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Biosynthesis, Characterization and Evaluation of Green Silver Nanoparticles against *Amrasca devastans* (Dist.) (Cicadellidae: Homoptera)

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A B S T R A C T

Biosynthesis of nanoparticles from plant-based sources has been proven to be cost efficient and eco-friendly. Due to its miniature size, nanoparticles have a higher surface area to volume proportion, which is very important for its insecticidal potentiality. Hence nanoparticles could be used as an important tool for pest management. The main objective of this study was to provide a better management strategy through analysing, evaluating and proving the bioefficacy of *Azadirachta indica* and *Pongamia pinnata* silver nanoparticles against 1st nymphal instars of *A. devastans* under laboratory conditions. Silver green nanoparticles were synthesized biologically from leaf extracts of *A. indica* and *P. pinnata* by Sunlight mediated exposure method and studied its bio efficacy on *A. devastans*. Characterization of the nanoparticles were done by UV-Visible spectroscopy for confirming the formation of nanoparticles and Particle Size Analyser (PSA) for determining size and distribution of particles. Surface Electron Microscope (SEM) was used for determining the surface topography of nanoparticles. The results obtained from Particle Size Analyzer (PSA) showed that *A. indica* and *P. pinnata* based $AgNo_3$ nanoparticles had an average diameter of 61.70 nm and 68.80 respectively. Even though both *A. indica* and *P. pinnata* based silver nanoparticles caused cent per cent nymphal mortality at 2000 ppm, *A. indica* based nanoparticles were found to be comparatively more efficient and having higher insecticidal activity against *A. devastans* at lower concentrations. Nymphal mortality was found to be positively correlated with increase in concentration of nanoparticles. Symptoms caused by silver nanoparticles were dehydration, inactiveness, brittleness etc.

Introduction

India is an agriculture-based country with predominant population dependent on agriculture. Among the crops cultivated through the country, cotton is one of the most important staple crops that is being domesticated for its fiber content. Pest attack is one of the major factors that results in productivity loss in major cultivated states. Up until few years before, chemical resort was the most available and widely implicated tool against insect pest management. Luxurious and injudicious application of chemicals resulted in pesticidal residues, destruction of soil structure, toxicity to non-targets, resistance development (Ahmad *et al.*, 1999), pest resurgence etc. which ultimately results in environmental and public harm (Subramanyam and Hagstrum, 1995; Okonkwo and Okoye, 1996).

This gap between pest management and availability of better alternative to chemical pest management is filled due to introduction and implementation of biorational tools in agriculture (Subashini *et al.,* 2004).

Nanotechnology has successfully proven to be an important biorational tool in the fields of agriculture, pharmacy, biotechnology etc. It has also offered modern and simple protocols to construct and design novel nanoparticles (1–100 nm) from different cheap sources of nanomaterials (Silver, Iron. Copper etc.) (Benelli and Lukehart, 2017; Ortigosa *et al.,* 2012). Nanoparticles are usually synthesized from microbes, fungi, plants etc.via low cost, single step and eco-friendly biological process (Mohanpuria *et al.,* 2008; Huang *et al.,* 2007).

Plant extracts are widely used for converting the metal oxides present in the nanoparticles to its ionic form. Green synthesis of nanoparticles from plant source can be beneficial over other numerous biological processes because it excludes the necessity of maintaining cell cultures. Further, nanoparticles could also be synthesized from plant sources at a large scale (Shankar *et al.,* 2004). Silver nanoparticles have been green synthesized using different plant part extracts like leaves, stem, seeds etc. from plants like Neem (*Azadirachta indica*) (Tripathi *et al.,* 2009), Soybean *(Glycine max*) (Vivekanandhan *et al.,* 2009), Tea (*Camellia sinensis*) (Begum *et al.,* 2009), *Euphorbia hirta* (Durga *et al.,* 2014) etc.

Among the important nanoparticles, silver nanoparticles were found to be having insecticidal, bactericidal, viral and fungicidal activities (Saxena *et al.,* 2010; Elumalai *et al.,* 2010; Fayaz *et al.,* 2010; Wen-Ru *et al.,* 2010; Ales Pana *et al.,* 2009). Insecticidal activity of nanoparticles was because of its small size and small surface area to volume ratio (Lu, 2011). Present study is carried out using silver nanoparticles synthesized from leaf extracts of *A. indica* and *Pongamia pinnata*. Even though synthesis of nanoparticles from plant extracts has been widely done and reported, investigation on its insecticidal activity against sucking pests in cotton is scanty. Hence, the present study was aimed to evaluate the insecticidal activity of green nanoparticles against cotton leaf hopper, *A. devastans* (Instar 1).

Cotton leafhopper/green jassid, *Amrasca devastans* is a homopterous polyphagous that usually attacks plants by sucking the sap from underside of the leaves of 25 species such as potatoes, beans, sorghum, maize, cotton etc. If left uncontrolled this pest will lead to curling and crinkling of leaves finally results in complete hopper burning of the leaves. This polyphagous pest has the potential to cause large scale loss of 100-120 kg/ha fibre (Ahmad *et al.,* 1999).

Materials and Methods

For the synthesis, silver nitrate powder was procured without further purification from Nanotech laboratory, UAS Dharwad, Karnataka. Other components such as distilled water was used for synthesis process and reduction of nanoparticle precursor.

Experimental procedure

Insect rearing

The nymphs of *A. devastans* for the biological assay were collected from the DCH 32 cotton field. Simultaneously, a low proportion of mass rearing was also done in the Research laboratory, Agricultural Research Station, Department of Agricultural Entomology, Sankeshwar, Karnataka, India, using 60 days old potted plants (covered with nets) at room temperature (28^{o}C) and relative humidity (75.00 %). Cotton plants required for culturing leafhopper population was raised in earthen pots. As the germination proceeds, the seedlings were covered with netlon of size 3 meters diameter and 4 meters height for preventing the entry of natural enemies and other. The potted cotton seedlings of60days old were inoculated with the leaf hopper adults and early nymphal instars

collected from fields of cotton and confined in the netted pots and the leafhopper culture was maintained continuously for 5-6 generations.

Preparation of plant extracts

Fresh leaves of both *A. indica* and *P. pinnata* were procured from trees found in the UAS Dharwad campus. Leaves were washed thoroughly with running tap water followed by distilled water to remove all the impurities. Five grams of the weighed leaves were crushed into paste form using mortar and pestle. To the crushed, 100 ml of distilled water was added to the crushed leaves and filtered with the help of filter paper (Whatman no. 1). This was then boiled at 80°C for 60 minutes. This extract was used for synthesizing silver nanoparticles and stored under refrigerated conditions (4 °C) for future use.

Green synthesis of silver nanoparticles from plant extracts

For synthesis of green nanoparticles, protocol proposed by Indrakumar (2016) using sunlight exposure method was used. Different concentrations of (1-10 ml) of plant extract were added to 90 ml aqueous solution of silver nitrate (AgNO3-1 mM) and kept at room temperature for further processes.

The colour change was observed from pale yellow to brown colour when exposed to direct sunlight for 3 hours (12.00 to 3.00 PM), which indicated that the green silver nanoparticles were synthesized due to the interaction between plant extract and silver metal ions. A test control was maintained without adding silver nitrate, which didn't show any colour changes. It indicated that the synthesis of green silver nanoparticles in the solution of reaction mixture where the colour variation took place.

Characterization of silver nanoparticles

Green nanoparticles were characterized using UV-Visible Spectrophotometer at wavelength ranging from 200 to 700 nm. The average size and distribution pattern of the green nanoparticles were measured using PSA (Particle Size Analyzer). SEM (Scanning Electron Microscopy) was also used to evaluate the surface topology of silver nanoparticles.

Bioassay of silver nanoparticles

First nymphal instars of *A. devastans* was tested for their susceptibility to different dosages of silver nanoparticles. At least four concentrations of each nanoparticle ranging from 500 to 2000 ppm was treated to obtain mortality that ranged from 10.00 to 100.00 per cent. Serial dilutions with distilled water were made on the day of experiment.

Excised leaf discs cotton with average diameter of 60 mm was dipped for about 30 seconds in different concentrations of each experiment. These leaf discs were then dried under shade for 60 minutes and placed on the Petri dishes (above cotton tissue papers). Preliminary dosages were then determined before commencing of tests for the specified treatments in order to establish mortality rates ranging from 10.00 to 100.00 per cent. Distilled water-treated leaf discs were served as controls for each treatment. Flonicamide 50WG was used as insecticidal check at 0.25 g/l. 10 nymphs of *A. devastans* were provided in each replication of every treatment. A minimum of three replications of all the concentration was included for each treatment.

Experimental treatments were further evaluated by observing the *A. devastans* mortality rate at 24, 48, 72 and 96 hours after treatment intervals. Any nymph that could not move when disturbed was considered dead. Percent reduction of *A. devastans* population over test control was done using formula

$$\text{Per cent nymphal mortality} = \frac{\text{Number of dead insects}}{\text{Total number of insects}} \times 100$$

$$\text{Corrected per cent mortality} = \frac{T - C}{100 - C} \times 100 \text{ (Abbott's formulae, 1925)}$$

T- Per cent mortality in nanoparticle treatment

C- Per cent mortality in test control

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

Synthesis and characterization of silver nanoparticles from *A. indica* and *P. pinnata*

The conversion of silver into silver ions in the green synthesized nanoparticle mixture after the incubating *A. indica* and *P. pinnata* leaves extract with silver nitrate solution was positively confirmed by the colour change occurred in the nanoparticle solution. Fresh suspension of silver nitrate and plant extract was pale greenish/yellow in colour. It turned dark brownish/black after completion of the green synthesis process through continuous exposure to direct sunlight (Fig. 1A and 1B)

Formation of silver nanoparticles using *A. indica* and *P. pinnata* was confirmed with UV Spectrophotometer which showed an absorption peak at 410 nm (Fig. 2A) and 425 nm (Fig. 2 B) respectively. After confirming the formation of silver in the nanoparticle solution using UV Spectrophotometer, size of the nanoparticles was determined using Particle Size Analyzer (Fig. 3 A and 3 B).

Mean diameter of *A. indica* based AgNPs was found to be 61.80 nm and that of *P. pinnata* based AgNPs was 68.80 nm. Further characterization of the nanoparticles was done using Surface Electron microscope (Fig. 4 A and 4 B) revealed that most of the nanoparticles were mostly in shape.

Bio-efficacy of nanoparticles against *A. devastans*

Biological efficacy of *A. indica* and *P. pinnata* encapsulated silver were evaluated against 1st nymphal instars of *A. devastans* (Table 1 and 2).

Effect of nanoparticles against nymphal mortality of *A. devastans*

The use of green based AgNPs against early instars inactivated the nymphs causing sluggishness, dehydration and brittleness. Mortality of nymphs was confirmed by noticing the inability to move when disturbed on its abdomen (Fig 4 A and 4 B).

The chemical which was used as an insecticidal control, Flonicamide 50 WG@ 0.25 gm/l was found to be significantly superior to all concentrations of nanoparticle treatments and showed highest nymphal mortality (100.00 %) of at 72 hours after treatment. Even though nanoparticle precursor ($AgNO_3$) was not found to be causing any nymphal mortality, both *A. indica* and *P. pinnata* extracts recorded 30.00 and 23.33 per cent nymphal mortality, respectively.

The bio efficacy *A. indica* and *P. pinnata* based AgNPs against 1st nymphal instars of *A. devastans* is presented in tables 1 and 2. After 24 hours of treatment, 2000 ppm of both *A. indica* and *P. pinnata* AgNPs documented 40.00 and 36.67 per cent nymphal mortality, respectively.

This was found to be significantly superior to all other concentrations. Next best results were recorded by 1500 ppm and 1000 ppm of *A. indica* based AgNPs with 30.00 per cent nymphal mortality, and was found to be on par with 1500 ppm of *P. pinnata* based AgNPs., followed by 500 ppm of *A. indica* based AgNPs and 1000 ppm of *P. pinnata* based AgNPs, by recording 20.00 per cent nymphal mortality. Lowest nymphal mortality of 13.33 per cent was recorded at 500 ppm of *P. pinnata* based AgNPs.

Table.1 Effect of green AgNPs synthesized from leaves of *A. indica* on nymphal mortality of *A. devastans*

Sl. No.	Concentrations	Per cent nymphal mortality at			
		24 HAT	48 HAT	72 HAT	96 HAT
1	AgNPs 500 ppm	20.00 (26.56)^{c}	30.00 (33.21)^{e}	60.00 (50.76)^{d}	73.33 (58.90)^{d}
2	AgNPs 1000 ppm	30.00 (33.21)^{b}	40.00 (39.23)^{d}	70.00 (56.78)^{c}	90.00 (71.56)^{c}
3	AgNPs 1500 ppm	30.00 (33.21)^{b}	50.00 (45.00)^{c}	73.33 (58.89)^{b}	93.33 (75.03)^{b}
4	AgNPs 2000 ppm	40.00 (39.23)^{a}	80.00 (63.43)^{b}	100.00 (90.00)^{a}	100.00 (90.00)^{a}
5	AgNO3 1mM	0.00 (0.25)^{f}	0.00 (0.25)^{g}	0.00 (0.25)^{f}	0.00 (0.25)^{f}
6	*A.Indica* extract-5.00 %	10.00 (18.43)^{e}	20.00 (26.56)^{f}	20.00 (26.56)^{e}	30.00 (33.21)^{e}
7	Flonicamide50 WG @ 0.30 g/l	30.00 (33.21)^{c}	83.33 (65.90)^{a}	100.00 (90.00)^{a}	100.00 (90.00)^{a}
8	Untreated control	0.00 (0.25)^{f}	0.00 (0.25)^{c}	0.00 (0.25)^{f}	0.00 (0.25)^{f}
	S.Em.±	0.71	0.96	0.78	2.30
	CV	5.39	4.89	2.90	7.55
	CD @ 1%	2.93	4.00	3.23	9.50

Note: Figures in the parenthesis are arcsine transformed values.
Incolumns, mean followed by same letter do not differ significantly by DMRT (p=0.01)
*HAT-Hours After Treatment
*AgNPs – Silver Nanoparticles

Fig.1A Colour change of *A. indica*-silver nitrate solution due to silver nanoparticle formation

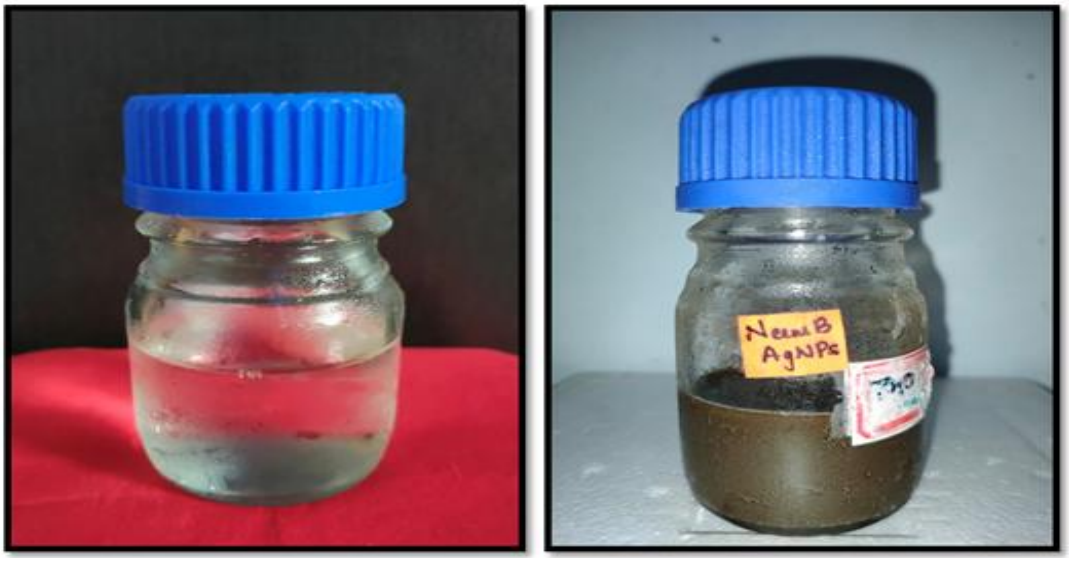


Table.2 Effect of green AgNPs synthesized from leaves of *P. pinnata* on nymphal mortality of *A. devastans*

Sl. No	Concentrations	Per cent nymphal mortality at			
		24 HAT	48 HAT	72 HAT	96 HAT
1	AgNPs 500 ppm	13.33 (21.41)^{d}	30.00 (33.21)^{d}	50.00 (45.00)^{d}	70.00 (56.78)^{d}
2	AgNPs 1000 ppm	20.00 (26.56)^{c}	30.00 (33.21)^{d}	56.67 (48.43)^{c}	80.00 (63.43)^{c}
3	AgNPs 1500 ppm	30.00 (33.21)^{b}	36.67 (37.26)^{c}	60.00 (50.76)^{b}	86.67 (68.59)^{b}
4	AgNPs 2000 ppm	36.67 (37.27)^{a}	60.00 (50.76)^{b}	100.00 (90.00)^{a}	100.00 (90.00)^{a}
5	AgNO3 1mM	0.00 (0.25)^{e}	0.00 (0.25)^{e}	0.00 (0.25)^{f}	0.00 (0.25)^{f}
6	*P. pinnata*-5.00 %	0.00 (0.25)^{e}	0.00 (0.25)^{e}	13.33 (21.41)^{e}	23.33 (28.88)^{e}
7	Flonicamide 50 WG @ 0.30g/l	40.00 (39.23)^{a}	70.00 (56.78)^{a}	100.00 (90.00)^{a}	100.00 (90.00)^{a}
8	Untreated control	0.00 (0.25)^{e}	0.00 (0.25)^{e}	0.00 (0.25)^{f}	0.00 (0.25)^{f}
	S.Em.±	0.95	0.70	1.17	1.24
	CV	8.29	5.05	4.70	4.33
	CD @ 1%	3.95	2.93	4.85	5.14

Note: Figures in the parenthesis are arcsine transformed values.
Incolumns, mean followed by same letter do not differ significantly by DMRT (p=0.01)
*HAT-Hours After Treatment
*AgNPs – Silver Nanoparticles

Fig.1B Colour change of *P. pinnata*-silver nitrate solution due to silver nanoparticle formation

Fig.2 A) UV visible spectroscopy of *Azadirachta indica* synthesized silver nanoparticles.
B) UV visible spectroscopy of *Pongamia pinnata* synthesized silver nanoparticles.

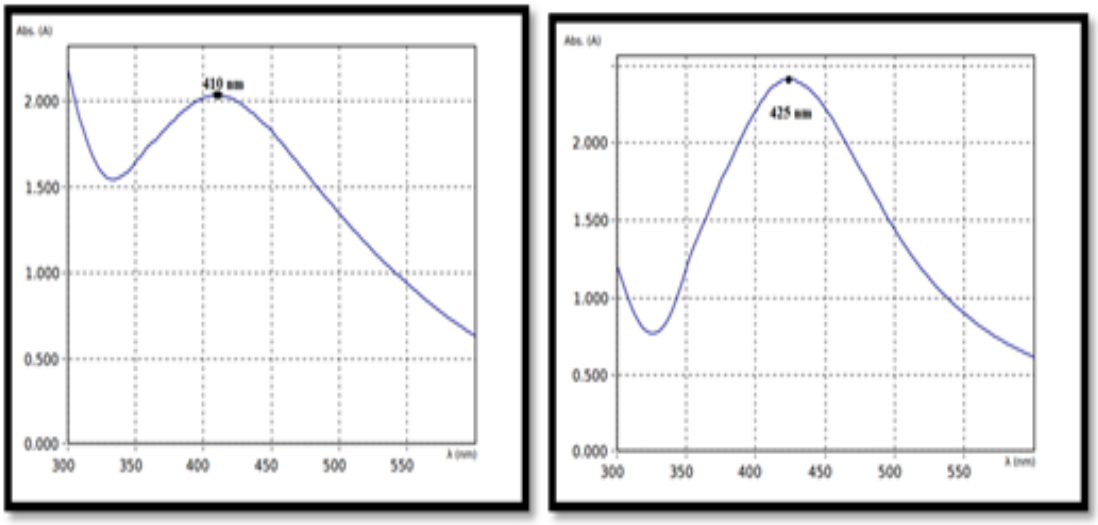


Fig.3 A) Particle Size Analyzer image of *A. indica* based AgNPs at 61.70 nm.
B) Particle Size Analyzer image of *P. pinnata* AgNPs at 68.80 nm.

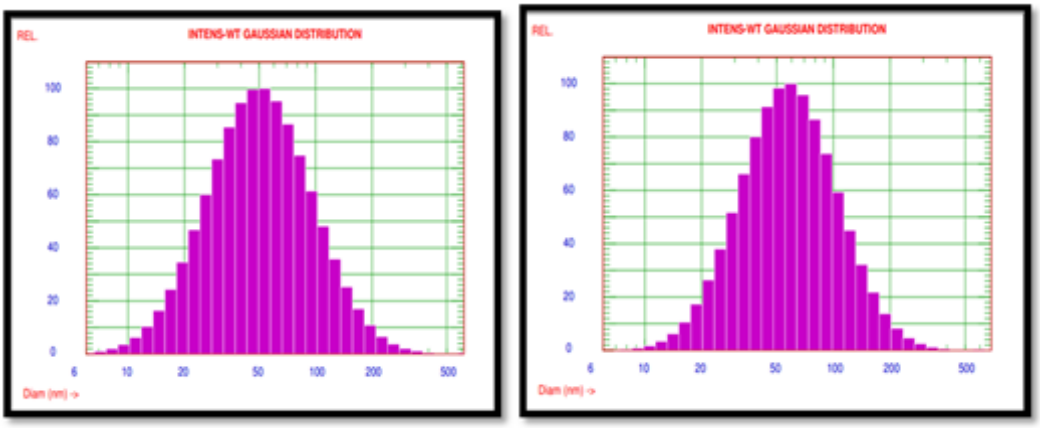


Fig.4 A) Scanning electron microscopic image of *A. indica* based AgNPs at 61.70 nm.
B) Scanning electron microscopic image of *P. pinnata* AgNPs at 68.80 nm.

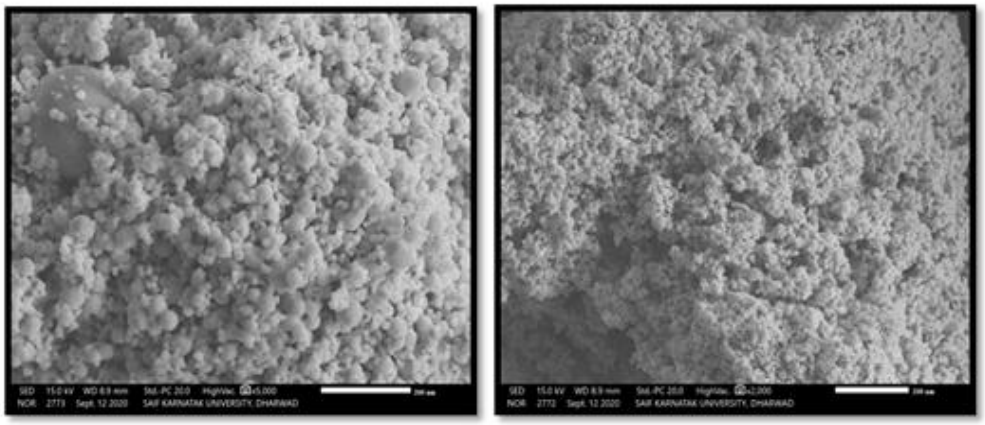


Fig.5A Effect of *Azadirachta indica* based AgNPs on 1st nymphal instars of *Amrasca devastans*

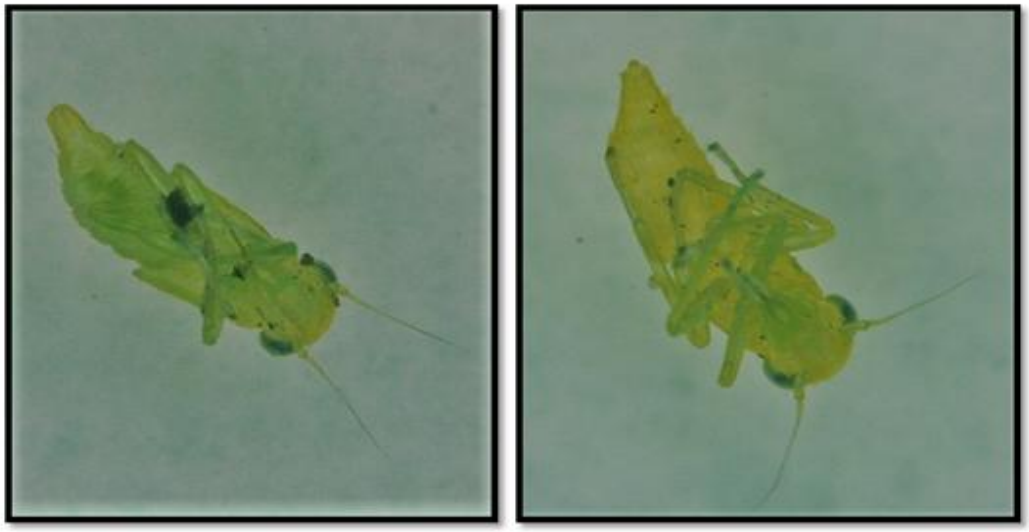
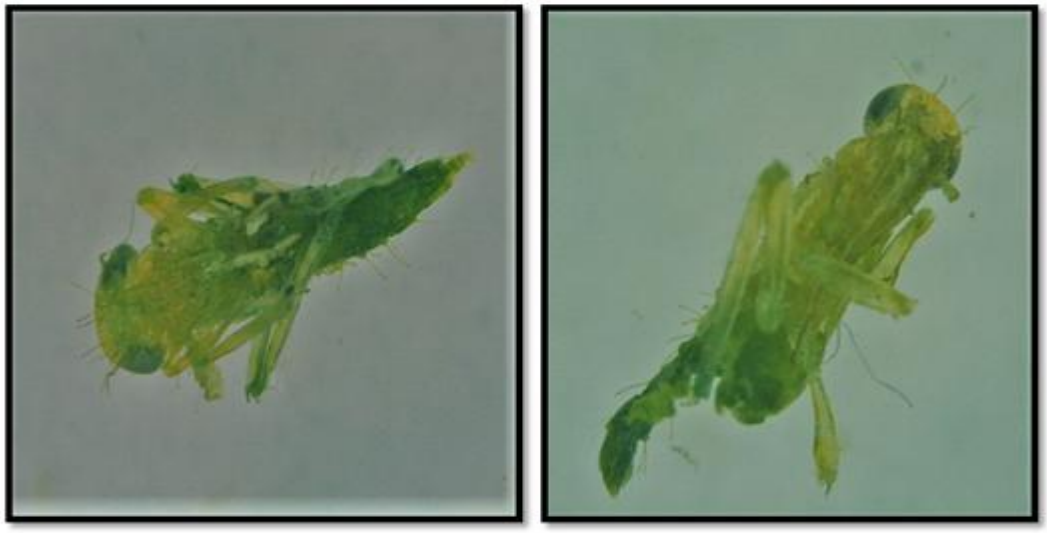


Fig.5B Effect of *Pongamia pinnata* based AgNPs 1st nymphal instars of *Amrasca devastans*



After 48 hours of treatment, 2000 ppm of *A. indica* based AgNPs were found to be significantly superior to all other concentrations with80.00 per cent nymphal mortality which was only next best to insecticidal check.

Next best nanoparticle treatment was 2000 ppm of *P. pinnata* based AgNPs, which recorded nymphal mortality of 60.00 per cent, followed by 1500 and 1000 ppm of *A. indica* based AgNPs with nymphal mortality of 50.00 and 40.00 per cent nymphal mortality, respectively. Lowest nymphal mortality of 30.00 per cent was recorded at 500 ppm of *A. indica* based AgNPs, which was on par with 500 ppm and 1000 ppm of *P. pinnata* based AgNPs. *A. indica* and *P. pinnata* based AgNPs@ 2000 ppm resulted in 100.00 per cent nymphal mortality after 72 hours of treatment, which was comparable to Flonicamide. *A. indica* based AgNPs@ 1500 ppm resulted in the second highest nymphal mortality of 73.33 percent,

followed by 1000 ppm *A. indica* based AgNPs by recording 70.00 per cent nymphal mortality. At 1000 and 500 ppm of *P. pinnata*-based AgNPs, the lowest nymphal mortality was 56.67 per cent and 50.00per cent were recorded, respectively.

At 72 hours following treatment, *A. indica* and *P. pinnata* based AgNPs@ 2000 ppm recorded 100.00 per cent nymphal death, which on par with Flonicamide. *A. indica* based AgNPs@ 1500 ppm recorded the second highest nymphal mortality of 73.33 percent, followed by 1000 ppm which recorded nymphal mortality of 70.00 per cent. *A. indica* based AgNPs registered 60.00 per cent nymphal mortality at 500 ppm, which was on par with1500 ppm of *P. pinnata* based AgNPs. At 1000 and 500 ppm of *P. pinnata* based AgNPs, lowest nymphal mortality was 56.67 and 50.00 per cent were recorded, respectively.

Similar trend of nymphal mortality was documented at 96 hours after treatment with nymphal mortality of 93.33 and 90.00 per cent at 1500 and 1000 ppm of *A. indica* based AgNPs, respectively.

This was followed by nymphal mortality of 86.67 per cent and 80.00 per cent at 1500 and 1000 ppm of *P. pinnata* based AgNPs, respectively. Lowest nymphal mortality of 70.00 per cent was recorded at 500 ppm of *P. pinnata* based AgNPs.

Results of this investigation are in agreement with Manoj *et al.,* (2018) who reported that cent per cent mortality against sucking pests like *Phenacoccus solenopsis, Aphis craccivora* and *Tetranychus urticae* at 750 and 1000 ppm of Soybean based AgNPs. All the nanoparticle treatments were found to be only next best to insecticidal check, Spiromesifen 240 SC and Buprofezin 25 SC. Similarly, Shanmugapriya *et al.,* (2017) synthesized green silver nanoparticles from peel extracts of *Raphanus sativus* and evaluated their insecticidal toxicity against mango leafhopper, *Amritodus brevistylus*. Results from bioassay revealed that LC_{50}value of 7.61 ppm at 48 HAT and was also found to be reducing its biological parameters including longevity, fecundity etc. Both *A. indica* and *P. pinnata* based AgNPs were efficient in managing *A. devastans* with cent per cent nymphal mortality documented at 72 and 96 hours after treatment.

At other exposure periods, *A. indica* based AgNPs were found to be slightly superior compared to *P. pinnata* based AgNPs. This is because of the lower size and spherical shape (lower surface area volume ratio) of *A. indica* based AgNPs

Author Contributions

All authors have contributed to the concept and execution of this experiment. Material preparation and analysis were carried out by M. M. Anees and S. B. Patil. First draft of the manuscript was written by M. M. Anees and all authors approved it.

Competing Interests

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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