in larval guts was countered by enhanced antioxidant enzyme which leads to prolonged development period of instars as being observed in the present study.

The present findings are in agreement with Chakravarthy *et al.*, (2012a) who used synthetic nanoparticles of CdS, Nano-Ag and Nano-TiO₂ against *S. litura*. Nano Ag caused maximum (56.89 %) mortality at 2400 ppm followed by 46.89 and 33.44 per cent mortality at 1200 and 600 ppm respectively. This more or less confirms the present finding in which 8000 ppm recorded cent percent mortality in 2nd instar larva at 72 HAT and 10000 ppm recorded cent per cent mortality in 3rd instar larva at 96 HAT. Further, the present study is also comparable with Chakravarthy *et al.*, (2012b) who tested the DNA tagged gold nanoparticles against *S.litura* at 200, 300, 400 and 500 ppm against

second instar *S. litura* larvae exposed to each concentration of DNA tagged gold nanoparticles for 30 seconds causing 50 per cent larval mortality above 500 ppm. As the particle concentration and days after treatment increased, larval mortality also increased and is in full confirmation with the present finding though the nanoparticles used is Silver.

It is concluded in the present study soya seed based AgNp were synthesized through sun light exposure method. soyabean seed extract was acts as both reducing and stabilizing agent for AgNp. The per cent mortality was highest (100 %) at 72 hours and 96 hours after treatment for 2nd and 3rd insta respectively. Increased concentration and exposure hours increased mortality. The soya seed based green Nano AgNps provides a promising novel approach for the large-scale industrial production of nanonmaterial's for pest management against 2nd and 3rd instars of *S.litura*.

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