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RESEARCH PAPER

Insecticidal efficacy of green synthesized silver nanoparticles, *Bacillus thuringiensis* and Triazophos against *Pectinophora gossypiella*

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Abstract

Pink bollworm (*Pectinophora gossypiella*) poses a significant threat to cotton crops worldwide, resulting in economic losses surpassing sustainable levels for farmers since 2011. This study examines different approaches to controlling pink bollworm infestations, specifically silver nanoparticles (AgNPs), bio-formulations of *Bacillus thuringiensis* (Bt), and a commercial insecticide (Triazophos), in both laboratory and field environments. AgNPs were synthesized using neem plant leaf extract (*Azadirachta indica*). The formation of AgNPs was confirmed through UV-visible spectroscopy, Fourier transform infrared (FTIR) spectroscopy, and a zeta sizer analyzer. In the laboratory, the effectiveness of individual and combined applications of AgNPs, Bt, and Triazophos was tested against 2nd and 4th instar pink bollworm larvae. Mortality rates were measured at 1, 2, and 5 days after application. The results showed that the treatment combining AgNPs and Triazophos (40ppm + 50ppm) achieved a larval mortality rate of over 90%, surpassing the other treatments. In field conditions, the combination of Triazophos and AgNPs at 40ppm (100ml + 50ml) proved most effective in managing *P. gossypiella* populations. These findings underscore the potential of nanoparticles as efficient larvicidal agents for controlling *P. gossypiella*. Furthermore, they emphasize the importance of reevaluating past management strategies for cotton crops given emerging alternatives to traditional insecticides. Future research directions may involve further optimizing nanoparticle formulations and investigating their broader ecological impacts on cotton ecosystems.

Keywords

Azadirachta indica, *Bacillus thuringiensis*, *Pectinophora gossypiella*, Silver Nanoparticles, Triazophos

Introduction

Cotton is a mainstay of textile and economic growth, ranking 4th in yarn production, 2nd in yarn exports, and 7th in cloth production worldwide (Sial et al. 2014; Wei et al. 2020). In Pakistan, cotton production decreased by 24.33% from 13.03 to 9.86 million bales between 2012–13 and 2018–19. Before the introduction of Bt cotton in Pakistan, comprehensive pesticide sequential sprays were used to control the cotton pest complex (Razzaq et al. 2013). More than half of the cotton-growing area worldwide is occupied by cotton containing *Bacillus thuringiensis* genes (Saeed et al. 2016; Shuli et al. 2018; Ali et al. 2019). As a result, there has been an increase in bollworm attacks due to poor control measures, leading to crop failures in various cotton-growing areas where farmers are facing socio-economic calamities as victims of pest infestations (Patil et al. 2010). The pink bollworm (Saunders) (Lepidoptera: Gelechiidae) is considered one of the most challenging pests to control due to its concealed feeding habits (Lykouressis et al. 2005; Mamoon et al. 2022). The parts of the cotton plant affected by *P. gossypiella* include flowers, bolls, buds, and seeds. *P. gossypiella* larvae begin feeding on flowers after emergence by twisting the flower petals together and feeding inside them. Such flowers are called as rosette flowers. Second-generation larvae of *P. gossypiella* bore into bolls to feed. This generation is very difficult to control. Chemical control becomes challenging as *P. gossypiella* larvae are inside the bolls feeding on seeds and lint. After destroying one seed, the larvae move on to another within the same boll. The bolls fail to open completely, with later-stage crop bolls remaining closed as *P. gossypiella* larvae hibernate within them (Sarwar 2017). To control this pest despite its challenging chemical control. Increased insecticide use leads to resistance in bollworms, especially against pyrethroids pesticides that are also very destructive to natural enemies (Dhaliwal and Arora 2003; Tabashnik and Carriè 2019). The control of this pest is based on different control strategies like integrated pest management (IPM) in which for control of a pest different control methods are adopted in a compatible manner (Damos et al. 2015; Ali et al. 2023a, 2023b). IPM modules have been failed for the control of various pests especially in cotton crop where farmers mostly rely on the use of synthetic insecticides. However, despite the use of chemicals, the expected control results are not obtained mostly due to lack of awareness among the farming community about effective insect pest management tactics (Sinzogan et al. 2004; Khan et al. 2015). An emerging technique to reduce the insecticide use has been developed as transgenic Bt cotton (containing *Bacillus thuringiensis*) as a component of integrated pest management (Gore et al. 2001; Tabashnik and Carriè 2019). This transgenic *Bt* cotton provided a very specific, safe and effective technique for the control of lepidopteran pests (Shelton et al. 2002). One of most recent way controlling insect pests is use of nanoparticles (NPs) which is an effective way to deal with notorious pests of cotton like *P. gossypiella*. The strategy has been used against various insect pests like *Euphorbia prostrata*, *Sitophilus oryzae*, *Haemaphysalis bispinosa*, *H. maculata* and in some lepidopteran insects (Zahir et al. 2012). The study aimed at evaluating the efficacy of some non-conventional materials against PBW and to determine the effect of the tested materials on some biochemical parameters under laboratory conditions.

Materials and methods

Preparation of silver nanoparticles (AgNPs)

Silver nanoparticles (AgNPs) were prepared using a green synthesis approach. Healthy fresh leaves of *A. indica* were collected from the research Farm of MNS University of Agriculture, Multan. The leaves were washed thoroughly with tap water and then with distilled water. Twenty-five grams of the leaves were cut into small pieces and placed in a 250 ml Erlenmeyer flask with sterile distilled water, heated at 90°C for 1 hour in a water bath. The aqueous plant extract was obtained by filtering the extract using Whatman filter paper No.1. A 0.01M AgNO3 solution was prepared by dissolving 169 mg AgNO3 in 1000 ml of double distilled water for further use in the green synthesis of AgNPs. The aqueous leaf extracts (10 ml) were added individually to 100 ml of 0.01M AgNO3 in an Erlenmeyer flask (250 ml), boiled at 90°C for 10–15 minutes, and stirred at 150 rpm at 30°C using a magnetic stirrer until the color changed from yellowish to dark brown, indicating the completion of AgNPs synthesis. After centrifugation at 6,000 rpm for 15 minutes and washing the pellet two to three times with sterile distilled water to remove residues, the resultant pellet was dried in a hot air oven overnight at 60°C to obtain a fine powder of nanoparticles.

Characterization of silver nanoparticles

The synthesis of AgNPs was confirmed through Ultraviolet-Vis spectroscopy analysis, measuring light absorbance in the range of 300 to 600 nm with a resolution of 1 nm. Fourier Transform Infrared Spectroscopy (FTIR) analysis was used to determine the functional groups of biomolecules present in the leaf extract. The particle size and distribution pattern of green-synthesized AgNPs were measured using a particle size analyzer (Horiba, SZ-100 Japan).

Collection and maintenance of insects

Field populations (larvae of 2nd and 4th instars) of *P. gossypiella* were collected from infested cotton fields and cultured at room temperature in the rearing laboratory of the Department of Entomology, MNS University of Agriculture Multan.

Test formulations

Several chemicals were evaluated under laboratory and field conditions including Silver Nanoparticles (AgNPs), Bio Care (16000 IU/mg), Triazophos 40% EC, Triazophos 20%+ AgNPs, and a Control with distilled water.

Bioassay

Bioassays were conducted at specific temperature and humidity conditions using second and fourth instar larvae collected from infested fields. Larval mortality was recorded at different time points after applications.

Field application

Field applications of treatments were carried out on cotton crops sown in the Research Area (C-Block) of MNS University of agriculture Multan. The study was laid out in a randomized complete block design with three replications.

Statistical analysis

Data on larvae mortality was analyzed using Minitab software for analysis of variance, with treatment significance determined by Tukey's HSD test at a 5% level of significance.

Results and discussion

AgNPs preparation and characterization

UV-Vis spectra were taken initially of plant extract of *A. indica* and aqueous solution of silver nitrate (Fig. 1a, b) indicating wave lengths at 300 nm and 413 nm. On addition of neem plant extract to silver nitrate aqueous solution, the color of reaction mixtures solution changed from yellowish to dark brown as a result of surface plasma resonance (SPR) excitation of AgNPs particles in the solution indicating the synthesis of AgNPs. SPR absorbance peak was found at wavelength 444 nm which was in the range of 430 to 460 nm as described in the previous studies. The green synthesized silver nanoparticles were further characterized by using Zeta particle sizer to determine the percentage of nanoparticles of Ag with average size in the synthesized solution. The recorded average sizes of nanoparticles in zeta sizer analysis were in range from 16 nm to 51 nm size where majority of particles existed in sizes 24 nm (27%), 21 nm (24%), 28 nm (20%), and 33 nm (11%).

Further, it was confirmed via UV-Vis spectrometer and recorded the wavelength of 444 nm (Fig. 2a). Since the prescribed wavelength of AgNPs range was 420–460 nm as a confirming the formation of AgNPs. The FTIR spectral analysis indicated the presence of potential functional groups of phytochemicals in the plant extracts utilized for

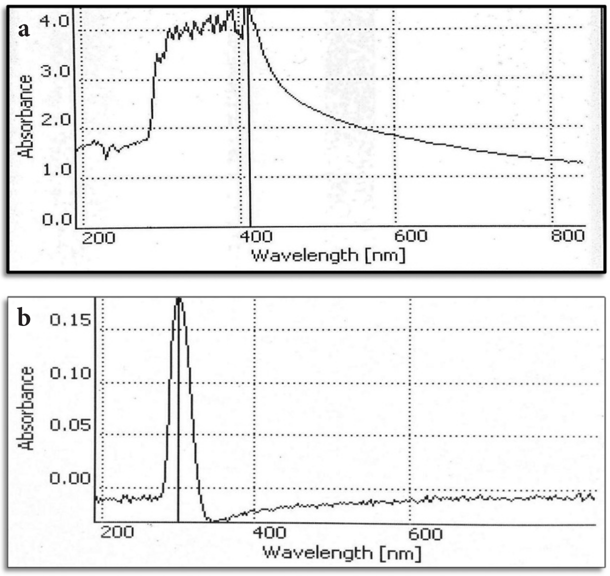

Figure 1. (**a**) UV-Viz spectra of leaves extract of *A. indica* (**b**) UV-Viz spectra of aqueous solution of $AgNO_3$.

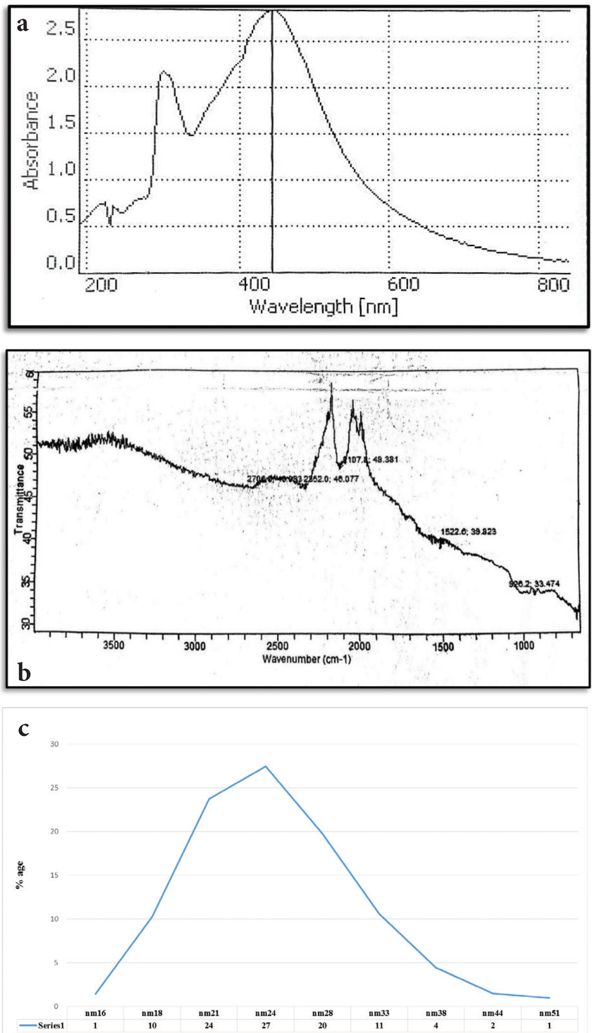

Figure 2. (**a–c**) UV-Viz spectra of green synthesized of AgNPs (**b**); FTIR analysis of green synthesized AgNPs (**c**) Particle size and percentage of AgNPs in Zeta sizer analysis.

AgNPs synthesis, following standard reference Infrared (IR) charts. Specifically, the synthesized AgNPs using leaf extract of *A. indica* displayed intense absorption peaks at 1641.34, 2360.75, and 3359.83 cm-1, corresponding to medium stretches of –C=C–alkenes. Additionally, medium and broad stretching observed at H–C=O:C–H indicated the presence of aldehydes and -OH alcohols and phenols, suggesting that biomolecules within the extracts functioned as reducing, capping, and stabilizing agents in the AgNPs development process (Fig. 2b). Furthermore, the particle size analyzer utilized to determine the average particle size of the synthesized AgNPs revealed an average size of 28.4 nm with plant extracts of *A. indica* (Fig. 2c) employed for the synthesis of AgNPs in this study. Since the particle sizes were very small, TEM studies were carried out to get to know the shape and size of the particles this was done using transmission electron microscope (JEOL JEM-1010). The particles in analysis were found mostly in circular shape with size from 12 nm to 56 nm with an average size of 27 nm (Fig. 3).

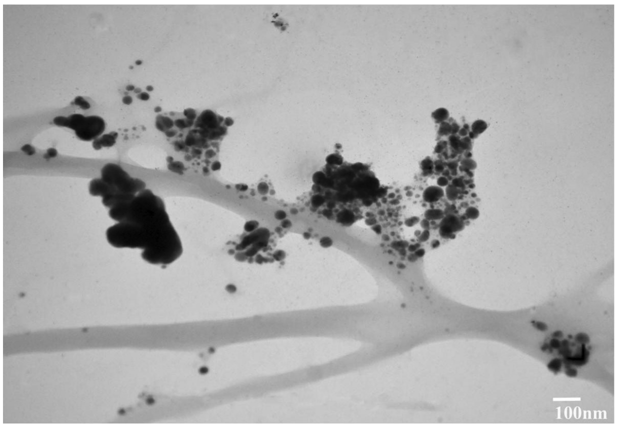

Figure 3. TEM images of synthesized silver nanoparticles showing the shape and size of synthesized AgNPs.

Laboratory bioassays against 2nd instar larvae of *P. gossypiella*

Five Treatment were used against 2nd instar larvae of *P. gossypiella*. The data recorded after 1d, 3d, and 5d respectively of each treatment and No. of dead and alive larvae were noted. After 5d, the results showed the treatment Triazophos+AgNPs-1 (combined) proved most toxic to larvae (90.39±5.78) followed by Triazophos (75.69±3.42). The least toxic treatment was proved Bt (33.23±1.83). The percent mortality of *P. gossypiella* larvae showed in (Table 1).

Laboratory bioassays against 4th instar larvae of *P. gossypiella*

In second bioassay, all five treatments were applied against 4th instar larvae of *P. gossypiella*. The recorded data after 1d, 3d, and 5d showed most effective treatment was Triazophos (47.22±3.64) followed by triazophos+AgNPs-1 (43.94±4.04). The least toxic treatments were proved AgNPs-1 and Bt (26.52±4.60 & 23.48±4.60) respectively. The percent mortality of *P. gossypiella* larvae showed in (Table 2).

Table 1. Mean mortality (%±SE) of Silver nanoparticles (AgNPs), *B. thuringiensis* (*Bt*) and Triazophos against 2nd instar larvae of *P. gossypiella* under laboratory conditions.

Treatments	Conc. (ppm	Mean mortality (% age)		
		After 1d	After 3d	After 5d
AgNPs-1	40	13.89±2.78abc	34.099±4.17c	45.19±2.99cd
AgNPs-2	80	22.22±2.77ab	42.67±3.96bc	63.13±6.80bc
B. thuringiensis	125	5.55±2.77bc	11.369±2.66d	33.23±1.83d
Triazophos	100	25.0±4.80a	54.0±4.29ab	75.69±3.42ab
Triazophos+ AgNPs-1	50+40	30.55±7.34a	68.69±2.02a	90.39±5.78a
Untreated	-	0.00±0.00c	3.03±3.03d	3.03±3.03e
P-value		0.00	0.00	0.00
F-value		8.39	52.3	52.9

Table 2. Mean mortality (%±SE) of Silver nanoparticles (AgNPs), *B. thuringiensis* (*Bt*) and Triazophos against 4th instar larvae of *P. gossypiella* under laboratory conditions.

Treatments	Conc. (ppm)	Mean mortality (% age)		
		After 1d	After 3d	After 5d
AgNPs-1	40	8.33±2.77ab	16.67±4.81ab	26.52±4.60cd
AgNPs-2	80	13.89±2.77ab	30.56±2.78ab	41.16±4.40bc
B. thuringiensis	125	2.78±2.77ab	11.12±4.81ab	23.48±4.60cd
Triazophos	100	19.44±0.00ab	44.41±2.77ab	47.22±3.64ab
Triazophos+ AgNPs-1	50+40	25.0±2.78a	36.11±7.34a	43.94±4.04a
Untreated	-	0±0.00b	0±0.00b	3.03±3.03d
P-value		0.04	0.03	0.000
F-value		3.25	3.47	20.7

Field application

Analysis of variance results revealed that pre-treatment population of *P. gossypiella* did not varied significantly from each other at P value (0.00). The results which are statistically analyzed are given below in Table 3. Boll infestation of *P. gossypiella* recorded from different plots was not significant. After application of treatments, the *P. gossypiella* infestation in collected bolls were recorded after 5 days. After analysis the results revealed that the reduction in boll infestation of *P. gossypiella* is different significantly from each other in different treatments. The maximum reduction of boll infestation as compare to pretreatment data was noted by Triazophos (58.7±0.88) followed by triazophos+AgNPs

Table 3. Percent reduction (%±SE) in pink bollworm infestation after first application under field condition.

Treatments	Dose/ acre (ml)	Pre-treatment (Infestation %)	Mean reduction (%)	
			5 Days after application	10 Days after application
AgNPs	100	14.3±3.92a	44.2±1.52b	62.8±1.20b
Triazophos	500	15.0±2.02a	58.7±0.88a	73.9±0.57b
Bt	250	13.6±2.60a	26.8±1.52b	46.3±2.60b
Triazophos + AgNPs	100+40	17.3±1.76a	54.7±0.57a	83.0±0.57a
Untreated	-	16.0±1.20a	0.0±1.45c	0.0±1.66c
P		0.84	0.001	0.000
F		0.34	14.34	18.15

(54.7±0.57) which showed good results. The minimum boll infestation reduction was noted by Bt as (26.8±1.52). After application of treatments, the *P. gossypiella* infestation in collected bolls was recorded after 10 days After analysis the results revealed that the boll infestation of *P. gossypiella* is different significantly from each other in different treatments. The maximum boll infestation reduction as compare to pre -treatment data was noted by triazophos+AgNPs (83.0±0.57) followed by Triazophos was (73.9±0.57) which showed good results. The minimum boll infestation reduction was noted by Bt as (46.3±2.60). For second application, Analysis of variance results revealed that pre-treatment population of *P. gossypiella* did not varied significantly from each other at P value (0.00). The results which are statistically analyzed are given below in Table 4. Population of *P. gossypiella* recorded from different plots was almost similar. After application of treatments.

Table 4. Percent reduction (%±SE) in pink bollworm infestation after second application under field condition.

Treatments	Dose/ acre (ml)	Pre-treatment (Infestation %)	Mean reduction (%)	
			5 Days after application	10 Days after application
AgNPs	100	11.3±2.33a	52.9±1.45b	67.6±0.88a
Triazophos	500	14.0±1.15a	66.7±1.20a	57.1±0.57b
Bt	250	19.0±1.15a	19.3±1.45bc	40.4±2.90b
Triazophos + AgNPs	100+40	15.3±1.45a	45.6±1.76b	69.6±1.20a
Untreated	-	12.0±1.73a	0.0±0.66c	0.0±0.88c
P		0.04	0.004	0.003
F		5.70	9.28	9.93

Discussion

Silver nanoparticles (AgNPs) prepared biologically from the leaf extract of *A. indica* show great potential for managing insect pests. The formation of silver nanoparticles was confirmed through UV-Vis spectroscopy analysis. The change in color from yellowish to dark brown in the aqueous solution after approximately 30 minutes indicates the formation of nano-sized particles when the leaf extract of *A. indica* is mixed with the aqueous AgNO3 solution. Previous studies by Shankar and Rhim (2015), Logeswari et al. (2015), and Ahmed et al. (2016) have described the confirmation of AgNPs formation through surface plasmon resonance (SPR) absorbance peak in UV-Vis analysis.

Studies by Al Shater et al. (2020) reported that a reduced bandwidth in the absorption peak indicates smaller, spherical-shaped particles with some agglomeration. The size of green-synthesized AgNPs typically ranges from 10 to 60 nm with a spherical shape and stability, as reported by Shah et al. (2009) and Ahmed et al. (2016). In this study, AgNPs synthesized using *A. indica* ranged from 16 nm to 51 nm, demonstrating the capping ability of phytochemicals in the extract for nanoparticle stability. The peaks observed in the characterization indicate aromatic rings, geminal methyl, and ether linkages, suggesting the presence of flavones and terpenoids responsible for stabilizing AgNPs. The crystallinity of silver nanoparticles is reflected by the intensity of peaks; however, the wide diffraction peaks indicate very small crystallite size.

Recent studies evaluating green-synthesized nanoparticles have highlighted their unique insecticidal properties. In this study, *A. indica*-mediated green-synthesized AgNPs were tested against 2nd and 4th instar larvae of pink bollworm under laboratory and field conditions, along with *B. thuringiensis* and Triazophos. The field study conducted during 2018–19 demonstrated that the application of AgNPs and other agents on cotton effectively reduced pink bollworm infestation. These nanoparticles are easy to prepare and use, showing no apparent resistance development in target insects. Additionally, their safety for non-target organisms further supports their potential use in field crops.

Conclusion

The results of this study clearly indicate that green-synthesized AgNPs possess significant insecticidal properties, offering a promising alternative to traditional pesticides. Furthermore, combining lower doses of rational pesticides with AgNPs showed excellent results even at reduced doses, potentially reducing pesticide usage, costs, and minimizing adverse effects on crops and the environment.

Author's contribution

All the above-mentioned authors have made a substantial contribution to the concept or design of the article; or the acquisition, analysis, or interpretation of data for the article.

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