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ORIGINAL RESEARCH ARTICLE

Larvicidal and biochechemical effects of methanol leaf extract and biosynthesized silver nanoparticles using *Nerium oleander* L. (Apocynaceae) against *Lucilia sericata* M. (Diptera: Calliphoridae)

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Abstract
Silver nanoparticles 'Ag NPs' appear to be a good choice to manage insect pests owing to their unique features. In addition, green-synthesized silver nanoparticles utilizing plant extracts have vital uses in the area of biology notably in pest control owing to their safety. This paper aims to explore the larvicidal activity of 'AgNPs' generated by the green chemistry procedure from *Nerium oleander* leaf extract and analyzed their effects on haemolymph proteins of *Lucilia sericata*. Phytochemical research was undertaken to identify the phytochemicals responsible for reduction and capping of green produced 'AgNPs'. Silver nanoparticles were effectively manufactured utilizing *N. oleander* leaf extract and silver nitrate, the aqueous plant extract function as a reducing as well as a stabilizing agent of 'AgNPs'. The produced 'AgNPs' were studied by UV–Vis spectra, Transmission Electron Microscope (TEM), Fourier Transforms Infra-Red (FT-IR) spectroscopy and X-ray diffraction (XRD) (XRD). Third instar larvae of *L. sericata* subjected to varied doses of *N. oleander* leaf extract and green-synthesized silver nanoparticles. GC–MS study indicated that crude leaf extract include 17 chemicals belong to the phytochemical classes; phenol, Sesquiterpen, sterol, ester, and heterocyclic compounds. UV–Vis spectra indicated a surface resonance peak at 430 nm corresponding to the production of 'AgNPs' and FTIR spectra demonstrated that the synthesized 'AgNPs' are capped with biomolecules which are responsible for reduction of silver ions. 'AgNPs' are spherical in form, the particle size varied from 4 to 32 nm and the average crystalline size was 27 nm. 100 percent mortality was reported in *N. oleander*-AgNPs treated larvae at 50 ppm ($LC_{50}=21$ ppm) while crude extract exhibited 80 percent mortality at 500 ppm ($LC_{50}=389$ ppm). Also, the examined substances substantially ($p<0.05$) lowered the overall protein contents of treated larvae and generated differences in the number of heamolymph protein bands with varied molecular weights as compared to control. The effectiveness in killing *L. sericata* larvae and stability of *N. oleander*-AgNPs has made this product a suitable candidate for the creation of a new biopesticide.

Keywords *Lucilia sericata* · *Nerium oleander* · GC–MS · AgNPs · Larvicidal · Protein profile

Introduction

One of the most widely employed ectoparasites for necrotic wounds is *Lucilia sericata* (Meigen), also known as the green bottle fly or the sheep blowfly (Nezakati et al. 2020). However, it may lead to myiasis in humans and animals, particularly sheep (Siwar et al. 2021) and (Mohamed et al. 2016). Itching, inflammation, erythema, eosinophilia, and sometimes secondary bacterial infections may result from myiasis in humans and animals. The larval diptera of the order diptera feed on living tissues of the host, usually at or near their natural orifices (Khater and Khater 2009; Elsheikha 2018; Akkol et al. 2020). (Demirel-Kaya et al. 2016). Attracting more pregnant females to the skin surface may result in significant cutaneous lesions, debilitation, and eventual death if left untreated (Broughan and Wall 2006), due to the long-term effects of chronic ammonia poisoning (Broughan and Wall 2006). (Khater and Khater 2009). *Mycobacterium avium* may be transmitted by *L. sericata* larvae and adults, in addition to the direct damage produced by eating (Fischer et al. 2004; Khater et al. 2011; Khater and Geden 2018). As a result, the regulation of the blow flies is

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critical. L. sericata is often treated with chemical pesticides. Insecticides may pose health and environmental risks, as well as the development of resistance (Naqqash et al. 2016; Abd El-Hamid et al. 2018). As a result, there is a pressing need for an environmentally sound alternative.

As a result of their vast range of uses, silver nanoparticles have recently garnered a lot of interest (Erick et al. 2017). It is possible that plant extracts could be more environmentally friendly than biological processes for the synthesis of nanoparticles. (Subbaiya et al. 2014; Govindarajan et al. 2016; Gul et al. 2016; Bharathi and Shanthi 2017; Banumathi et al. 2018; Ga'al et al. 2018; Nalini et al. 2019; Parthiban et al. 2019; Pilaquinga et al. 2019; Suleiman 2020; Kitherian et al. 2021). Controlling *L. sericata* using plant extracts has been demonstrated to be successful (El-Khateeb et al. 2003; Khater and Khater 2009; Khater et al. 2011; Mohamed et al. 2016; Khater and Geden 2018). The Apocynaceae family includes the oleander, a beautiful toxic plant. Scabies, ringworm, dermatitis, abscesses, eczema, and sores are just a few of the conditions for which *N. oleander* has been used to treat (Santhi et al. 2011). *N. oleander* extracts of different plant parts and its silver nanoparticles have been shown to be effective against a broad variety of pests, according to several studies (Roni et al. 2013; El-Akhal et al. 2015; Behravan et al. 2017; Semiz 2017; Raveen et al. 2017; El-Monairy et al. 2020; Zaid et al. 2021). The objective of this investigation is to introduce an ecofriendly method for myiasis control by providing new insight on the activity of 'AgNPs' of *N. oleander* compared to the leaf extract against the 3rd instar larvae of *L. sericata* under laboratory conditions. In addition, haemolymph protein content was determined as a measure of their activity on the defense components.

Materials and Method

Plant specimens collection

Plant nurseries in Benha District (300 27′ 38" north, 310 11′ 15" east) in the Qalyubiyya Governorate, Egypt, provided the fresh leaves used in this study (Fig. 1). Benha University at Benha, Egypt's Department of Botany, Faculty of Science, verified the classification of plants.

Preparation of crude extract

Delicately cleaned and air-dried for seven days at room temperature, then manually ground into powder using an electric blender, the leaves were then ready to be used. A Soxhlet extraction device was used to extract 100 g of finely powdered leaves (500 ml methanol) for 48 h (Freedman et al. 1979). Using a Labo-Rota C311 rotary evaporator (40 oC water bath) for 2–3 h, the extract was concentrated. It was then weighed and stored in screw-cap vials at -4 oC for later investigation.

Fig. 1 *Nerium oleander* (whole plant)

Gas Chromatography/ Mass Spectrometry 'GC–MS'

A Shimadzu GC–MS-QP 2015 plus system (Kyoto, Japan) (Fig. 2) compressed a Hewlett Packard chromatograph model 5970 equipped with a flame ionisation detector (FID) and HP capillary column (50 m 0.2 mm) coated with DB-5 bonded phase (0.25 m film thickness) to analyse the main components of *N. oleander* leaf extract. The detector and injector were set at 250 °C and 200 °C, respectively, for optimal performance. At 60 °C, the oven temperature was first set to 200 °C at 3 °C/min, then kept at that temperature for 25 min. Carrier gas helium was employed at a flow rate

Fig. 2 UV- 3600 Shimadzu spectrophotometer

of one millilitre per second (mL/s). Drizzled one microliter of 1 percent (v/v) sample into the split mode in the ratio of 15:1. The GC peak area was used to estimate the extract composition %. 280 °C interface, 200 °C ion source, 70 eV EI mode, 35–500 Da scan range were the MS operating conditions. The National Institute of Standards and Technology's database was used to compare the retention times and mass spectra of the compounds (NIST).

N. oleander silver nanoparticles synthesis and characterization

According to Jafer and Annon (2018), the 'AgNPs' synthesis method was followed. *N. oleander* leaf extract was used to effectively produce silver nanoparticles. The production of 'AgNPs' was indicated by a shift in colour from green to dark brown. Researchers may get insight into the formation and stability of "AgNPs" through the UV–Vis spectroscopy study. By using a UV–Vis spectrophotometer (Shimadzu UV-3600PC Series) with a wavelength range of 200–700 nm, the 'AgNPs' were proven to be biologically produced. The shape and size of the produced 'Ag NPs' were studied using high-resolution transmission electron microscopy JEOL (JEM-2100 TEM). For studying the 'AgNPs' constituent capping and reducing agents, an FT-IR spectrum was recorded using an FT-IR spectrophotometer (8300 FT-IR Shimadzu, Japan). Shimadzu Maxima-X-7000 XRD instrument, Kyoto, Japan (40 kV, 30 mA current, Cu K radiation, 0.15418 nm wavelength) was used for the experiment. Diffraction patterns were recorded in continuous mode with a step size of 0.02 throughout a 2 range of 5–40.

Scherrer's equation was used to determine the crystalline size of Ag nanoparticles.

$$D = K\lambda/\beta\cos\theta$$

Constant (0.94) K: X-ray wavelength,: breadth at half maximum of (111) reflection at 2θ, D: average crystallite size, θ: Angle of diffraction.

Obtaining and Maintaining *L. sericata* specimens

Using fly netting, we gathered adults of *L. sericata* M. in Egypt's Qalyubiyya Governorate's Mitt Kenana Village and Toukh Center. An open area was baited with beef to catch flying insects. Flies were taken to the Medical Entomology Insectary, part of the Entomology Department of Benha University's Faculty of Science. Flies were raised in a lab under carefully monitored circumstances (27 °C 2 °C, 70% 5% relative humidity, and 12 h of light followed by 12 h of darkness). Milk powder and cotton pads soaked in 10% sucrose solution were fed to larvae, while the adults were given milk powder and sucrose-soaked cotton pads (Khater and Geden 2018).

Assays of larvae

Larvicidal activities of methanol leaf extract and biosynthesized 'AgNPs' of *N. oleander* were evaluated against *L. sericata* according to Khater and Geden (2018). *L. sericata* third-instar larvae were exposed to five concentrations of the examined materials: 100, 200, 300, 400 and 500 ppm for crude leaf extract and 10, 20, 30 40 and 50 ppm for 'AgNPs'. The procedures for the ingestion experiments were carried out as follows: For each concentration and for the control group, the procedures were repeated five times. Each replicate was made up of twenty larvae (100 larvae for each concentration). One ml of the test materials was added to 100 cm 3 plastic cups, which contained 25 g liver (Fig. 3). One millilitre of distilled water was added to the samples in the control groups. A rubber band was used to secure gauze around the plastic cups. Under these circumstances, all trials were conducted (27 2 ^{0}C, 70% RH, and 12:12 h light: dark cycle). Every day until pupation, the mortality rate of the larvae was recorded.

Biochemical assays

The LC_{50} of each investigated substance was applied to the larval medium in order to determine the most toxic concentration. *L. sericata* larvae of the early third instar (100 larvae) were transferred from the rearing medium to each concentration. Samples were taken for biochemical testing after two days of therapy.

Collection of haemolymph

The larvae were refrigerated for 15 min at 4 ^{0}C prior to collecting the haemolymph. The larval abdomen was punctured with a sterile needle to extract the haemolymph. Eppendorf tubes were immediately used to store the haemolymph that had leaked out of the patient's body. Phenylthiourea crystals were placed in the tubes to avoid melanization. For hemocyte-free haemolymph, samples of haemolymph were centrifuged for 10 min at 20.000 g at 4 ^{0}C and the supernatant was kept at -18 ^{0}C until needed (Mahmoud et al. 2020).

Determination of haemolymph proteins

According to Bradford (1976), Coomassie Brilliant Blue G-250 was employed to measure the total protein content of the larval haemolymph. 5 ml of protein reagent was combined with 0.1 ml of diluted haemolymph sample. As a reference, we utilised bovine serum albumin. Absorption was measured at 595 nm.

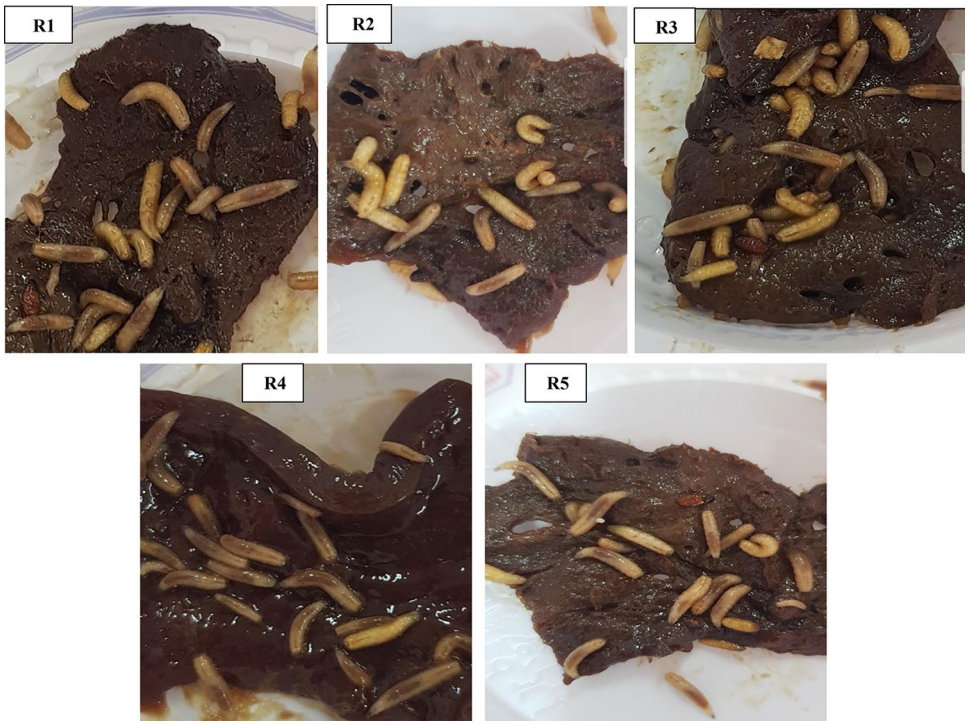


Fig. 3 Bioassay images**, R; replicate**

Analysis of haemolymph protein of larvae of *L. sericata* by electrophoresis

Treatment buffer (0.125 mol/L tris–HCl pH 6.8, SDS 4%, 2-mercaptoethanol 10%, glycerol 20%) was used to dilute ten microliters of haemolymph, which was then heated to 95 degrees for one minute in a water bath before being cooled on ice until use. The denatured haemolymph was then examined using Omni PAGE vertical protein electrophoresis system SDS–polyacrylamide gel slabs. According to Mahmoud et al. (2020), the electrophoresis conditions and methods were followed. The molecular weights (MWs) of the separated protein bands were determined using a mixture of protein standards (markers) ranging from 212 to 7 kDa (Sigma) that had been dissolved in distilled water, diluted in the treatment buffer, and treated in the same way as the samples. For 60 min, electrophoresis was performed at room temperature using a 90 V supply. For two hours, the resolving gel was stained with Coomassie blue R-250 after it had been fixed in fixing solution. Destaining was accomplished using a 45 percent methanol/10 percent acetic acid solution. Gel Doc VILBER LOURMAT was used to scan, photograph, and analyse the gel.

Analytical Methods

ANOVA was used to compare the means of the five different chemical concentrations and the control group means statistically. Means were separated using Duncan's test ($p < 0.05$). LC50 values and 95 percent confidence limits were calculated by probit analysis using the computer application PASW Statistics 2009, as specified by Finney (1971). (SPSS version 22). Mean standard deviation was used to represent biochemical data (SD). The student "t-test" for paired observations was used to examine the statistical significance of differences in mean values. If you're looking for minor, significant, or extremely significant levels of importance, you'll find them here. The following equation was used to determine change percentages:

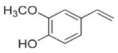
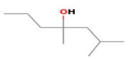
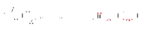
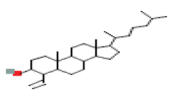
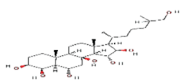
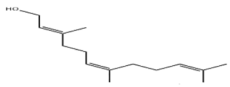
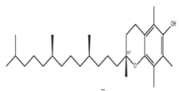
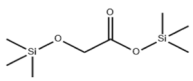
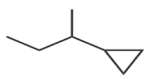
$$[(\text{test} - \text{control})/\text{control}] * 100 \text{ is the change percentage}$$

Results

GC–MS study of *N. oleander* extract to characterise its chemical composition

GC–MS was used to examine the phytochemical components in a methanol leaf extract of *N. oleander* in this investigation. There were a total of 17 chemical compounds found in the leaf crude extract (Table 1 and Fig. 4). The major compounds were Tert-Butyldimethyl-Silyl 14-acetoxy-3,6,9,12 tetraoxatetradecan-1-oate (24.56 percent), 4-Heptanol, 2,4 dimethyl (13.59 percent), Thiophene,2 butyltetrahydro (11.498%), Methacrylic acid hexadecyl ester (10.8%) and Bicyclo 3,1,1 heptane, (2.6.6)

Table 1 Chemical characterization of *N. oleander* leaf extract through GC/MS analysis

S. No	Compounds	R Time	Peak Area %	M Weight	M. Formula	Nature	Structure
1	Thiophene,2 butyltetrahydro	8.56	11.498	102.2	$C_5H_{16}S$	Phenyl propanoid	
2	2-Methyoxy-4-vinyl phenol	10.59	0.7678	105.17	$C_9H_{10}O_2$	Phenol	
3	M-Iso propylaniline	10.69	0.5814	135.21	$C_9H_{13}N$	Hydrocarbon	
4	Bicyclo-3,1,1-heptane (2.6.6) trimethyl,1 alpha, 2 beta, 5 alpha	11.61	7.046	138.25	$C_{10}H_{11}$	Hydrocarbon	
5	Bicyclo 3,1,1 heptane, (2.6.6) trimethyl-1 alpha, 2 beta, 5 alpha	11.839	8.458	138.25	$C_{10}H_{11}$	Hydrocarbon	
6	Tert-ButyldimethylSilyl 14- acetoxy-3,6,9,12 tetra oxatetradecan-1-oate	12.64	24.56	144.0	$C_{12}H_{32}N_2Si_2$	Sterol	
7	4-Heptanol, 2,4 dimethyl	12.72	13.59	144.25	$C_9H_{20}O$	Sterol	
8	Tetrahydropyran-2-yl 12,28,10 dodecadienoate	12.92	2.37	276.43	$C_{17}H_3O_3$	Sterol	
9	Methacrylic acid hexadecyl ester	13.01	10.80	310.52	$C_{20}H_{38}O_2$	Ester	
S. No	**Name of the compound**	**R Time**	**Peak Area %**	**M Weight**	**M. Formula**	**Nature**	**Structure**
10	17-Dimethyl Hexyl 10, 13-dimethyl 4-vinyl hexadec-ahydrocyclopenta a-phenanthren,3-0 1	13.75	5.16	328.28	$C_{10}H_{22}O$	Phenol	
11	5-alpha Cholestane, 6-alpha, 7-alpha, 8-beta, 15-alpha, 16-beta, 26-heptaol	13.86	2.29	384.664	$C_{27}H_{48}O_7$	Phenol	
12	2,6,10Dodecatrien-ol, 3,7,11 trimethyl	15.74	0.644	344.5	$C_{11}H_{32}O_3$	Phenol	
13	Vitamin E	17.86	3.42	430.71	$C_{29}H_{50}O_2$	Sesquiterpen hydrocarbon	
14	Trimethyl silyl trimethyl Siloxyacetate	19.9	1.964	438.18	$C_8H_{20}O_3Si_3$	Heterocyclic compound	
15	Butane 2-cyclopropyl	20.9084	3.810	98.19	C_7H_{14}	Hydrocarbon	
16	Cedradiol	21.11	1.8632	238.37	$C_{15}H_{26}O_2$	Phenol	
17	2.2 Dimethyl propanoic acid, 2, 6 dimethyl 1-en-3yn-5-yl- ester	23.07	1.14	294.47	$C_{16}H_{26}O_2$	Ester	
	Total Peak Area (%)		99.9624				

trimethyl-1 alpha, 2 beta, 5 alpha (8.458 percent). This category of substances includes phenol, sterol, sesquiterpen, ester and heterocyclic compounds; they are all phytochemicals. For 40.52 percent of the total, sterol predominates, followed by phenol (22.222 percent).

Silver nanoparticles were synthesised and characterised

Ag ions are reduced to 'AgNPs,' as seen by the colour shift from green to dark brown over time (Fig. 5). The absorbance peak of

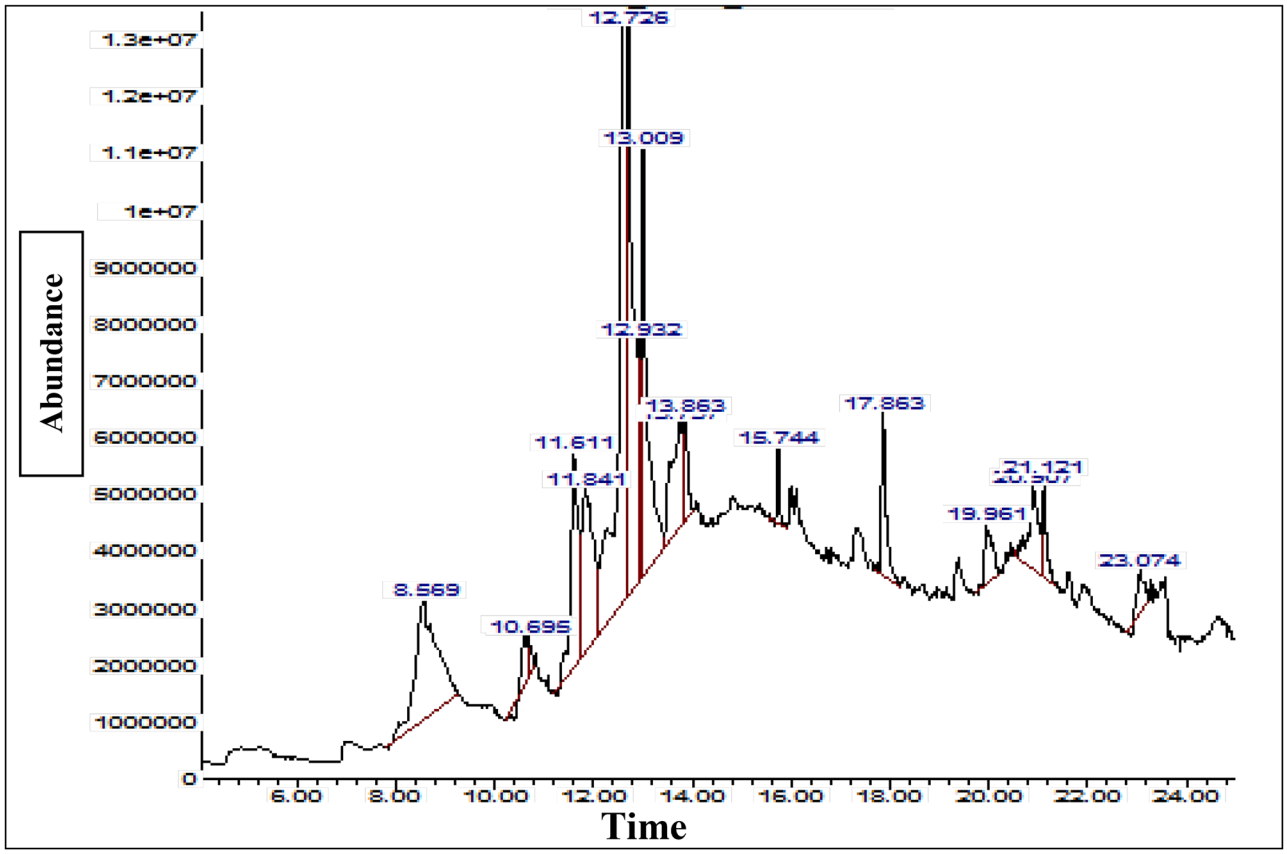


Fig. 4 GC–MS chromatogram of *N. oleander* leaf extract

430 nm was seen in the UV–Vis spectra of *N. oleander*-AgNP samples (Fig. 6). According to TEM analysis, the biosynthesized 'AgNP' particles have spherical shape with average diameter of 4–32 nm and exhibit little to no aggregation (Fig. 7).

N. oleander leaf extract was used to generate "AgNPs," which were then analysed using an FT-IR spectrophotometer (Fig. 8). The results indicate many distinct transmittance peaks. There was an O–H/N–H stretching peak at 3400 cm^1, a terpenoid characteristic C-H stretching peak at 2850 cm^1, an N–H (bend) amide peak at 1603 cm^1, and a phenol stretching band at 1288 cm^1 that revealed the existence of C = O stretching. There are peaks at 1078 cm^1 and 519 cm^1 that correspond to C–H bend alkenes in alcohols, carboxylic acid, and ester.

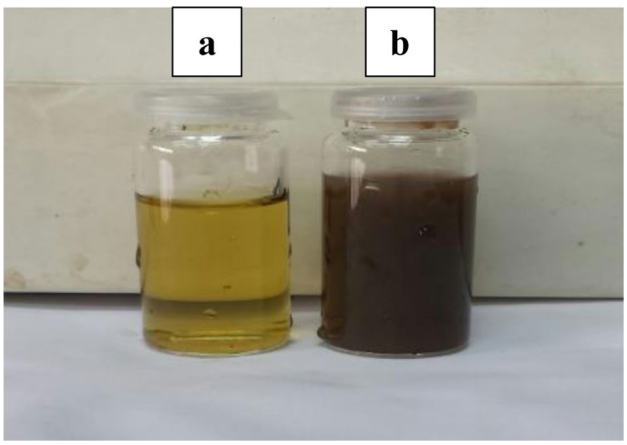


Fig. 5 Preparation of *N. oleander*-AgNPs **a**; crude leaf extract, **b**; AgNPs

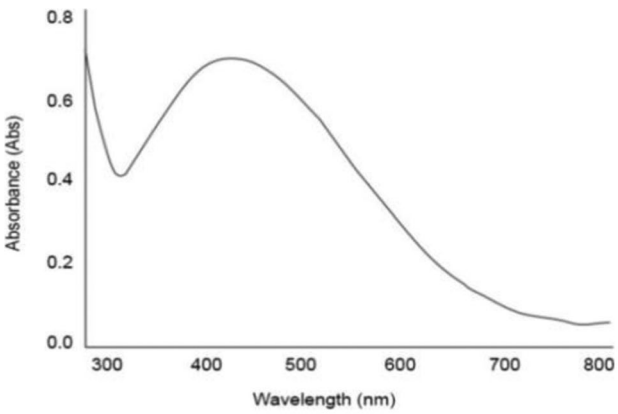


Fig. 6 UV–Vis spectroscopy of *N. oleander*-AgNPs

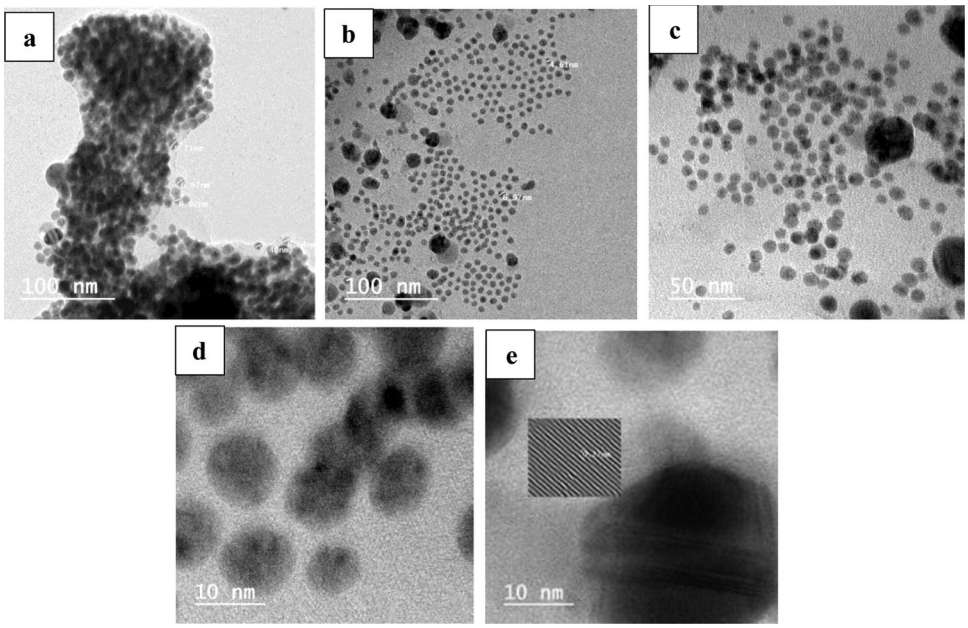


Fig. 7 TEM image of *N. oleander*-AgNPs: (**a**-**c**); higher magnification of the image, **d**; lower magnification, **e**; Tem image of a single particle

XRD analysis was used to study the crystalline structure of the green synthesised *N. oleander* 'AgNPs,' and the pattern produced is shown in Fig. 9. Each peak corresponds to one of the five primary planes of Ag in the XRD spectrum: (111), (120), (200), (202), or (300) planes of the metal. The Scherrer's equation predicted the 'AgNPs' crystal size to be 27 nm based on the whole width at half maximum of the (111) peak of Ag.

Larvicidal effects of *N. oleander* crude extract and 'AgNPs' against *L. sericata*

Table 2 shows the results of the *L. sericata* 3rd instar larvae susceptibility tests to the investigated compounds. The greatest concentration of *N. oleander* crude extract (500 ppm) killed 80% of the larvae 48 h after treatment. 'AgNPs' enhance the larvicidal effectiveness of *N. oleander* leaf extract, resulting in 100% mortality at 50 ppm in larvae treated 48 h after treatment. In larvae treated with *N. oleander*-'AgNPs', the calculated LC_{50} value was 21.0 ppm, which is nearly 18 times more hazardous than pure leaf extract (LC_{50} = 389 ppm). It was shown that the mortality of the larvae was significantly correlated with the concentration of the tested materials. 'AgNPs' treatment had the maximum slope, as demonstrated by the positive slope values (1.168). A larger concentration-mortality response is indicated by steeper slopes.

Effects on biochemistry

Total protein content

Larva haemolymph protein content 48 h after treatment was studied and the findings are reported in Table 3. For the control larvae, the protein level was 87.63 mg/ml haemolymph.

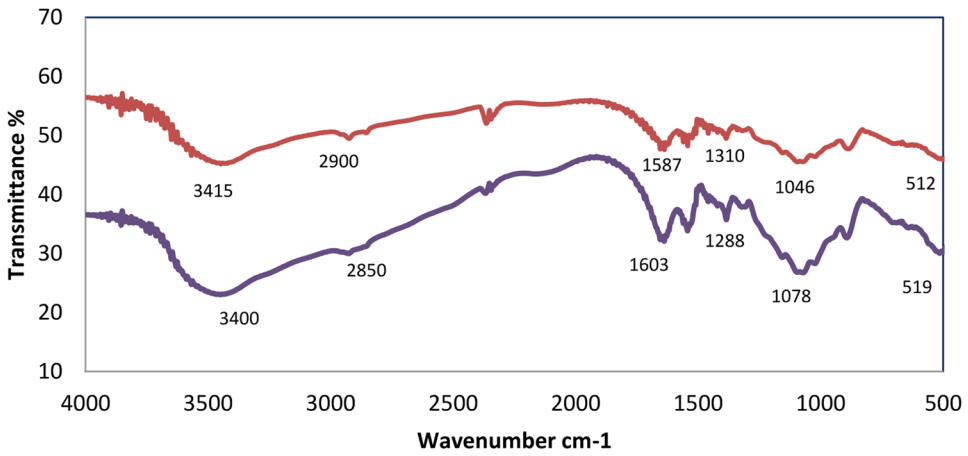


Fig. 8 FTIR spectra of leaf extract (dark red) and 'AgNPs' (dark blue) of *N. oleander*

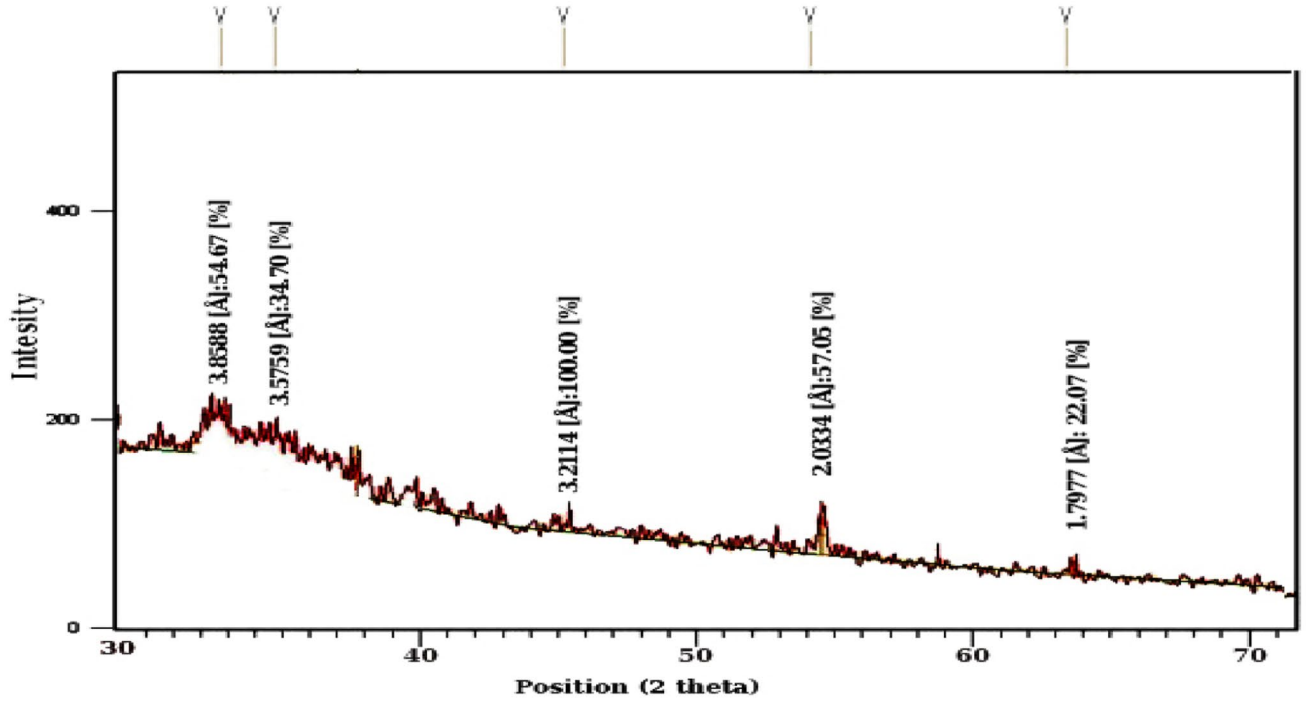


Fig. 9 X-ray diffraction pattern of 'AgNPs' synthesized by *N. oleande*r

The total protein content of larvae treated with *N. oleander* leaf extract decreased by 26.38 percent ($p < 0.05$) when compared to the control value. There was a substantial ($p < 0.01$) reduction in total protein content in larvae treated with *N. oleander*-'AgNPs, which was 41.14 percent lower than the protein level in control larvae.

Haemolymph protein pattern

Protein bands of *L. sericata* 3rd instar larvae fed with LC_{50} concentrations of each investigated substance are shown in Table 4 and Plate (1). It was found that the greatest number of protein bands seen in the control was 16, with

Table 2 Larvicidal activities of leaves extract and biosynthesized 'AgNPs' of *N. oleander* on *L. sericata* 48 h post treatment

Leaf extract				***N. oleander*-AgNPs**			
Conc. (ppm)	**No**	**M % ± SD**	***p*-Value**	**Conc. (ppm)**	**No**	**M % ± SD**	**p-Value**
100	100	17 ± 1.14^{a}	ns	**10**	100	27 ± 1.67^{b}	0.030
200	100	23 ± 0.89^{a}	ns	**20**	100	48 ± 4.16^{b}	0.021
300	100	33 ± 2.30^{a}	ns	**30**	100	68 ± 4.72^{c}	0.011
400	100	$52. \pm 4.61^{b}$	0.04	**40**	100	87 ± 3.29^{d}	0.000
500	100	80 ± 3.94^{c}	0.01	**50**	100	100 ± 0.00^{e}	0.000
Control	100	0.0 ± 0.00^{a}	ns	**Control**	100	0.0 ± 0.00^{a}	ns
Slope	1.157			1.168			
LC_{50}	389			21.0			
95%Confidence limits (LCL-UCL)	(368–401)			(18.0–24.0)			
Relative efficacy	1			18.52			

a, b, c, d, e: mean the significant differences between the percentages of mortality (M%)

LCL lower limit, *ns* non-significant, *SD* standard deviation, *UCL* upper limit

Table 3 Effect of LC_{50} of the tested compounds on haemolymph protein content of *L. sericata* 3rd instar larvae 48 h post treatment

Treatment	Mean[a] (mg/ml haemolymph)	SD[b]	Change %	*t-test*	
				p-value	Significance level[c]
Control	87.63	6.11	-	-	-
Leaf extract	64.51	3.98	-26.38	0.04	*
NO-AgNPs[d]	51.58	4.59	-41.14	0.006	**

[a] Mean of three replicates

[b] Standard Deviation

[c] *: Significant ($P<0.05$) and **: Highly Significant ($P<0.01$)

[d] *N. oleander*-AgNPs

molecular weights ranging from 197.53 to 6.35 kDa. The molecular weights of the protein bands in the marker ranged from 212 to 7 kDa, with a total of nine bands. In both control and treated larvae, the five protein bands (1, 2, 5, 6, and 10) with molecular weights of 197.53, 171.934, 113.01, 98.27, and 65.75 kDa were found to be present. 82.79 and 42.78-kDa molecular weight bands were seen in all treated samples, but the control samples did not have these bands. A single band with a molecular weight of 25.295 kDa was only found in leaf extract-treated samples, and it was not present in either the control or any other treated samples. At 48 h after exposure to 'AgNPs' of *N. oleander*, numerous protein bands in the untreated haemolymph vanished in the treated samples. All of the molecular weights of these bands; 3, 4, 7, 8, 9, 11, 12, 13, 14, 15 and 16 are between 157.329 and 6.35 kDa. Protein bands of molecular weights 145.49, 55.144, and 38.37 kDa were found in the haemolymph of samples that had been treated with 'AgNPs', but not in those that had been treated with leaf extract.

Table 4 Molecular weight analysis of haemolymph proteins of *L. sericata* treated with LC_{50} of leaf extract and 'AgNPs' of *N. oleander* 48 h post-treatment using SDS-PAGE

M	No. of band	Control	Extract	AgNPs	Frequency	Polymorphism
212		-	-	-	-	-
	1	197.53	197.53	197.53	1	Polymorphic
	2	171.934	171.934	171.934	1	Polymorphic
	3	157.329	157.329	-	0.67	Monomorphic
		-	-	145. 49	0.33	Unique
	4	126.67	126.67	-	0.67	Monomorphic
120		-	-	-	-	-
	5	113.01	113.01	113.01	1	Polymorphic
	6	98. 27	98. 27	98. 27	1	Polymorphic
97		-	-	-	-	-
	7	92.51	-	-	0.33	Unique
	8	85.17	-	-	0.33	Unique
		-	82.79	82.79	0.67	Monomorphic
	9	73.71	-	-	0.33	Unique
66		-	-	-	-	-
	10	65. 75	65. 75	65. 75	1	Polymorphic
		-	-	55.144	0.33	Unique
	11	51.75	51.75	-	0.67	Monomorphic
45		-	-	-	-	-
		-	42.78	42.78	0.67	Monomorphic
	12	39.28	-	-	0.33	Unique
		-	-	38.37	0.33	Unique
	13	33.19	33.19	-	0.67	Monomorphic
31		-	-	-	-	-
	14	29.19	-	-	0.33	Unique
		-	25.295	-	0.33	Unique
20		-	-	-	-	-
	15	19.29	-	-	0.33	Unique
14		-	-	-	-	-
7		-	-	-	-	-
	16	6.35	-	-	0.33	Unique

KDa Kilo Daltons, *M* marker, *MW* molecular weight

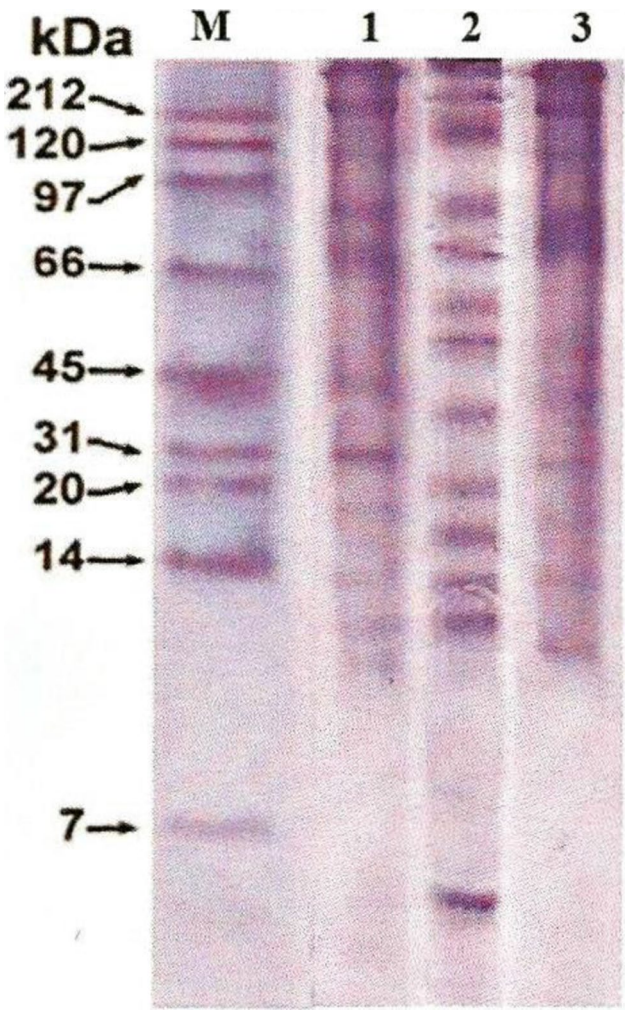


Plate 1 Haemolymph protein banding patterns of *L. sericata* 3rd instar larvae control and treated 48 h post treatment. **Lane 1:** protein bands of larvae treated with 'AgNPs' of *N. oleander*. **Lane 2:** protein bands of control. **Lane 3:** protein bands of larvae treated with *N. oleander* leaf extract

Discussion

N. oleander leaf extract included 17 components, which accounted for 99.9624 percent of the total extract, according to our research. Phenol, hydrocarbon, sterol, ester, and heterocyclic compound are all phytochemicals. Sterol was found to be the most prominent component, with phenol following close after. Other researchers have also found sterol in the leaves of *N. oleander* (El-Akhal et al. 2015; Chaudhary et al. 2015; Redha 2020). Furthermore, phenols were also discovered by several scientists in a phytochemical investigation of the *N. oleander* leaf extract (Aboud 2015; Sinha and Biswas 2016; Bameta et al. 2017). It was found that eight hydrocarbon compounds were detected in the *N. oleander* methanol leaf extract by GC–MS analysis (Hameed et al. 2015). The GC–MS study of *N. oleander* methanol leaf extract has previously shown the presence of ester and a heterocyclic molecule (Mishra et al. 2018).

In the presence of *N. oleander* leaf extract, the colour of the silver nitrate solution changed from green to dark brown, providing the first indication that the reduction of Ag ions by reducing agents in the plant extract results in 'AgNPs'. *N. oleander*-AgNPs had an absorbance peak at 430 nm in the UV–Vis spectrum, which suggested the presence of 'AgNPs' in the sample. Surface Plasmon Resonance (SPR) absorption bands are formed owing to the simultaneous vibration of the electrons of the metal nanoparticles in resonance with the light wave (Kumar 2018). SPR excitation in 'AgNPs' may be the cause of the prominent 430 nm resonance. Subbaiya et al. (2014) also found a similar outcome in their research. 'AgNPs' with spherical shapes were clearly visible in the electron micrograph, as were relatively few aggregates. They were not in direct touch with one other, suggesting that the silver nanoparticles had been stabilised by some kind of capping agent (Kumar 2018). It has been reported that the capping components of the plant extract are responsible for the bio-reduction of silver ions to "AgNPs" by Roni et al. (2013) and Jafer and Annon (2018). Proteins may create a protective barrier around silver nanoparticles, preventing them from aggregating and stabilising them in the medium, according to Kumar (2018), who found that the carbonyl group of amino acid residues has a significant affinity for metal ions. The hydroxyl (–OH), amine (–NH), and carboxyl (–C = O) groups of *N. oleander* leaf extract were shown to be the most important in the production of 'AgNPs' by Shawuti et al. (2021). The phenolic groups, terpenoid, and ester contained in the leaf extract aid in the synthesis of 'AgNPs'. Results that are in line with those of Roni et al. (2013) and Subbaiya et al. (2014). Diffraction peaks at 2 theta in the X-ray diffraction pattern of biosynthesized 'AgNPs' represent the patterns of the face-centered cubic and crystalline structure of the biosynthesized 'AgNPs. *N. oleander* extracts from leaves and flowers yielded biosynthesized "AgNPs" with peak shapes similar to those found in *N. oleander* (111), (200), and (220) (Hadi et al. 2020; Shawuti et al. 2021). 'AgNPs' have an average crystallisation size of 27 nm. The observed result was in line with Kumar (2018) and Hadi et al. (2020) findings.

The mortality and concentration of the materials tested in larvicidal assays against *L. sericata* were shown to be positively correlated. Many scientists believe that the larvicidal properties of *N. oleander* leaf extract are due to its primary components, such as those found in GC–MS analysis. Using *N. oleander* leaf extract to treat a variety of insect pests yielded similar results (Behravan et al. 2017; Semiz 2017; El-Monairy et al. 2020; Zaid et al. 2021). LC_{50} readings showed an 18-fold increase in toxicity of *N. oleander*-AgNPs when compared to the methanol

leaf extract alone. 'AgNPs' synthesised from plant extracts have a high larvicidal efficacy, which may be explained by their ability to permeate insect exoskeletons and then enter insect cells, where they restrict macromolecules like DNA and proteins, changing their structure and thereby their function, ultimately leading to cellular function loss and cell death (Benelli 2016). *N. oleander*-AgNPs are more efficient against *Anopheles stephensi* and *Musca domestica* than the crude extract, according to studies by Roni et al. (2013) and El-Monairy et al. (2020).

Proteins are the building blocks of life and must be present in order for the organism to function. Proteins, unlike carbohydrates and lipids, are used to build and maintain structures. In addition, proteins play a significant part in a wide range of biological processes, including hormone regulation, biochemical reaction catalysis, cell structure, and detoxification (Hammad 2020). The protein content of the treated larvae was lower than that of the control. It is possible that the anabolic activity of the treated insects was suppressed by the investigated materials, and that the metabolic activity was mostly catabolic (Eldeeb et al. 2018). Also, Abd El-Wahab (2002) found similar effects with *Spodoptera litura* treated with *N. oleander* extracts. Other insect species treated with other botanical compounds had their protein content reduced as well (Ibrahim and Mahmoud 2018; Hammad 2020). According to these findings, the treated larvae had varying numbers of heamolymph protein bands. Insect protein production and utility may be determined by comparing the haemolymph protein bands of various treatments (Ismail and Abd El-Gawad 2018). Amer et al. (2019) revealed that the number of protein bands that separated from *L. sericata* larval (ES) products were 15 bands with MWs ranging from 20.35 to > 150 kDa. These results are consistent with the findings. There have been no published findings to date on the effects of 'AgNPs' therapy on the haemolymph protein profile of *L. serecata*. SDS-PAGE analysis of samples treated with various treatments has shown the emergence of distinct protein bands and the elimination of others, compared to controls in many other studies (Mahmoud et al. 2016, 2020; Kitherian et al. 2021).

Conclusion

Silver nanoparticles can be easily and affordably synthesised according to the findings of this research. Phenol and proteins, which are thought to be reducing and capping agents for the "AgNPs," were shown to be bioactive molecules by FT-IR analysis. It was shown that the biosynthesised 'AgNPs' had an 18-fold higher larvicidal activity against *L. sericata* than pure extract because they were spherical in shape, hydrophilic, dispersed evenly in water, and were very durable. Compared to untreated larvae, the investigated substances lowered overall haemolymph protein levels and altered protein profiles. These findings show that the tested compounds may interfere with the larvae's internal defence mechanisms. These alterations may also serve as biochemical indications of treatment stress. Green larvicides like *N. oleander* leaf extract might be a viable eco-friendly option for the quick biological manufacture of silver nanoparticles needed to control blow flies. The safety and efficacy of the synthesised 'AgNPs' need to be tested in the field before they can be put into use.

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Declarations

Conflicts of interest The author declares no competing interests.

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