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ORIGINAL RESEARCH ARTICLE

Biosynthesis of Silver Nanoparticles using *Borago officinslis* leaf extract, characterization and larvicidal activity against cotton leaf worm, *Spodoptera littoralis* (Bosid)

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Abstract

Silver nanoparticles (Ag NPs) was achieved by using leaf extract of *Borago officinalis* plant as eco-friendly reducing and capping agent. The results obtained from ultraviolet-visible spectrophotometer, dynamic light scattering, scanning & transmission electron microscopy, energy dispersive X-ray, and fourier transformed infrared confirmed the biosynthesis and the physical characterization of the produced nanoparticles. The synthesized Ag NPs was greatly more toxic to *Spodoptera littoralis* compared to crude extract. The LC50 values of the crude extract, and synthesized Ag NPs were 22.6 and 0.33 mg/g while LC90 values were 969.0 and 1.7 mg/g, respectively. Ag NPs and plant extract caused a significant elongation in larval period (18.02 and 18.82 days), respectively) compared with (15.78 days) in the control treatment. Whereas, there was no significant elongation in the pupal period in both males and females in case of Ag NPs and plant extract compared to the control treatment. Our results declared that the leaf crude extracts of *B. officinalis* and green synthesis of silver nanoparticles using it have the potential to be used as an ideal eco-friendly approach for controlling S. littoralis. This is the first report on the pesticide activity of this plant extract and its synthesized silver nanoparticles to control this insect pest.

Keywords *Borago officinalis* · Larvicides · Silver Nanoparticles · *Spodoptera littoralis*

Highlights
- Green biosynthesis of silver nanoparticles using *Borago officinalis* leaf extract.
- Characterization of silver nanoparticles using various advanced techniques.
- Toxic effect of the tested plant extract and the synthesized silver nanoparticles.
- Determination of LC_{50} of the tested plant extract and the synthesized silver nanoparticles.
- Potential of the tested plant extract and the synthesized silver nanoparticles on various biological aspects of *Spodoptera littoralis* after treatment with LC_{50}.

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Introduction

The cotton leaf worm *Spodoptera littoralis Boisd.* (Lepidoptera: Noctuidae) is considered the most serious pest in a wide range of crops and vegetables cultivation in many regions around the world (El-Aswad et al. 2003). New potent insecticides became essential nowadays where this insect has a resistance to conventional chemical insecticides (Boraei et al. 2014). Different groups of nano material act as insecticides, fungicides and herbicides. Recent advances in nano science with materials have unique properties than their macroscopic or bulk counter parts (Matsumoto et al. 2009). Silver nanoparticles (Ag NPs) are effective nanomaterials as compared with various metallic nanoparticles that are implicated in biomedical applications (Wei et al. 2015; Zhang et al. 2016) due to their specific and genuine chemical, biological, and physical properties (Burduşel et al. 2018). Ag NPs proved to have promising features and impressive potential for the development of novel antimicrobial agents (Du´ran et al. 2010; Iravani 2011; Vasquez-Munoz et al. 2014; Szweda

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et al. 2015; (Xue et al. 2016); Das et al. 2016). Hence, there is a severe demand for developing environmentally ecofriendly methods especially green synthesis of silver nanoparticles that have toxic properties against insect pests (Song and Kim 2009). Green synthesis of nanoparticles using plant extracts have been implied as ecofriendly selections. By removing the complicated process of maintaining cell cultures, it can be profitable over other biological procedures (Iravani 2011).. Lately, silver nanoparticles have been synthesized using several plant extract such as *Nelumbo nucifera* (Santhoshkumar et al. 2011), *Pongamia pinnata* (Rajesh et al. 2010), *Azadirachta indica* (Tripathi et al. 2009), *Caesalpinia ferrea* (Soares et al. 2018), *Cinnamon zeylanicum* (Sathishkumar et al. 2009) and *Moringa oleifera* (Al-Kalifawi 2016). Biosynthesis of silver nanoparticles (Ag NPs) using the medicinal plant, starflower *B. officinalis* (Fam: Boraginaceae) was successfully effective as antibacterial and anticancer agent (Singh et al. 2017). Reports showed that the active ingredients in *B. officinalis* are chiefly phenolic profiles such as flavonoids (Zemmouri et al. 2019), which seem to be responsible for the toxicant activity to insects (Onyilagha et al. 2004). Accordingly, our hypothesis depends on using Ag NPs synthesized by *B. officinalis* leaf extract as a novel control agent against S. *littoralis*.

Materials and methods

Tested insects

The culture of the tested insects, *S. littoralis* was initiated from egg-masses collected from infested cotton field at Qualubia Governorate. These egg-masses were surface sterilized with formalin vapor treatment as suggested by David et al. (1972). The newly hatched larvae were fed on fresh castor oil plant leaves, *Ricinus communis* and kept in glass jars (1600 cc) covered with muslin cloth under laboratory conditions of 25 $\pm 2^0$C and 60–70% R.H. The late third instar larvae were taken and used in the laboratory bioassay.

Preparation of plant extracts

The fresh leaves of *Borago officinalis* were collected from the ornamental gardens of Atomic Energy Authority and washed several times with tap water and rinsed with distilled water to remove any dust and dirty particles. The cleaned leaves were dried in shade for 2–3 weeks at room temperature and ground in an electric mill into fine powder. 25 g of the dried leaves powder was soaked in 250 ml of acetone in a 300-mL Erlenmeyer flask for 72 h) .The flasks was then shaken for 30 min in a shaker and its contents were filtered through whatman no. 1 filter paper. The filtrate was stored in amber colored air tight bottle at 4 °C and tested within 5 days.

Biosynthesis of silver nanoparticles (Ag NPs)

Silver nitrate (AgNO3, 99.9%, with average molecular weight of 169.87) and acetone as a solvent were purchased from Lab Chemicals Trading Company, Egypt. Following the method reported by Dinesh et al. (2015) but with some modifications, 10 ml acetone extract of *B. officinalis* was added to 90 ml of 1 mM aqueous AgNO3 solution in an Erlenmeyer flask and kept aside for 24 h at room temperature for complete bio reduction and saturation of Ag NPs. The primary detection of synthesized Ag NPs was carried out in the reaction mixture by observing the color change of the medium, which became reddish brown in color. The reaction mixture was dried at 40 °C and the product was burned at 600 °C in oven under vacuum to prevent the oxidation process. This Ag NPs were further used for characterization. Leaves extract and distilled water without $AgNO_3$ solution were used as a control.

Characterization of silver nanoparticles

UV-VIS spectral analysis

Preliminary characterization of the silver nanoparticles was carried out using UV–visible spectroscopy (JASCO-Japan-model V- 560) at a resolution of 1 nm. The reduction of silver ions was monitored by measuring the UV–visible spectra of the solutions from 300 to 800 nm. Dilutions with distilled water were made from colloidal solutions obtained from the synthesis process in the ratio of 1:10. The nanoparticle solution showed maximum absorbance at 420 nm.

Optimization of silver nitrate concentration and pH value

Silver nitrate concentration was optimized and monitored using different concentration of silver nitrate (0.5, 1 and 3 mM). The reaction pH was maintained at 5, 7, 9 and 11, respectively by using 0.1 N HNO_3 and 0.1 N NaOH.

Dynamic light scattering (DLS)

Average particle size and size distribution was determined by the dynamic light scattering (DLS) technique (PSS-NICOMP 380-ZLS, USA). Before measurements, the samples were diluted 10 times with deionized water. 250 µl of suspension were transferred to a disposable low volume cuvette. After equilibration to a temperature of 25 °C for 2 min, five measurements were performed using 12runs of 10 s each.

Transmission Electron Microscopy (TEM)

The particle size and shape were observed by TEM (Sanghi and Verma 2009), (JEOL electron microscope JEM-100 CX) operating at 80 kV accelerating voltage. The prepared silver

suspensions were diluted 10 times with deionized water. A drop of the suspension was dripped into coated copper grid and allowed to dry at room temperature.

Fourier Transform Infrared Spectroscopy (FTIR)

To determine (FTIR) pattern of the sample, silver nanoparticles were diluted with potassium bromide in the ratio of 1:100, dried (at room temperature), and compressed to form a disc. The discs were later subjected to FTIR spectroscopy measurement. These measurements were recorded on a JA SCO FT-IR -3600 and spectrum was collected at a resolution of 4 cm^{-1} in wave number region of 400 to 4000 cm^{-1}. For comparison, *B. officinalis* plant extract was measured by the same process.

Energy Dispersive X- ray (EDX)

The Energy Dispersive X- ray (EDX-model-OXFORD) Spectroscopy was performed on Scanning Electron Microscope (SEM- JEOL-JEM- 5400) equipped with an EDX detector and EDX spectrum was measured at 10KV accelerating voltage. Solid sample was prepared by drying of silver nanoparticles solution on plastic disc and dried at room temperature.

X-ray diffraction (XRD) The crystalline structure of the synthesized Ag NPs was examined through powder X-ray diffraction using (The XRD-6000 series, including stress analysis, residual austenite quantitation, crystallite size / lattice strain, crystallinity calculation, materials analysis via overlaid X-ray diffraction patterns Shimadzu apparatus using nickel-filter and Cu Kα target, Shimadzu Scientific Instruments (SSI), Kyoto Japan. Founded in 1875) operating with a Cu anode at 40Kv and 50 mA in the range of 2θ value between 20° and 100° with a speed of 2°/min. 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 crystallite size was calculated using line broadening profile and Debye Scherrer's formula (Lei and Fan 2006), $D = K\lambda / \beta\cos\theta$ for peak broadening from size effects only. Where λ is the wavelength of X-rays used (1.5418 ˚A), β is the full width at half maximum (FWHM) intensity of the diffraction line, θ is the Bragg angle for the measured hKl peak and K is a constant equal to 0.9 for Ag0.

SEM analysis

Scanning Electron Microscopy (SEM) was performed to determine the morphological features of the Ag NPs. Few drops of solution were put on the small thin aluminum sheet. It was heated till the liquid phase evaporated. The samples were characterized by SEM at a voltage of 20 kV.

Larvicidal activity

Larvicidal activity of the produced Ag NPs was determined according to WHO protocol (2005). The experiments were done using leaf dipping method (Kasmara et al. 2018). Before testing, the *S. litoralis* larvae were starved for 3 h. The late 3rd instar larvae of *S. littoralis* were fed on fresh leaves of castor oil plant treated with acetone extract of *B. officinalis* and the synthesized Ag NPs. The crude Extract and the synthesized Ag NPs were prepared in the concentrations ranging from (12.5, 25.0, 50.0, 100.0 mg/g) and (0.1, 0.2, 0.4, 0.6, 0.8, 1.0, 2.0, 4.0 mg/g), respectively. Each mixture was continuously stirred for one hour on a magnetic stirrer for complete dissolution. The solutions of nanoparticles turned brownish in color and were kept as such overnight. Each concentration was replicated five times (ten larvae in each replicate). All bioassays were performed at 25±2^{0}C and 60–70% R.H. The control larvae were fed on fresh leaves of castor oil treated with tap water. The larval mortality was observed daily until the end of larval period. The percentage of pupation, adult emergence, and survival were recorded for the average of five replicates.

Data analysis

Lethal concentration (LC_{50}, LC_{90}) and their associated confidence intervals were estimated using Probit analysis, slope and LC_{50} were calculated according to Finney (1971). Data of the biological studies were analyzed using (ANOVA) technique and the means were analyzed using Duncan s multiple range test (P = 0.05) (Steel and Torrie 1960).

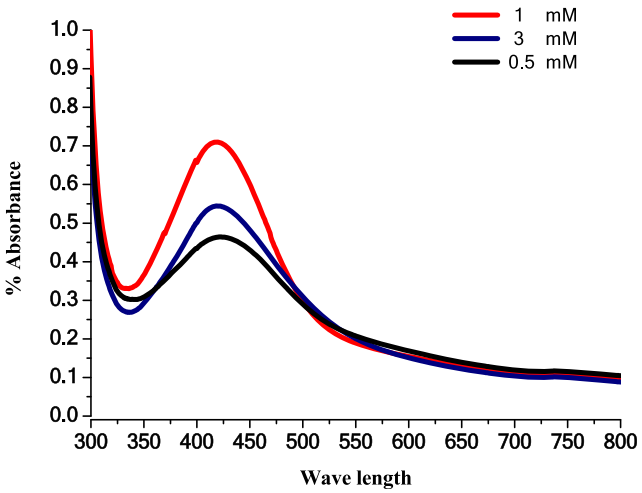

Fig. 1 UV–vis absorption spectra of Ag NPs synthesized by *Borago officinalis* leaf extract at different $AgNO_3$ concentration

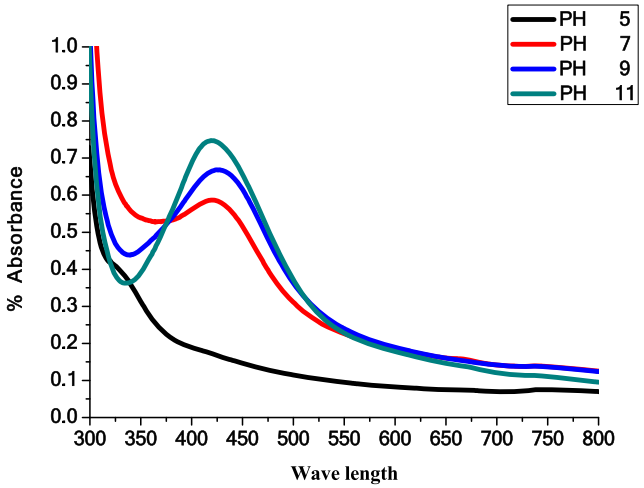

Fig. 2 UV–vis absorption spectra of Ag NPs synthesized by *Borago officinalis* leaf extract at different PH

Results

Characterization of silver nanoparticles

UV-VIS spectral analysis

The addition of *B. officinalis* leaf extract to silver nitrate solution produces a color change of reaction mixture from pale yellow to reddish-brown which indicated the formation of silver nanoparticles. Characteristically, the synthesized silver nanoparticles displayed a maximum absorption peak with the λ max around 420 nm. The physicochemical factors such as the concentration of silver nitrate and pH variations were inquired to optimize the synthesis of Ag NPs. It was noticed that the height of the absorption peak of UV-Vis spectrum around 420 nm intensifies as the concentration of silver nitrate increased, the maximum peak intensity was obtained at 1 mM of $AgNO_3$ (Fig. 1). Peaks of different heights at 420 nm confirm the production of Ag NPs with no change in wavelength. Furthermore, the Ag NPs were synthesized at varying pH ranges (5.0, 7.0, 9.0 and 11.0), and the results are illustrated in (Fig. 2). The UV-vis spectrum clears that the increasing in peaks intensity provides evidence for the enhanced formation of Ag NPs at neutral pH. Whereas the formation of nanoparticles strapped at low pH (pH = 5) .At an elevated pH (pH = 11), a great number of Ag NPs with smaller sizes are produced.

DLS analysis

To deliberate the size distribution profile, polydispersity of the synthesized Ag NPs in colloidal phase, DLS analysis was accomplished. Light is scattered by nanoparticle randomly following the Brownian motion in all directions disturb the intensity of the light, this causes fluctuation in intensity of measured light. The size of the synthesized Ag NPs from plant extract was determined to be 40.50 nm (Fig. 3).

Transmission Electron Microscopy (TEM)

TEM micrograph of Ag NPs solution prepared by *B. officinalis* leaf extract is shown in (Fig. 4) .TEM micrograph of Ag NPs was taken after 72 h of reaction time. The synthesized Ag NPs were more or less uniformly shaped and sized. The particle size was found to be between 20 and 60 nm with average size 40 nm. Few irregularly shaped Ag NPs were also noticed.

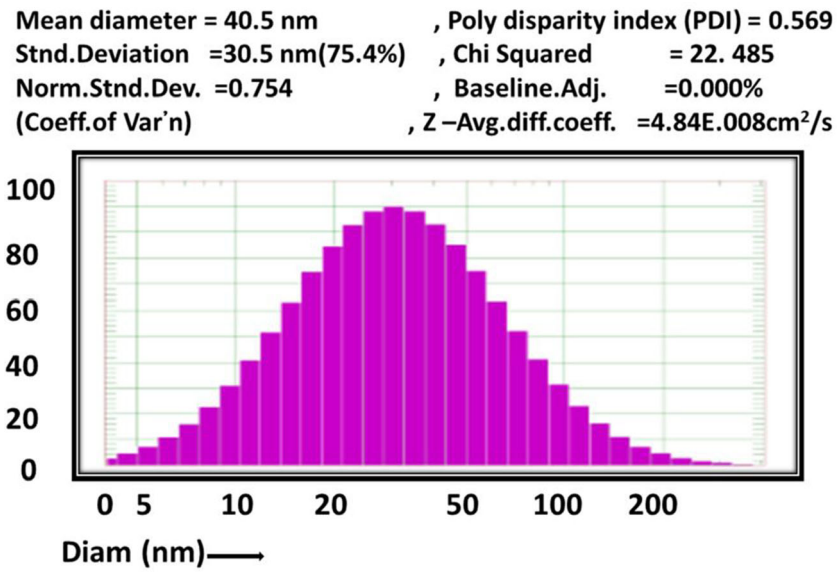

Fig. 3 DLS graph of synthesized AgNPs using *Borago officinalis* leaf extract

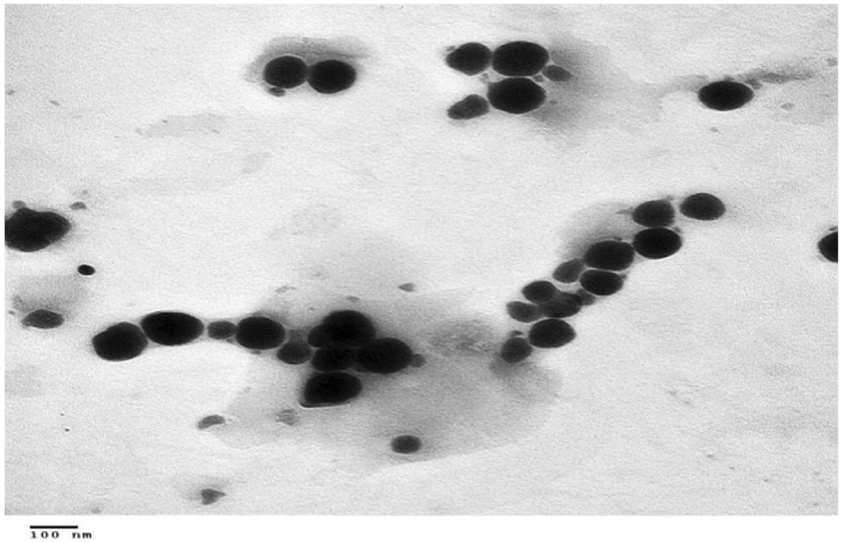

Fig. 4 Transmission electron microscopy (TEM) of green-synthesized silver nanoparticles using *Borago officinalis* leaf extract

Fourier Transform Infrared Spectroscopy (FTIR) analysis

FTIR spectrum of *B. officinalis* – Ag NPs was reported to identify the possible interactions between silver and the active ingredient involved in the synthesis of nanoparticles. The obtained spectrum investigated varying absorption peaks at 3671.63, 2978.77, 2110.44, 1794.10, 1413.21, 1097.97, 871.78, 612.60 and 564.23 cm^{-1} (Fig. 5). The band vibrations shown at 3671.63, 2978.77, and 2110.44 cm^{-1} correspond to the secondary amines bonds and the phenolic hydroxyl groups which are involved in the formation of Ag NPs by reduction of Ag^{+} to Ag^{0}. The peak at 1794.10 cm^{-1} is due to C = O stretching vibrations of the carbonyl group, while 1413 cm^{-1} denoted the aromatic stretching of –C–N. The existence of aromatic ring or C = C is due to the presence of 1413 cm^{-1} band. Stretching mode C–O–C is observed at 1097.97 by an aromatic ether linkage. The band at 612.60 cm^{-1} is due to aromatic C-H stretch. Peaks at 871.78 and 564.23 cm^{-1} represent aromatic groups (C = H) and C ≡ C groups for alkene. Consequently, Ag NPs exhibit a strong binding harmony to different functional groups in the plant compounds, such as total phenolic, total flavonoids, total flavonols, total tannins, total anthocyanins and proteins; these functional groups form a layer coating Ag NPs. As a result, they essentially act as capping agent, providing stability and preventing agglomeration of Ag NPs.

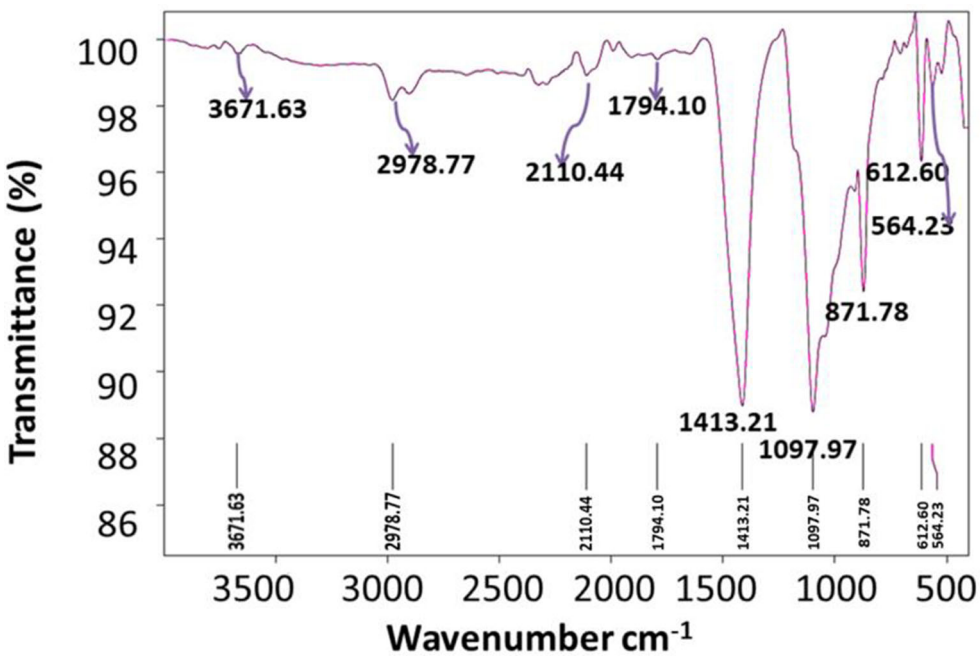

Fig. 5 FTIR spectra of synthesized silver nanoparticles using *Borago officinalis* leaf extract

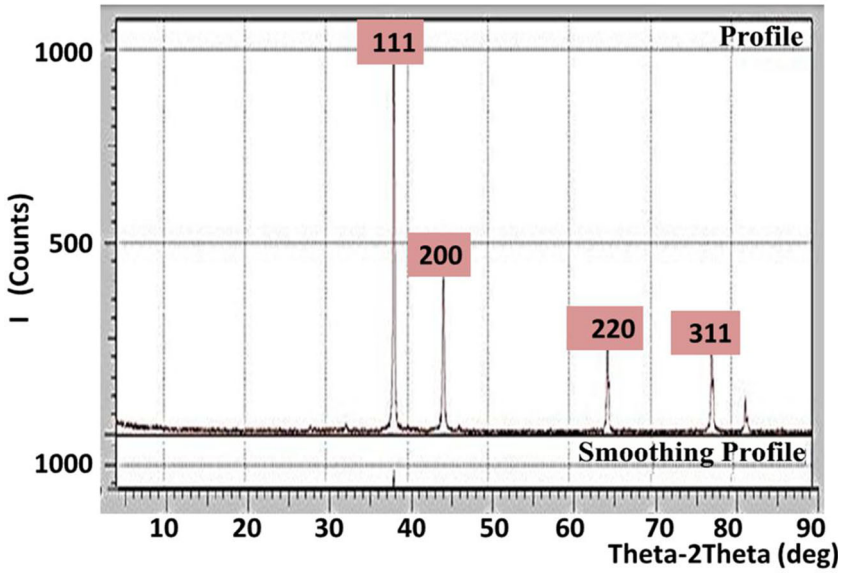

Fig. 6 X ray diffraction of Silver nanoparticles synthesized by *Borago officinalis* leaf extract

X-ray diffraction analysis

The X-ray diffraction (XRD) spectra of the silver nanoparticles showed four main characteristic Bragg's diffraction peaks. The peaks were positioned at 2θ of 38.30º, 44.49º, 64.63º and 77.58º. These reflections corresponded to (111), (200), (220) and (311), respectively facets of the face centered cubic (fcc) structure of silver nanoparticles synthesized by aqueous extract of ***B. officinalis*** leaf extract. The diffraction peaks indicated that the synthesized nanoparticles were of pure crystalline nature (Fig. 6).

EDX analysis of Ag-NPs

The qualitative and quantitative elemental profile involved in the development of nanoparticles was determined using EDX analysis. EDX analysis exhibited higher counts at 3 keV due to silver, thus confirming the development of silver nanoparticles (Fig. 7).

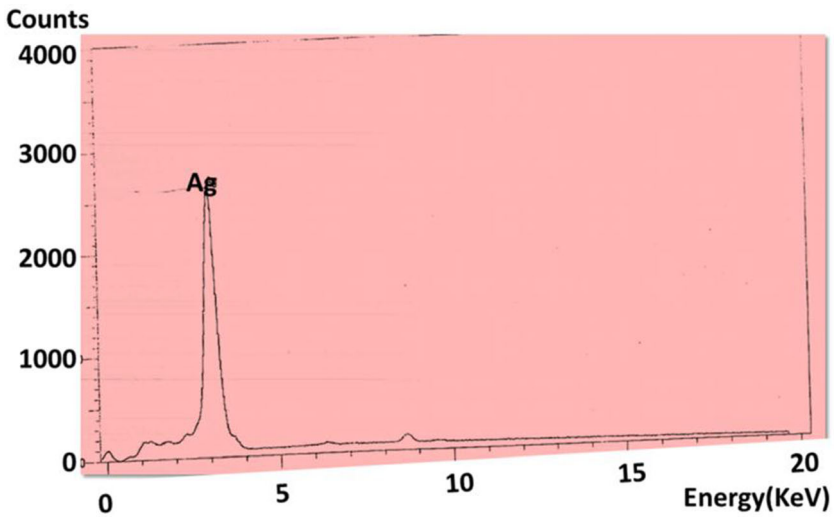

Fig. 7 Energy dispersive X-ray (EDX) profile of silver nanoparticles synthesized by *Borago officinalis* leaf extract

SEM analysis of silver nanoparticle

To study the surface morphology, scanning electron microscopy (SEM) was utilized. The contour of the observed Ag NPs was relatively spherical in nature as shown by SEM. The average size of Ag NPs synthesized from *B. officinalis* leaf extract was measured to be 45 nm (Fig. 8) .The nanoparticles were not in direct contact, even within the aggregation which indicating stabilization of the nanoparticles by the capping agent.

Larvicidal activities

Larvicidal activity of the plant extract and Synthesized Ag NPs

The larvicidal activity of *B. officinalis* leaf extract and *B. officinalis*–Ag NPs were investigated under laboratory conditions. They were evaluated against the 3rd instar larvae of S. *littoral*is after 24 h exposure. As shown in (Fig. 9), the

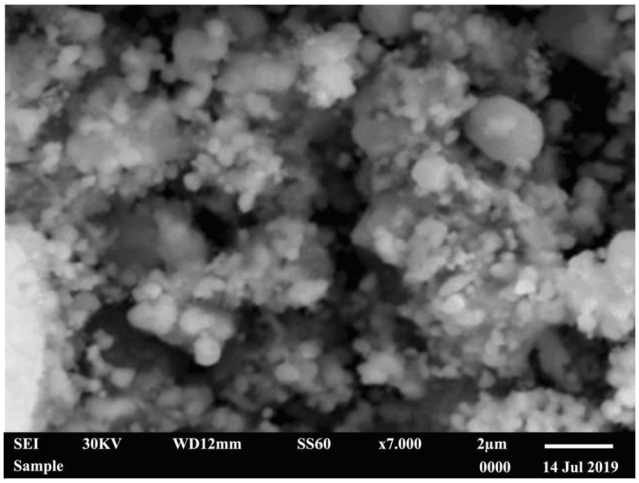

Fig. 8 Image of scanning electron microscopic observation of synthesized silver nanoparticles

B. officinalis leaf extract recorded 72% mortality at the concentration 100 mg/g, while, it recorded 46% mortality at 12.5 mg/g. Furthermore, in case of *B. officinalis*-Ag NPs, 100% mortality was recorded at 4.0 mg/g concentration whereas 0.1 mg/g caused 30% mortality at the end of larval period compared to 6% mortality in the control treatment (Fig. 10).

Data embody the LC_{50} and LC_{90} values of *B. officinalis* leaf extract and *B. officinalis* - Ag NPs Table 1. The LC_{50} and LC_{90} values calculated were 0.33 and 1.7 mg/g, respectively for the *B. officinalis* - Ag NPs as compared to 22.6 and 969.0 mg/g, respectively, for the *B. officinalis* leaf extract.

Latent effect of Ag NPs on *Spodoptera littoralis* development

(Fig. 11) expresses the graphical representation of Larval and pupal periods of *S. littoralis* treated with LC_{50} of the *B. officinalis* extract and *B. officinalis* - Ag NPs. The treatment caused a significant elongation in larval period (18.02 and 18.82 days), respectively compared with (15.78 day) in the control treatment, whereas there was no significant elongation in the pupal period in both males and females in case of the tested plant extract and its synthesized Ag NPs compared to the control treatment. As shown in (Fig. 12), both *B. officinalis* leaf extract and *B. officinalis* - Ag NPs caused a significant increase in pupal mortalities as it recorded (23 and 37.33%), respectively as compared to (2%) in the control treatment. From the view point of pupation, adult emergence and survival potential, a significant reduction in the pupation percentage caused by *B. officinalis* extract and *B. officinalis* - Ag NPs as it recorded (48 and 50%), respectively, compared to 98% in the control treatment. The percentage of adult emergence significantly decreased to 62.67% in case of *B. officinalis* - Ag NPs compared to 98% in the control, while, there is no significant difference between the *B. officinalis* extract 77% and the control group. Moreover, *B. officinalis* extract and *B. officinalis* - Ag NPs caused a significant reduction in the survival percentage as it recorded (36 and 32%), respectively, compared 92% in the control treatment (Fig. 13).

Discussion

Biosynthesis of nanoparticles using biological agents has been an important approach for the synthesis of different forms of nanoparticles like copper, iron, platinum, silver, and zinc, etc., Rasheed et al. (2017). Clarification the mechanism of plant-mediated synthesis of nanoparticles is a very promising area of research (Kumar and Yadav 2009). In the present study, the formation of silver nanoparticles using *B. officinalis* leaf extract was confirmed by various advanced techniques. The addition of the *B. officinalis* leaf extract to the silver nitrate solution produces a color change of the reaction mixture from pale yellow to reddish-brown due to reduction of silver ion; which indicated the formation of silver nanoparticles. Similarly, Krishnaraj et al. (2010) reported that the fabricated silver nanoparticles exhibit yellowish brown color in aqueous solution due to the excitation of surface Plasmon vibrations in silver nanoparticles. The maximum absorption peak of *B. officinalis* –Ag NPs was found at 420 nm, which indicates the fabrication of Ag NPs. Nakkala et al. (2014), reported a comparable UV-visible spectral profile with silver

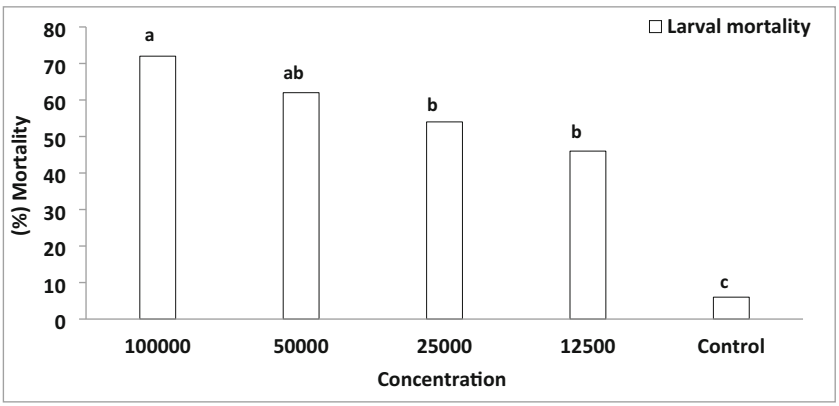

Fig. 9 Effect of *borago officinalis* leaf extract on cumulative mortality (%) of *Spodoptera littoralis* larvae

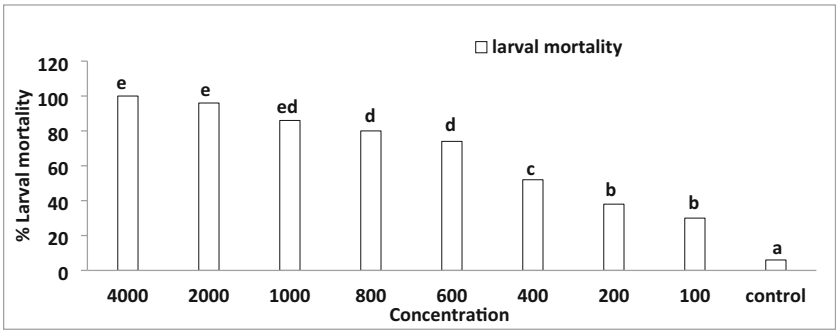

Fig. 10 Effect of synthesized silver nanoparticles using *borago officinalis* leaf extracts on cumulative mortality (%) of *Spodoptera littoralis* larvae

nanoparticles developed from *Acorous calamus* rhizome extract displaying a broader peak around 400 nm. Similarly, several other reports demonstrated that the absorption peak of the silver nanoparticles appears to be around this region (Zahir and Rahuman 2012; Thatoi et al. 2016). The concentration of silver nitrate and pH variations were investigated to optimize the synthesis of *B. officinalis* –Ag NPs .The absorption peak intensity of silver nanoparticles became distinct with increasing the concentration of $AgNO_3$ (0.5, 1, 3 mM) with no change in the wavelength, the maximum peak intensity was obtained at 1 mM of $AgNO_3$ .The reason for the decrease in absorption peak intensity with increasing $AgNO_3$ concentration may be because $AgNO_3$ forms a coat on growing particles (Gurunathan et al. 2009). Additionally, the absorption intensity was increased when the pH value increased and pH 11 is the most favorable pH for the synthesis of Ag NPs using *B. officinalis* leaf extract. These results may be due to the effects of pH on the dissociation, agglomeration, isolation, interfacial free energy and net charge of a complexing agent. In case of acidic medium, the accumulation of silver nanoparticles to form larger nanoparticles was supposed to be preferred rather than to nucleate and develop new particles. At an elevated pH, a great number of Ag NPs with smaller sizes are generated (Iravani and Zolfaghari 2013). Mock et al. (2002) cleared that the pH is an important factor in the formation of colloidal nanoparticles and deliberated that the size and the shape of the biosynthesized nanoparticles fluctuate with the pH variations. Moreover, Veerasamy et al. (2011) during working on mangosteen leaves extract found that elevated pH expedites the nucleation and subsequent synthesis of a greater number of nanoparticles with a reduced diameter. However, at acidic pH, silver nanoparticles preferably tend to aggregate than that to nucleate. The size of the *B. officinalis* -AgNPs from plant extract was determined to be 40.50 nm using DLS analysis. Further, TEM analysis helped to determine the surface properties and size of the *B. officinalis* –Ag NPs .Observations reveal nanoparticles of spherical shape having a diameter of 20 to 60 nm with average size 40 nm. These results are consistent with the shape of the SPR peak in the UV/VIS spectra. These findings are supported by similar reports in recently published studies (Wang et al. 2018). According to the FTIR spectral analysis, it can be expected that the characteristic functional groups expressly phenols, alkenes, aromatic amines and carbonyl groups from *B. officinalis* leaf extract carried out the reduction, stabilization and capping of *B. officinalis* –Ag NPs. *B. officinalis* leaf extract has been observed to contain various biologically active phytoconstituents including phenols, tannins, flavonoids, α-terpineol, flavones, terpenes fenchone, 4-terpinenol and polysaccharides (Bora and Sharma 2011) and this supports our FTIR results. The Observations from XRD analysis of *B. officinalis* –Ag NPs peak values at 38.30º, 44.49º, 64.63º and 77.58º conforming (111), (200), (220), and (311) lattice structures are indicating the presence of face-centered cubic structure of Ag NPs. This result was in conformity with many studies reporting the crystalline cubic nature of biosynthesized Ag NPs (Krishna et al. 2016 and Balaji et al. 2009).The compositional elemental profile was determined using EDX analysis of numerous distinct particles. The EDX analysis of *B. officinalis* -AgNPs demonstrated higher counts at 3 keV due to silver, thus confirming the development of silver nanoparticles. This 3 keV peak has been similar to published in other reports (Fouad et al. 2018 and Kalimuthu et al. 2017). SEM image intended that the synthesized *B. officinalis* –Ag

Table 1 Toxicity of *Borago officinalis* leaf extract and the synthesized silver nanoparticles against the 3rd instar larvae of *Spodoptera littoralis*

Extract	$^{LC}50$ ^{Value} (mg/g)	$^{LC}90$ ^{Value} (mg/g)	95% Confidential limit		Chi-square $\chi 2$	Slope
			LCL	UCL		
Leaf	22.6	969.0	1279.8	9386.8	0.4960	0.7853
AgNps	0.33	1.7	3.1	9.2	42.9735	1.7765

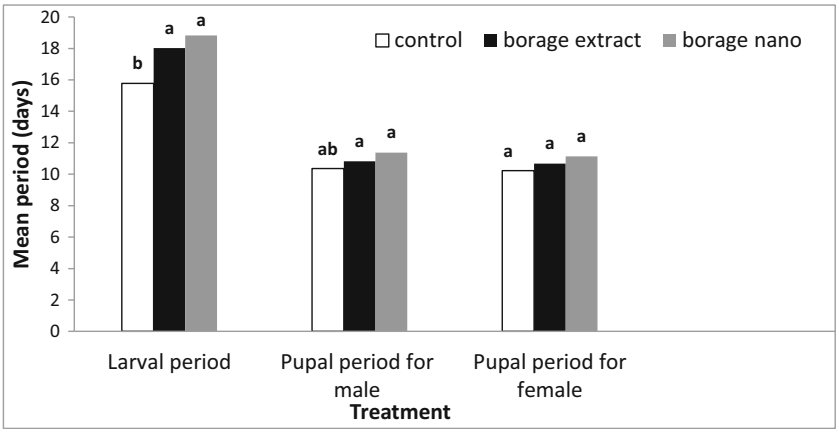

Fig. 11 Effect of LC_{50} of synthesized silver nanoparticles using *Borago officinalis* leaf extract on the developmental periods of *Spodoptera littoralis*

NPs were spherical in shape with an average size of 45 nm which proved the natural character of silver (Basu et al. 2016, Ga'al et al. 2018 and Shameli et al. 2012).

The toxicity of the *B. officinalis* leaf extract and the synthesized Ag NPs against the 3rd instar larvae of *S .littoralis* was increased by increasing the concentration and the time of exposure. The toxic effect of the plant extract may be due to the secondary metabolites present in this plant (Isman 2006 and Shekari et al. 2008). Additionally, it is obvious from these results that the synthesized silver nanoparticles showed effective entomotoxic potential than *B. officinalis* leaf extract against *S. littoralis* larvae where the LC_{50} for the synthesized Ag NPs was 0.33 mg/g compared with 22.6 mg/g at the *B. officinalis* leaf extract. These findings are contradicted with Vani and Brindhaa, (2013) who reported that nanoparticles are more reactive than their bulk counterpart because of their increased surface to volume ratio. These larvicidal activities might be due to the denaturation of the sulfur-containing proteins or phosphorous containing compound like DNA that, leads to the denaturation of organelles and enzymes (Choi et al. 2008). Furthermore, the small size of Ag NPs, facilitate the easily penetration across the insect cuticle and even into individual cells of the digestive tract, where they interfere with physiological processes (Suresh et al. 2018). The toxicity of Ag NPs against insects comparing with aqueous plant extracts was observed by many investigators as (Abduz Zahir et al. 2012) who found that synthesized Ag NPs using *Euphorbia prostrata* were more effective than the aqueous extract and $AgNO_3$ solution against *Sitophilus oryzae.* Also, (Sujitha et al. 2015) and (Murugan et al. 2016) reported that the *M. oleifera* seed extract -synthesized Ag NPs is toxic than *M. oleifera* seed extract against larvae and pupae of *Ae. aegypti* and *Cx. quinquefasciatus*. On contrary, (Yasur and Pathipati 2015), found that the PVP coated Ag NPs did not cause any mortality but had disrupted the other metabolic enzymes and their activities and consequently lead to death.

In the present study, the tested plant extract and its synthesized Ag NPs significantly elongated the larval period of *S. littoralis*, whereas, the pupal period of both male and female not affected compared with the control group. Our results are coincident with (Ibrahim and Ali 2018) who mentioned that Silver nanoparticles caused a significant increase in the larval period (17.2 days) than control (16.8) treatments. Moreover, Panacek et al. (2011) stated that the developmental time of *Drosophila melanogaster* was prolonged from 14 days to 16 days compared to that found for the control sample at a concentration of 40 mg /Lof silver nanoparticles. Silver concentrations equal to 60, 80, and 100 mg/ L significantly

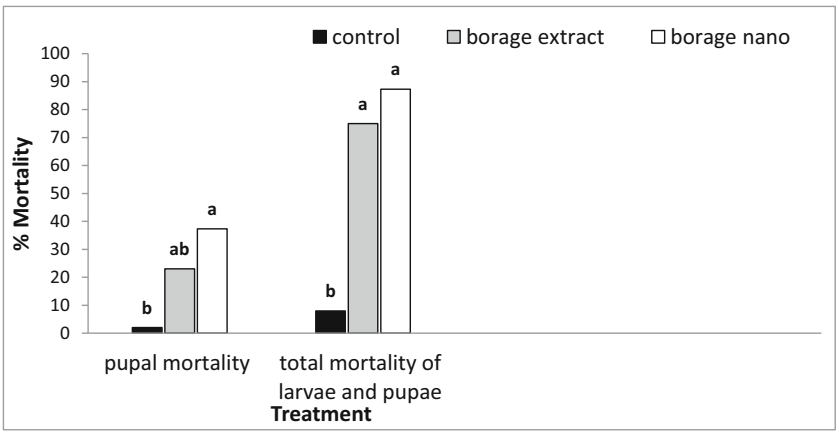

Fig. 12 Effect of LC_{50} of synthesized silver nanoparticles using *Borago officinalis* leaf extract on the pupal mortality of *Spodoptera littoralis*

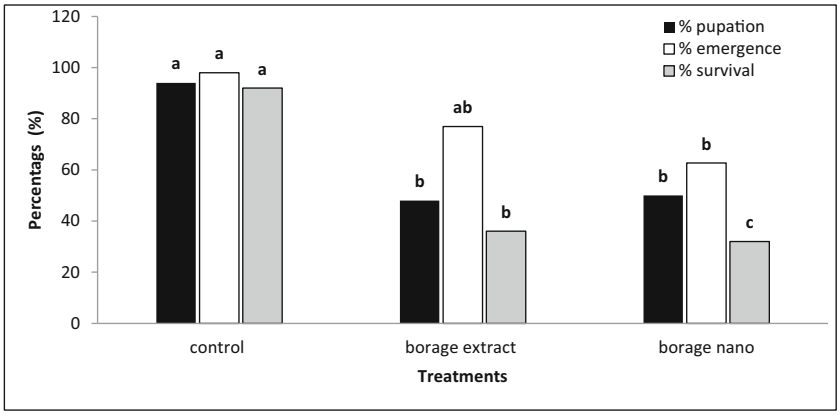

Fig. 13 Effect of LC_{50} of synthesized silver nanoparticles using *Borago officinalis* leaf extract on the percentage of pupation, emergence and survival of *Spodoptera littoralis*

influenced developmental stages in the phase of larvae development Meanwhile, Araj et al., (2015) indicated that none of the tested nanoparticles, silver nanoparticles (Ag NPs) and sulfur nanoparticles (SNPs) applied against the fruit fly *Drosophila melanogaster* has a significant effect on pupal longevity.

The obtained results demonstrated that both Ag NPs and the tested plant extract caused a significant increase in pupal mortalities. These results are in contact with that obtained by Meng et al., (2017) who stated that higher concentrations of silver nanoparticles caused larval and pupal mortality. Moreover, Afrasiabi et al., (2016) ; Mao et al., (2018) mentioned that silver nanoparticles caused developmental alteration in Drosophila melanogaster, *Heliothis virescens* and *Trichoplusia ni* larvae in the form of reduced or retarded pupation rate. Data also revealed that the percentage of pupation, emergence and survival of *S. littoralis* was reduced in a significant manner after treatment with LC_{50} of both *B. officinalis* leaf extract and the synthesized Ag NPs. These findings were similar to those obtained by Panacek et al., (2011) who reported that the application of a concentration 20 mg / L silver nanoparticles against the fruit fly *Drosophila melanogaster* led to 50% of the tested flies were unable to leave the pupae, and they did not finish their developmental cycle, while the concentrations 60, 80, and 100 mg / L induced a strong toxic effect leading to a significant decrease in the number pupae. Drosophila development cycle was not finished in such high silver concentrations and the individuals were unable to leave the pupae cases. At 100 mg /L there were no pupae formed. Also, Kantrao et al.., (2017) reported that feeding the third instar Larvae of *Helicoverpa armigera* on artificial diet containing *Ficus benghalensis* - Ag NPs, *Ficus religiosa - Ag NPs* (6.6 µl/g diet) and permethrin (100 µM/g diet) inhibited larval growth and survival rate compared with control larvae. Comparatively, the survival rate was significantly reduced in a dose-dependent manner with increasing concentrations of Ag NPs. Moreover, (Dass and Mariappan 2018) denoted that the green synthesized silver nano-particles (Ag NPs) using *Coleus aromatics* and *Wrightia tinctoria* leaf extract induced a significant reduction in adult emergence of *Culex quinquefasciatus*. Though there is a reduction in the rate of adult emergence as a function of concentration, there is no significant difference among the two plants silver nanoparticles. Raj et al., (2017) mentioned that ingestion of Ag NPs in *Drosophila* during larval stage causes a significant reduction in the percentage of larvae reaching adulthood (survival) and the percentage of adult emergence in a dose dependent manner. Our results suggest that the synthesized Ag NPs more effective for controlling S. *littoralis* than the *B. officinalis* leaf extract and also more consistent to protect the environment than the chemical pesticides.

Conclusion

In the present study, we focused on green synthesis of silver nanoparticles using *Borago officinalis* leave extract as reducing and capping/stabilizing agents. The physical property of synthesized silver nanoparticle was characterized using relevant techniques. *B. officinalis* - Ag NPs were mostly spherical in shape, crystalline in nature with face-centred cubic geometry and their mean size ranged between 20 and 60 nm. Further we demonstrated the possible application of *B. officinalis* - Ag NPs as a substitute of insecticide to show *Spodoptera* larvicidal activity. The fabricated Ag NPs showed significant larvicidal activity against *spodoptera littoralis* larvae even at low doses. The maximum mortality (100%) was produced at a concentration of 4.0 mg/g while the minimum mortality (30%) was produced at a concentration of 0.1 mg/g. Therefore, the *B. officinalis* - Ag NPs can be an important component of an integrated pest management programme against *S. littoralis*.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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