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ORIGINAL ARTICLE

Biogenic silver nanoparticles from *Trichodesma indicum* aqueous leaf extract against *Mythimna separata* and evaluation of its larvicidal efficacy

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Abstract

The present exploration is focused on the bio-fabrication of silver nanoparticles (Ag NPs) using *Trichodesma indicum* aqueous leaf extract as a reducing agent. The synthesized Ag NPs were productively characterized by UV-vis spectroscopy, XRD, and TEM studies. The photosynthesis of Ag NPs was done at room temperature for 24 h and at 60°C. The green synthesis of spherical-shaped Ag NPs bio-fabricated from *T. indicum* with a face centred cubic structure showed average particle sizes of 20–50 nm, which is inconsistent with the particle size calculated by the XRD Scherer equation and TEM analysis. We further explored the larvicidal efficacy of biosynthesized Ag NPs with leaf extracts of *T. indicum* against *Mythimna separata*. The results showed that Ag NPs (20–50 nm) of *T. indicum* possess good larvicidal activity against *M. separata* with an LC_{50} of 500 ppm. Thus, we can advocate that Ag NPs of 20–50 nm size extracted from *T. indicum* may be considered in the pest management programme of *M. separata* in future.

Key words: insect pest control, larvicidal, *Mythimna separata,* nanoformulation, silver nanoparticles (Ag NPs)

Introduction

The oriental armyworm, *Mythimna separata* (Lepidoptera: Noctuidae) is a serious pest of cereals in Asia and Australia (Li *et al.* 2015). In nature, it has been recorded feeding on 33 plant species and grasses belonging to eight families (Sharma and Davies 1982). This pest has caused grave harm at regular intervals on sorghum (*Sorghum bicolor*), pearl millet (*Pennisetum glaucum)*, rice (*Oryza sativa*), maize (*Zea mays*), wheat (*Triticum aestivum*) and sugarcane (*Saccharum officinarum*), because more area is under irrigation, and there have been changes in farming systems due to the introduction of high yielding varieties, increased fertilizer use and continuous cultivation (Rashid *et al.* 2013; Wang *et al.* 2015). Yield losses are inflicted mainly by the larval stage and the gregarious behaviour of larvae. Sometimes, their population outbreaks result in complete crop loss (Ishiwaka and Masuda 2008). The occurrence of *M. separata* outbreaks has been attributed to extensive use of fertilizers and manure, trash mulch, drought following rains, floods resulting from heavy rainfall, initiation of migrant populations entering a geographic area, as well as temperature and humidity preceding and also during the outbreaks (Ishiwaka and Masuda 2008; Ensaf *et al.* 2016). The use of chemical insecticides has been a most important tool for pest control. However, there are serious consequences such

as intoxication of people and animals, contamination of water, air and soil, which results in residues in food, high persistence in the environment, and resistance in pests. They also have an impact on beneficial insects (Regnault-Roger *et al.* 2005; Abdallah *et al.* 2016; Zhu *et al.* 2016). To prevail over this situation, different forms of insecticides have been advocated.

Recently, nanotechnology has been embraced in the world of pesticides and the processes of insect pest control. It has the potential to revolutionize modern day agriculture (Harper 2010). Plants provide a better platform for nanoparticle synthesis since they are free of toxic chemicals and provide natural capping agents. The use of plant extracts also reduces the cost of microorganisms' isolation and culture media thereby enhancing the cost competitive feasibility over nanoparticle synthesis by microbes (Prasad 2014). Nanostructure materials show unusual physical, chemical and biological properties, which are completely distinct from their bulk materials and individual molecules (Prasad *et al.* 2014; Bhattacharyya *et al.* 2016). Nanotechnology includes encapsulation and entrapment of nanoagrochemicals such as nanofertilizers, nanopesticides, nanoherbicides, plant growth stimulating nanoscale biomolecules and other active substances by using polymers, dendrimers, surface ionic attachments etc. (Prasad *et al.* 2016; Jampilek and Kralova 2016). Moreover, other mechanisms may be used for controlled and slow release of agrochemicals which allow the slow uptake of active ingredients. This in turn reduces the amount of agrochemical application by minimizing input and waste (Chen and Yada 2011). Nanomaterial release arrangement in agriculture is important because of the better solubility and stability to degradation in the environment (Chen and Yada 2011; Chowdappa and Gowda 2013). The nanoscale materials, released from vehicles, increase the effectiveness of the insecticidal properties by binding firmly to the plant surface. They reduce the amount of agrochemicals by preventing run off into the surroundings (Johnston 2010; Chowdappa and Gowda 2013). Nanotechnology can be used for combating plant diseases either by controlled delivery of functional molecules or as a diagnostic tool for disease detection. Several nanoparticles such as nanoporous zeolites, nanocapsules and nanosensors may be used in insect pest suppression (Hallberg 2010). The use of nanoparticles as pesticides is an alternative strategy to combat pests which have become resistant to conventional pesticides. There are very few reports pertaining to the larvicidal activity of silver nanoparticles against insect pests. Therefore, there is a need to explore the use of green silver nanoparticles against pests in order to devise management programmes. Hence, the objective of the present study was to investigate the influence of green nanosilver materials (with different doses) against the second instar larvae of *M. separata*.

Materials and Methods

Test organism

Different larval instars of *M. separata* were collected from infested fields in the Srinagar and Budgam districts of Kashmir, India. The culture was maintained in the Entomology Laboratory of the Department of Zoology, University of Kashmir at 24±2°C and relative humidity 60–65%. Fresh maize leaves (*Zea mays*) were provided for larval feeding. The second instar larvae were taken from the F_1 generation and used for bioassay in the laboratory.

Plant collection

Fresh, healthy leaves of *Trichodesma indicum* were collected from the Bharathiar University campus, Coimbatore, India. The *T. indicum* leaves were washed several times with tap water to remove dust particles and finally rinsed with distilled water. The cleaned leaf material was dried in the shade at room temperature and stored for further use.

Preparation of silver nanoparticles (Ag NPs) solution

The Ag NPs used in the present study were extracted from *T. indicum* leaves according to Prabha *et al.* (2015). Twenty grams of fresh leaves were washed with double distilled water and were transferred to a 500 ml beaker containing 100 ml double distilled water and then boiled for 10 min. The obtained extract was filtered through Whatman No.1 filter paper. Five ml of the plant extract was added to 45 ml of 1 mM $AgNO_3$ solution for reduction into Ag^+ ions. The appearance of a reddish brown colour indicated the formation of Ag NPs, which was preliminarily confirmed spectrophotometrically. The reaction mixture was then stored at room temperature for 24 h for total formation of the nanoparticles. The Ag NPs solution thus obtained was purified by repeated centrifugation at 5,000 rpm for 20 min. The supernatant was discarded and the pellet was dissolved in double distilled water. The Ag NPs were confirmed by colour change.

Characterization of Ag NPs

The produced nanoparticles were subjected to UV-vis absorption spectra and measured in a 1-cm quartz cuvette using a Shimadzu, UV-3600 spectrophotometer, Japan. XRD (X-ray diffraction) analysis of the prepared nanoparticles was done using a diffractometer with Cu Kα radiation operated at 40 kV/30 mA. The morphology and size of Ag NPs were investigated using a JEOL-JEM-2100 transmission electron microscope

(TEM). A sample for TEM study was prepared by placing 0.05 ml of distributed silver solution onto a carbon film, supported on a copper grid followed by solvent evaporation.

UV-visible spectroscopy studies

The Ag NPs show plasmon resonance at 400 to 450 nm in the UV-visible spectrum. The UV-visible absorption spectra of synthesized Ag NPs was analysed with a spectrophotometer Shimadzu, UV-3600 (Japan).

X-ray diffraction (XRD) analysis

The crystalline structure of the synthesized Ag NPs was examined through powder X-ray diffraction using a Rigaku Multiflex X-ray powder diffractometer with Cu Kα radiation operating between 10° and 80° at the scanning rate of 2° per minute. The Ag NPs were spread over a glass slide and the solvent was evaporated to form a thin film of Ag NPs for XRD analysis. The crystalline size was calculated using line broadening profile and Debye Scherrer's formula ($D = 0.94\lambda / B \cos\theta$).

Transmission electron microscope analysis

A TEM was used to visualize the morphology of the Ag NPs. TEM grids were prepared by placing 0.05 ml of the Ag NPs solution on carbon-coated copper grids and drying under a lamp. Furthermore, the additional presence of metals in the sample was analyzed with a TEM (JEOL).

Determination of lethal concentration through the Ag NPs

The lethal concentration of the synthesized nanoparticles (20–50 nm size) was determined by solutions of a series of dilutions ranging from 200, 300, 400 to 500 ppm. The solutions were prepared by the addition of 0.05 ml of ethanol (for clear solution). The solutions were continuously stirred for one hour with the blunt end of a glass rod for complete dissolution of nanoparticles in the media. The solutions turned brownish in colour and were kept as such overnight. Thereafter they were ready for the experiment.

Larvicidal activity

Larvicidal activity was determined with different doses viz. 200, 300, 400 and 500 ppm of nanoparticle solution with the addition of 0.05 ml ethanol for cleanness of the solution. The stock solution was prepared and different doses were applied on second instar larvae, which were on the surface of maize leaves. The prepared nanomaterial solutions were then sprayed uniformly over the entire surface of the leaves. Ten second instar larvae of *M. separata* were released onto treated leaves. The first observations were made after 20 min of surface treatment with 200, 300, 400 and 500 ppm. Another control diet was maintained where leaves were treated with distilled water with 0.05 ml of ethanol. Larvae were provided with fresh treated maize leaves every 24 h. Observations were taken from the first day onwards. Larval mortality was recorded at 24 h intervals.

Statistical analysis

Statistical analysis was performed using SPSS version 20.0 for Windows. The larval mortality data were analysed using one way ANOVA to compare the effects of the treatments. Significant differences between treatments were determined using LSD test at 5% level.

Results and Discussion

Silver nanoparticles synthesised from *T. indicum* leaves exhibited unique optical properties due to the possessions of surface plasmon resonance (SPR). Silver nanoparticles showed more intense SPR at 445 nm (Fig. 1). The prepared nanoparticles initially remained colourless and later turned reddish brown – a characteristic of Ag NPs. After that, these nanoparticles showed no additional alterations in colour implying the achievement of response. This constancy resulted from a potential deference that develops from an antagonism of the reaction and also weak Van der Waals forces of attraction and electrostatic repugnance.

The XRD spectrum confirmed the crystalline characteristic pattern of the prepared Ag NPs and clear

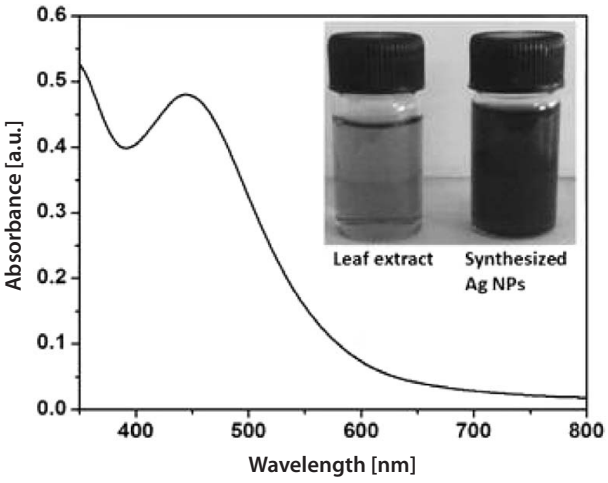

Fig. 1. Shows UV-vis spectra formation of the Ag NPs from leaf extract of *Trichodesma indicum*. Spectrum of Ag NPs formed due to addition of *T. indicum* leaf extract to silver nitrate solution

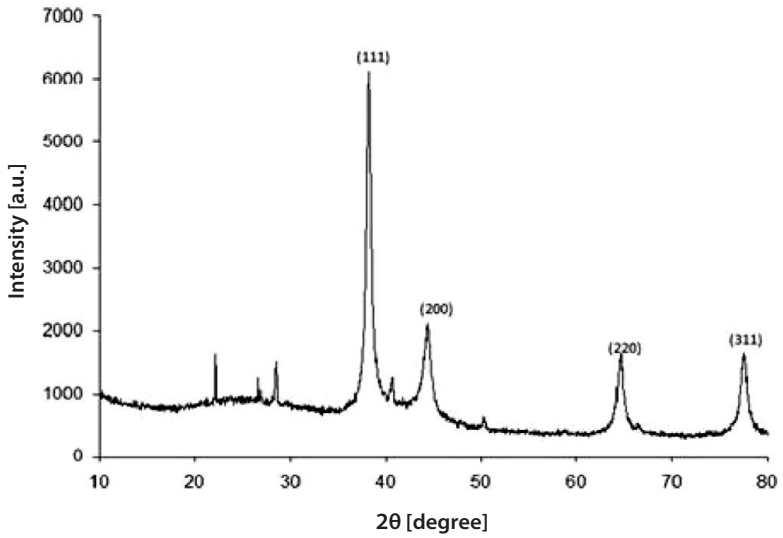

Fig. 2. X-ray diffraction (XRD) spectrum of silver nanoparticles (Ag NPs) extracted from *Trichodesma indicum*

peaks at 38.20°, 44.39°, 64.52° and 77.43° in the 2θ range corresponding to (111), (200), (220) and (311) Bragg's reflection planes were exactly indexed to the face centred cubic structure of silver nanoparticles (JCPDS 04-0783) (Fig. 2). Generally, the broadening of peaks in the XRD patterns of solids is attributed to particle size effects. Broader peaks signify smaller particle size and reflect the effects due to experimental conditions on the nucleation and growth of the crystal nuclei.

The TEM images of the prepared nanoparticles are shown in Figure 3. The spherical shaped nanoparticles were 20–50 nm in size, including the diameter. Most of the prepared nanoparticles were well spread out, while some were aggregated.

This study clearly demonstrated that Ag NPs possessed a tempting effect on the larval stage of *M. separata*. It has been reported that silver nanoparticles act as plant growth stimulators and reduce unwanted microorganisms in soils and hydroponic systems (Sharon *et al.* 2010). Moreover, it may be mentioned here that silver was usually used as an antimicrobial agent in early civilizations. Silver nitrate has been used extensively due to its broad spectrum and multiple modes of antimicrobial activity in nature (Wei *et al.* 2009). Ag NPs are the most studied and utilized nanoparticles in bio-systems because of their strong inhibitory effect on certain diseases (Kim *et al.* 2008). Moreover, it is recognized that there is antifungal efficacy of a colloidal nanosilver solution against rose powdery mildew (Jo *et al.* 2009). Various forms of Ag NPs have been tested to examine their antifungal activity especially on two plant pathogenic fungi: *Bipolaris sorokiniana* and *Magnaporthe grisea*. In recent studies (*in vitro* and *in vivo*) on the efficacy of Ag NPs against powdery mildew before and after a disease outburst in plants under diverse cultivation circumstances, there was significant inhibition of the growth of fungal hyphae with the Ag NPs (Lamsal *et al.* 2011; Alghuthaymi *et al.* 2015; Megeed *et al.* 2015). Therefore, metal nanoparticles could be an improved option for insecticides. It has been reported that nanomaterials possess insecticidal properties (Simonian *et al.* 2005; Biju 2007; Rai *et al.* 2012; Chakravarthy *et al.* 2012). Furthermore, metal nanoparticles are essential components of new biosensors and self-assembled nanodevices (Levy *et al.* 2006; Hsing *et al.* 2007; Bhattacharyya *et al.* 2010).

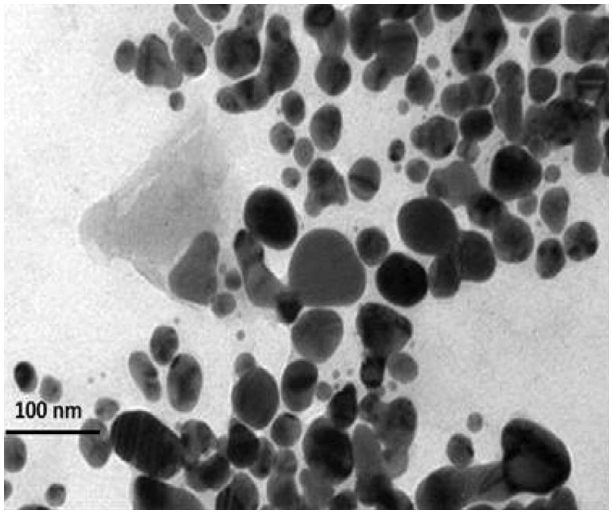

Fig. 3. TEM image of Ag NPs extracted from *Trichodesma indicum*

In this study, Ag NPs caused considerable mortality at all treatment concentrations of second larval instars of *M. separata* (Table 1, Fig. 4). Mortalities of 46.67% (±6.67) and 83.33% (±3.33) were achieved at 200 and 300 ppm concentrations, respectively, on the fourth day of treatment. At 400 ppm concentration, the mortality was 46.67% (±3.33), 76.67% (±8.81) and 100% (±0.00) on the second, third and fourth days, respectively. At 500 ppm concentration, mortality reached

Table 1. Effect of different concentrations of Ag NPs on 2nd larval instar of *Mythimna separata* after treatment

Treatment [ppm]	Percentage of larval mortality days after treatment*			
	1st	2nd	3rd	4th
200	0.0 (±0.00) a	0.0 (±0.00) a	23.34 (±3.33) a	46.67 (±6.67) a
300	0.0 (±0.00) a	26.66 (±3.33) b	46.67 (±3.33) b	83.33 (±3.33) b
400	0.0 (±0.00) a	46.67 (±3.33) c	76.67 (±8.81) c	100.0 (±0.00) c
500	16.66 (±3.33) b	66.67 (±6.67) d	100.0 (±0.00) d	–
Control	0.0	0.0	0.0	0.0

* mean of 10 larvae/replication/treatment; figures in parentheses are standard error (±SE); means followed by same letters in each column are not significantly different by LSD at 5%

16.66% (±3.33), 66.67% (±6.67) and 100% (±0.00) on the first, second and third days of treatment, respectively. When the data were subjected to ANOVA (Table 1), there were statistically significant differences between the treatments required for 50% mortality of the larvae. On the first, second and third days of treatment, the 400 and 500 ppm concentrations produced significantly higher mortality than 200 and 300 ppm treatments. Moreover, on the fourth day 300 and 400 ppm produced higher mortality than 200 ppm treated concentration.

At a lower concentration of Ag NPs, the behaviour of the larvae was normal on the first day. At the highest concentration (500 ppm), feeding was reduced, larvae had sluggish movement and were black on the first day after treatment (Fig. 4b). On the second day after treatment, the larvae became stiff (Fig. 4c) and on the third day lysis of the larvae tissues was observed at the same concentration (500 ppm) (Fig. 4d). There was no mortality of the insects treated with distilled water, with 0.05 ml of ethanol, in the control experiment (Fig. 4a). The above experiment clearly established that Ag NPs

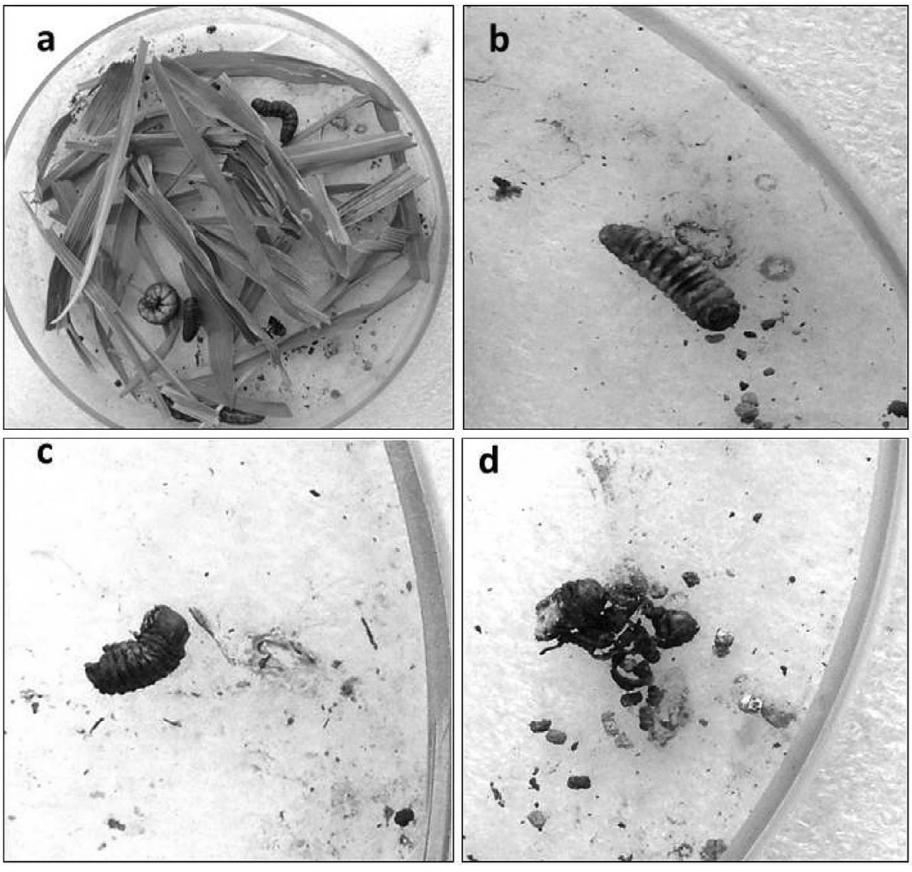

Fig. 4. Larvicidal experiment: a – *Mythimna separata* larvae in the control, b – 2nd instar *M. separata* larva on 1st day after treatment with 500 ppm concentration, c – 2nd instar *M. separata* larva on 2nd day after treatment with 500 ppm concentration, d – 2nd instar *M. separata* larva on 3rd day after treatment with 500 ppm concentration

should be tested at concentrations higher than 300 ppm to determine the effective dose for 50% larval mortality. Recent studies demonstrated that Ag NPs induce embryonic injuries and reduce survival in zebra fish *Danio rerio* (Asharani *et al.* 2008; Griffitt *et al.* 2008). Moreover, nanosilver is also toxic in mammalian systems (Chen and Schluesener 2008). Therefore, our results clearly indicate that the proposed green silver nanomaterials have a tremendous effect on *M. separata* that leads to mortality at specific doses. Toxicity occurs because Ag^+ ions are released from the surface of the nanoparticle by oxidation processes and interact with biological molecules (e.g. insect proteins) upon entering the insect physiological processes (Park *et al.* 2010). Ag NPs are also known to interact with thiol groups of proteins and endorse their denaturation (Johnston *et al.* 2010), thus leading to larval mortality. It may be due to necrosis of the said insect cell that leads to mortality of the targeted insect pests. However the actual mechanism of toxicity is inadequately understood and more experiments are needed in this direction. This is the first report about the control of larvae of *M. separata* with Ag NPs.

Conclusions

We have developed a fast, eco-friendly and convenient method for biogenic synthesis of Ag NPs from silver nitrate using *T. indicum* at room temperature. From the experiment it can be concluded that Ag NPs, 20–50 nm in size, obtained from *T. indicum* possess good larvicidal activity against *M. separata*. The maximum mortality (100%) was produced at a concentration of 500 ppm within the shortest period of time, i.e. on the third day after treatment. Therefore, these Ag NPs can be an important component of an integrated pest management programme against *M. separata*.

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