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Pesticidal activity of green synthesized silver nanoparticles using *Nerium olender* (L.) leaves extract against *Leucinodes orbonalis* (G.)

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Abstract
Eggplant fruit and shoot borer, *Leucinodes orbonalis* Guenee (Lepidoptera: Pyralidae) is a key insect pest of eggplant in all eggplant growing areas of India and Southeast Asia. Green synthesis of pesticides from plant extract is pioneer approach for pest control. The present study was carried out for two years 2015-16 and 2016-17 to establish the larvicidal activity of synthesized silver nanoparticles (AgNP's) using plant leaf extracts from *Nerium olender* (L.). Synthesis was confirmed through UV-Vis spectrophotometer in wavelength range of 200-700 nm. The peak was recorded at 410 nm which confirmed the formation of silver nanoparticles, zeta potential values were found to be -13.7 mV, FT-IR analysis showed strong peaks at ranges of 800-4000cm^{-1} which exhibited presence of different types of functional groups *viz.,* (O-H, H-H, C-H, C-C and N-H). DLS analysis showed that the biosynthesized nanoparticles have average particle size of 27.64 nm. Efficacy of green synthesized silver nanoparticles (AgNP's) from *Nerium olender* (L.) against *Leucinodes orbonalis* (G.) was evaluated in dilutions range 200, 300, 400 and 500 ppm. Larval mortality of *L.orbonalis* was found to be 76.66% and 80 % in both the years 2015-16 and 2016-17. The LC_{50} was also calculated both in 2015-16 and 2016-17; the lowest LC_{50} value was recorded to be 289.17 ppm after fifth day of the treatment. As the nanoparticles concentration and days after treatment increased, larval mortality also increased. There were statistically significant differences in larval mortality between concentrations. The result suggested that the use of plants for synthesis of silver nanoparticles may be considered as environmentally safer and greener approach for pest control.

Keywords: Nanotechnology, *Leucinodes orbonalis*, *Nerium olender*, Silver nanoparticles, Green synthesis

1. Introduction

Brinjal fruit and shoot borer, *Leucinodes orbonalis* Guenee (Lepidoptera: Pyralidae) is a destructive and first ranked biotic constraint of eggplant in almost all the eggplant growing areas of the world including Nepal [1]. This insect has gained the potential status of pest due to larva's unique nature of feeding on monophagous diet aided by homing and tunnelling behaviour ultimately enables insects to face the challenges of chemical pesticides [2]. Farmers rely exclusively on use of synthetic insecticides in order to combat the pest. Farmers apply insecticides 10-12 times in winter and 25 to 30 times or even more in summer and rainy season crop[3]. The dose of insecticide was used much higher than recommended level during fruiting and harvesting time. Ultimately it results into development of pest resistance and environmental contamination [4]. The environmental hazards posed by synthetic pesticides provide an impetus for investigations into some eco-friendly and bio rational alternatives [5]. Nanotechnology has become one of the most promising new approaches for pest control in the recent years [6]. Nanoparticles represent a new generation of environmental remediation technologies that could provide cost-effective solution to some of the most challenging environmental cleanup problems. Silver has been used in many applications in pure free metal or in compound form because it possesses antimicrobial activity against pathogens, yet it is nontoxic to humans [7].

Biosynthesis of insecticides from plant extracts is currently under exploitation. Plant extracts are very cost effective and eco-friendly and thus can be an economic and efficient alternative for the large-scale synthesis of synthetic and other chemical insecticides. In this study *Nerium olender* (L.) has been used on the basis of its larvicidal properties. *Nerium oleander* (L.) is a member of family Apocynaceae and is native to the Mediterranean regions of Europe and

Asia. Oleander is an extremely poisonous plant and contains numerous toxic compounds, such as oleandrin and neriine, classified as cardiac glycosides [8]. The cardiac glycosides peruvoside from yellow oleander is used medicinally for the treatment of cardiac insufficiency [9]. The present study was designed with a novel, rapid and cost effective route of biosynthesis of AgNP's using *Nerium oleander* (L.) leaf extract. Hence, the synthesis of AgNPs, reducing the silver ions present in the solution of silver nitrate by the cell-free aqueous leaf extract of *Nerium oleander* (L.) and tested against *Leucinodes orbonalis*(G.).

2. Materials and Methods

2.1 Rearing of *Leucinodes orbonalis* (G.*).*

To get sufficient amount of test larvae of *Leucinodes orbonalis* (G.), the insect was reared naturally on standing crops with net caging on brinjal crop in Assam Agricultural University, Jorhat during 2015-2016 and 2016-2017. In the month of November to last week of March insect pests attack were observed. Net caging of heavily infested plots was done to facilitate un-interrupted supply of sufficient stock of larvae.

2.2 Collection and preparation of plant leaves extract

Fresh plant leaves of *Nerium oleander* (L.) was collected and washed several times with distilled water to remove the dust particles and then sun dried to remove the residual moisture. The leaf extracts used for synthesis of nanoparticles were prepared by placing 10 gram of the washed, dried and fine cut leaves in 200 ml glass beaker along with 100 ml of sterile distilled water. The aqueous mixture was then boiled for 5–10 minutes until the colour of aqueous solution changes to light yellow or brown. Then the aqueous extracts were cooled to room temperature and filtered with Whatman filter paper No. 1 before centrifugation at 1200 rpm for 5 minutes to remove the heavy biomaterials. The leaf extracts were stored at room temperature in order to be used for further studies as per the method [10].

2.3 Synthesis of silver nanoparticles

Silver nanoparticles were synthesized by modified method [10]. For synthesis of silver nanoparticles 5-10 ml of plant leaf extract *Nerium oleander* (L.) was added separately into 100 ml of 1 mM aqueous solution of silver nitrate ($AgNO_3$) (Sigma Aldrich) with stirring magnetically at room temperature. After adding 0.0017gm/ml of silver nitrate solution in 100 ml of sterile distilled water which contained 5-10gm of plant extract the flasks were put into the dark condition for 1-2 days. After 1-2 days the resulting light solution changed to dark brown in colour, indicating the formation of silver nanoparticles (AgNPs).

2.4 Characterization of green synthesized silver nanoparticles (AgNPs) of *Nerium oleander* (L.)

Characterization of green synthesized AgNPs of *Nerium oleander* (L.) were done by the following equipments *viz.*, UV-Vis spectrophotometer, Zeta potentiometer and Fourier transform infrared spectroscopy. The silver nanoparticles formation were confirmed by measuring the wave length of reaction mixture in the UV-Vis spectrum of the Shimadzu UV 1601 spectrophotometer at a resolution of 1 nm (from 300 to 700 nm) in 2 ml quartz cuvette with 1 cm path length. Zeta potential was used for determined the surface charge of nanoparticles in order to study their coagulation properties of green synthesized nanoparticles. Zeta potential of the silver nanoparticles was determined by Malvern Zetasizer ZEN 3600. Fourier transform infrared spectroscopy (FT-IR) was used for the characterization of functional groups on the surface of AgNPs by plant extracts. The spectra were scanned in the range of 4000–400 cm^{-1} range at a resolution of 4 cm^{-1}. The samples were prepared by dispersing the AgNPs uniformly in a matrix of dry KBr, compressed to form an almost transparent disc.

2.5 Efficacy of green synthesized silver nanoparticles against *Leucinodes orbonalis* (G.)

The experiment was conducted with 5 treatments and 5 replications for synthesized AgNPs from *Nerium oleander* (L.). To determine lethal concentration of green synthesized nanoparticles solution, serial dilution ranging from 200, 300, 400 and 500 ppm were prepared. The required amount of larvae were directly collected from field and put into the clean, air dried petriplates. The 3 hrs starved different larvae were fed with different concentrations of silver nanoparticles treated and untreated plant parts [11]. After every 24 hrs, the uneaten plant parts were removed and placed with fresh treated and untreated ones. Then the percent larval mortality data were subjected to ANOVA analysis.

2.6 Observations

Corrected Larval Mortality (%)

Mortality rates (MR) were measured [12] for larvae of *Leucinodes orbonalis* (G.). Series of control *Leucinodes orbonalis* (G.) larvae were allowed to complete their development under the room conditions of light and temperature. Then the MR were estimated as,

$$\text{Larval mortality (\%)} = \frac{\text{Number of dead larvae}}{\text{Initial number of larvae}} \times 100$$

2.7 Statistical analysis

Data were analyzed by Completely Randomized Design (CRD). Result with $P > 0.05$ was considered to be statistically significant. The data sets were subjected to Analysis of Variance (ANOVA) and the data sets were also used for Probit Analysis[13] to determine the LC_{50} of the above mentioned synthesized nanoparticles using, (SPSS, Inv., version 20.00).

3 Results and Discussion

3.1Characterization of green synthesized silver nanoparticles (AgNPs) of *Nerium oleander* (L.)

For synthesis of silver nanoparticles*, Nerium olender* (L.) leaves extract were exposed to 1 mM aqueous solution of silver nitrate and observed that colour of plant extracts changes from light colour to dark brown colour at 72-96 hrs of reaction. This colour changed from light to dark which confirmed the synthesis of silver nanoparticles. Gradual increase in colour development from light to dark brown was observed in synthesized materials, which indicated the formation of silver nanoparticles [14]. In the present study characteristics SPR absorption peak (band) was observed in plant extract of *Nerium olender* (L.) treated with 1 mM $AgNO_3$ at 410 nm. Green synthesis of silver nanoparticles using *Nerium olender* (L.) and found the absorbance peak at 410 nm, silver nanoparticles exhibits dark yellowish brown colour in the aqueous solution due to the SPR phenomenon [15]. Zeta potential was determined and recorded the charge of green synthesized silver nanoparticles of *Nerium olender* (L.)

was -13.7mV. It indicated that the silver nanoparticles were stable and did not have ability to agglomerate.

FT-IR analysis of silver nanoparticles synthesized using *Nerium olender* (L.) extract displayed strong absorption peaks at 3480 cm^{-1} - 3300 cm^{-1} result from stretching of N-H band of amino groups or inductive bond of O-H hydroxyl group. The absorption peaks at 1560cm^{-1} - 1100cm^{-1} indicates fingerprinting region of O-H group and C-O stretching vibration of alcohol. The FT-IR spectra of aqueous silver nanoparticles prepared from the *N. oleander* leaf extract showed transmittance peak at 509.12 cm^{-1} (C–H band alkenes), 1,077.05 cm^{-1} (C–O stretch alcohols). 1,600.63 cm^{-1} (N– H bend amines), 2,736.49^{-1} and 2,479.04 cm^{-1} (O–H stretch carboxylic acids), and 3,415.31 cm^{-1} (N–H stretching due to amines group). These peaks indicated that the carbonyl group formed amino acid residues and that these residues capped the silver nanoparticles to prevent agglomeration, thereby stabilizing the medium. *Nerium olender* nanoparticles and found strong bands at 3698.28cm^{-1} had an O-H functional group and the type of vibration is Stretch and free, 3403.05cm^{-1} has O-H functional group, vibration was stretched [15]. Bands at 1500.39cm^{-1} has a C=C functional group and the type of vibration was stretched 1529.52cm^{-1} had N-O functional group and it was also stretchable.

Plate 1: Plant extracts, (A) before addition of $AgNO_3$ (B): after formation of silver nanoparticles

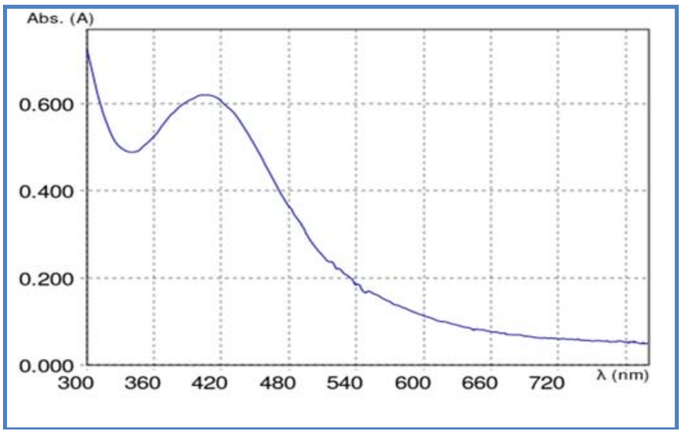

Plate 2: UV –Vis absorption spectra obtained from synthesized silver nanoparticles using *Nerium olender (L)*

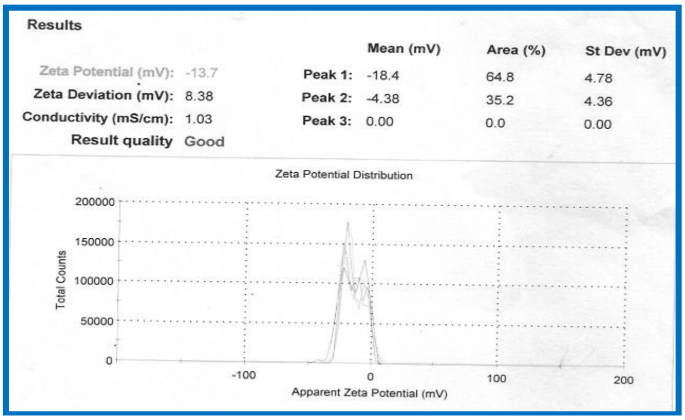

Plate 3: Zeta potential analysis of green synthesized silver nanoparticles from *Nerium olender* (L.)

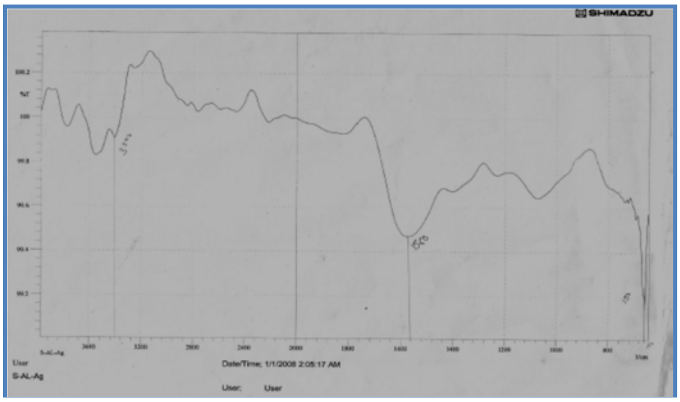

Plate 4: FT-IR analysis of silver nanoparticles extracted from *Nerium olender* showing different absorption peaks

Efficacy of green synthesized silver nanoparticles from *Nerium olender* (L.) against *Leucinodes orbonalis* (G.)

Efficacy of green synthesized AgNPs from *Nerium olender* (L.) at different concentration (200, 300, 400, 500 ppm and control) was tested against *Leucinodes orbonalis* (G.) for both the year 2015-16 and 2016-17.*Nerium olender* (L.) AgNPs gave mortality of 50%, 60% and 76.66% respectively after first, third and fifth day of spraying at 500 ppm concentrations during 2015- 16. In 2016-17, *Nerium olender* AgNPs gave mortality of 50%, 63.33% and 80% after fifth day of the treatment, respectively at 500 ppm concentration. Larval mortality increased as the concentration and days after treatment increased, after first day of spraying at 200 ppm concentration mortality was 33.33% whereas at fifth day after spraying mortality was increased to 63.33%. The results of larvicidal activity clearly indicated that the percentage of mortality being directly proportional to the concentration of the extract. Smaller size of the nanoparticles gave higher efficacy. There were highly significant differences between treated and control larvae. *Nerium olender* against *Anopheles stephensi* showed result viz., at 100-ppm concentration, the larval mortality was 27.6 % whereas at 500-ppm concentration, it was increased to 99.2 %, as concentration increased then mortality also get increased [16]. It has been suggested that nano meter-sized Ag particles possess different physical and chemical properties from their macro scale counterparts that affect their interaction with biological structures and physiological processes [17].

Nanoparticles extracted from *Nerium olender* (L.) gave the lowest LC_{50} value of 289.12 ppm against fourth instar larvae of *Leucinodes orbonalis* (G.) after fifth day of the treatment during 2016-17, while in 2015-16 LC_{50} was 302.12 ppm after fifth day of the treatment. Maximum LC_{50} 468.30 ppm after first day of the treatment during 2015-16,while in 2016-17 probit value 464.35 ppm respectively, after first day of the treatment. Probit analysis showed variations in virulence. These LC_{50} values had minimum fiducial limits. Chi-square test revealed statistically non-significant differences. This indicates that the probit analysis supplemented the ANOVA result. Concentration plays important role in larvicidal activity. Present findings are in agreement with earlier reports, bio efficacy of inorganic nanoparticles CdS, Nano-Ag and Nano-TiO_2 against *Spodoptera litura* [18]. The LC_{50} (216.91 to 938.95) was increased as the larval age increased. Probit analysis showed variations in virulence. DNA tagged gold nano particles gave the lowest LC_{50} of 256.31 ppm against 2nd instar *S. litura* larvae on the 5th day and the maximum LC_{50} of 645.75 ppm on the 3rd day after treatment. However, on the 4th day, the LC_{50} of 377.21 ppm was realized.

Table 1: Effect of green synthesized silver nanoparticles from *Nerium olender* (L.) on per cent mortality of *Leucinodes orbonalis* (G.) under laboratory conditions during 2015-16 and 2016-2017

Treatment (AgNP's)	Dose (PPM)	Per cent larval mortality at different days after treatment		
		1 DAT	3 DAT	5 DAT
Nerium olender $AgNO_3$ (2015-16)	200	33.33 (35.24)	40 (39.23)	63.33 (52.71)
	300	36.66 (37.23)	50 (45)	66.66 (54.70)
	400	40 (39.23)	53.33 (46.89)	70 (56.79)
	500	50 (45)	60 (50.77)	76.66 (61.07)
S.Ed ±		2.39	2.74	2.96
CD (5%)		5.01	5.77	6.08
Nerium olender $AgNO_3$ (2016-17)	200	33.33 (35.24)	36.66 (37.23)	63.33 (52.71)
	300	40 (39.23)	46.66 (43.05)	66.66 (54.70)
	400	46.66 (43.05)	53.33 (46.89)	73.33 (58.89)
	500	50 (45)	63.33 (52.71)	80 (63.44)
Control (Water spary)	-	0.0	0.0	0.0
S.Ed ±		2.68	2.92	3.12
CD (5%)		5.64	5.90	6.20

Mean of 10 larvae/replication/treatment; figures in the parentheses are angular transformed values, DAT= Days after treatment

Table 2: LC_{50}value on per cent mortality of *Leucinodes orbonalis* (G.) on exposure to different concentrations of green synthesized AgNPs, during 2015-16 and 2016-17

AgNP's from Plants	DAT	X^2(n-1)	Regression equation	LC_{50} (PPM)	Fiducial Limits (con. at 95% CL)
Nerium olender $AgNO_3$ (2015-16)	1	0.51	Y=-2.93+1.09x	468.30	379.25 to 979.37
	3	0.78	Y=-3.49+1.34x	388.96	331.49 to 511.22
	5	1.10	Y=-2.67+1.07x	**302.26**	223.66 to 391.26
Nerium olender $AgNO_3$ (2016-17)	1	0.68	Y=-3.50+1.31x	464.35	387.48 to 730.26
	3	0.85	Y=3.67+1.42x	381.52	357.69 to 483.81
	5	1.32	Y=-3.71+1.43x	289.17	226.01 to 336.29

LC_{50}= Lethal concentration that kills 50% of the exposed larvae, UCL = Upper confidence limit, LCL= Lower confidence limit, x^2 = Chi-square values, significant at P<0.05 level, DAT= Days after treatment.

3.3 Behavioural changes in *Leucinodes orbonalis* (G.) larvae after treatment with green synthesized silver nanoparticles synthesized from *Nerium olender* (L.)

After exposure of larvae to the test concentrations dilutions of 200, 300, 400 and 500 ppm of green synthesized AgNPs from *Nerium olender* (L.) several changes have been noticed. At 200-300 ppm concentration, larvae started to become colourless. Larvae went into pupal condition within three to four days of the treatment at 400-500 ppm concentration. At fifth day after treatment pupa turned into black colour and died inside in the pupal case. DNA tagged gold nanoparticles against *S. litura* [19] which showed cessations of active movement in larvae, larval skin and entire body became stiff, hard and oozing of the body content (fleshy white), lysis after third day of the treatment. Four days after treatment, the body became swollen, pulpy and fragile. It attained almost a 'C'-shaped and body turned into dark brown. Five days after treatment, the larvae showed premature moulting and the body became discoloured and turned brown. Leaf aqueous extract and synthesized silver nanoparticles of *Nerium oleander* against *Anopheles stephensi* became restlessness, sluggishness, tremors, and convulsions followed by paralysis after exposure to different test concentrations [16].

Plate 5: Behavioural changes in *Leucinodes orbonalis* larvae 3DAT with different concentrations of *Nerium olender* (l.) AgNPs

Plate 6: Behavioural changes in *Leucinodes orbonalis* larvae at 500 ppm concentration *Nerium olender* (L.)AgNPs 5 DAT

Plate 7: Untreated *Leucinodes orbonalis* larvae 5 DAT

4. Conclusions

Inspired by the thought that little is known regarding the effects of AgNPs on *Leucinodes orbonalis* (G.) pests, the result suggested that, the use of *N. olender* AgNPs found to be very effective. Biosynthesis of silver nanoparticles may be considered as environmentally safer and greener approach for pest control. Green synthesis of silver nanoparticles can be used as a valuable tool in pest management strategies against *Leucinodes orbonalis* (G.). However the environmental tracking of silver nanoparticles needs to be assessed. More detailed study require to understand the actual mechanism of effect of silver nanoparticles inside the insect's body.

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