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Impact of nanosilver-profenofos on cotton leafworm, *Spodoptera littoralis* (Boisd.) larvae

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Abstract

Background: The cotton leafworm, *Spodoptera littoralis* (Boisd.), is one of the most dangerous pests in Egypt and Africa, causing significant economic loss of cotton crop yield. The frequent use of insecticides to control this insect has led to the development of a generation's resistance to them. The need of a new, safe control method and effective insecticides has become necessary after the recent increases in environmental pollution and insect resistance. This study is devoted to developing a novel synthetic scheme to produce a pesticide nanocomposite of very high efficiency compared to the originals. The nature of its chemical binding has been investigated via Fourier transform-infrared (FT-IR) and transmission electron microscopy (TEM) techniques.

Results: In this work, the method is based on using silver nanoparticles (AgNPS) as a pesticide carrier by loading the organophosphorus pesticide profenofos on to their surface. The profenofos, AgNPS alone and nanocomposite profenofos (AgNPS@P) have been tested against second- and fourth-instar larvae of laboratory and field cotton leafworm. Our findings indicate that the AgNPS@P is more effective on cotton leafworm larvae than each of profenofos and nanosilver alone. The activity of AgNPS@P (LC_{50} = 0.94 and 5.15 ppm) was increased to 85 and 69 times more than that of profenofos (LC_{50} = 79.52 and 356.97 ppm) against second- and fourth-instar larvae of field cotton leafworm.

Conclusions: This method may be successful for reducing environmental pollution and the resistance of this pest to many pesticides.

Keywords: Cotton leafworm, *Spodoptera littoralis*, Organophosphorus pesticide, Profenofos, Nanosilver, Nanocomposite

Background

The loss of agricultural production by pests is estimate to be about 14–25% of the total production. Weeds have a direct effect on the size, quantity and quality of food security, which decreases agricultural crop production. Some insect pests are vectors of many diseases which cause serious problems to human health and others damage crops (Salahuddin et al. 2004).

The noctuid moth of the cotton leafworm, *Spodoptera littoralis* (Boisad.) (Lepidoptera: Noctuidae) is found widely in Mediterranean Europe and Africa. This insect has been record in the UK at least six times, where it is also renowned either as the Mediterranean brocade, an immigrant or as a casual import. It often feeds on fruit, vegetables, flowers and field crops. Generally, the larvae prefer young leaves and, while they are consuming these, they are also feeding on other parts of the plant. Infestation frequently leads to complete defoliation and devouring of the leaves. The larvae interfere with plant development by destroying growth points and flowers as well as hollowing out the seed bolls, which often causes them to wilt and drop (Croft 1990). The Egyptian cotton leafworm, *S. littoralis*, is the most destructive pest of several crops such as cotton (*Gossypium hirsutum* (L.)),

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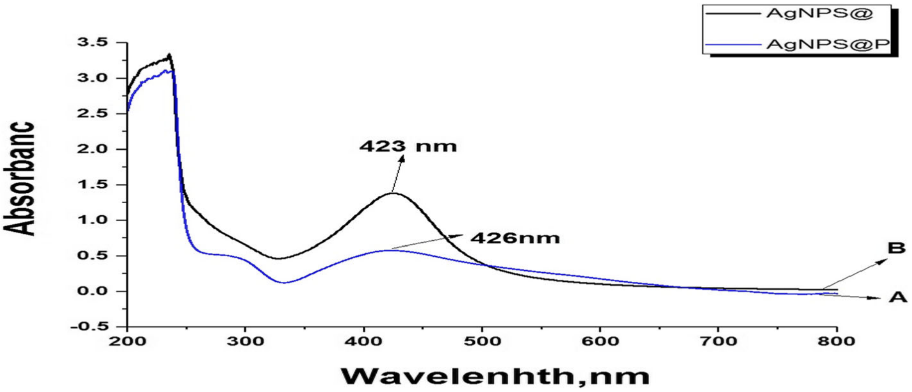

Fig. 1 The ultraviolet-visible (UV-VIS) spectrum of (A) AgNPS@P and (B) AgNPS only

peanut (*Arachis hypogaea* (L.)), soybean (*Glycine max* (L.)) and various vegetables in Europe, Africa and Asia (El-Aswad et al. 2003).

Insect resistance is a major problem generated by the frequent use of the conventional pesticides for controlling the insect pests (Nkya et al. 2014). The development of cotton leafworm resistance to the use of synthetic pyrethroids, organophosphorus, carbamates and other chemical insecticides has been correlated with the appearance of cross-resistance in many cases (El-Zemaity et al. 2003). On the other hand, the use of pesticides causes an increase in costs and many problems of environmental and personal exposure which has led to the contamination of ground waters, plants, soil, animals and damaging beneficial non-target organisms (Kuzma et al. 2006). In addition, many insecticides are not soluble in water, so large quantities of organic solvents are required and most of these solvents contaminate the environment (Sanni and Mutta 2014).

Profenofos (IUPAC: 4-bromo-2-chloro-1-[ethoxy(propylsulfanyl) phosphoryl]oxybenzene is also called phosphorothioic acid, *O*-(4-bromo-2-chlorophenyl) *O*-ethyl-*S*-propyl ester (free encyclopedia CAS Number 41198-08-7 from the pesticide manual). The profenofos mechanism of action is via inhibition of the acetylcholinesterase enzyme. Profenofos can be used on a variety of crops such as maize, potato, soybean, sugar beet, cotton and other vegetables. In the United States, it is used exclusively on cotton and is primarily used against lepidopteran insects. Profenofos can be used against the cotton mealybug, cabbage caterpillar, diamondback moth and asparagus caterpillars, as well as against wheat and cabbage aphids (FAO and WHO 2009). However, profenofos is toxic to birds, small mammals, bees, fish and aquatic invertebrates. Several fish-killing incidents have occurred in which profenofos exposure, primarily due to runoff, has been implicated as a probable cause (Onwuka 2015). Based on a study of patients poisoned with profenofos and its close chemical relative, prothiofos, the compounds have been described as moderately severe toxins that cause respiratory failure. Differences in chemical structure that distinguish these two compounds from more common organophosphorus pesticides – namely, the presence of the *S*-alkyl group on the phosphorus atom where most organophosphorus compounds

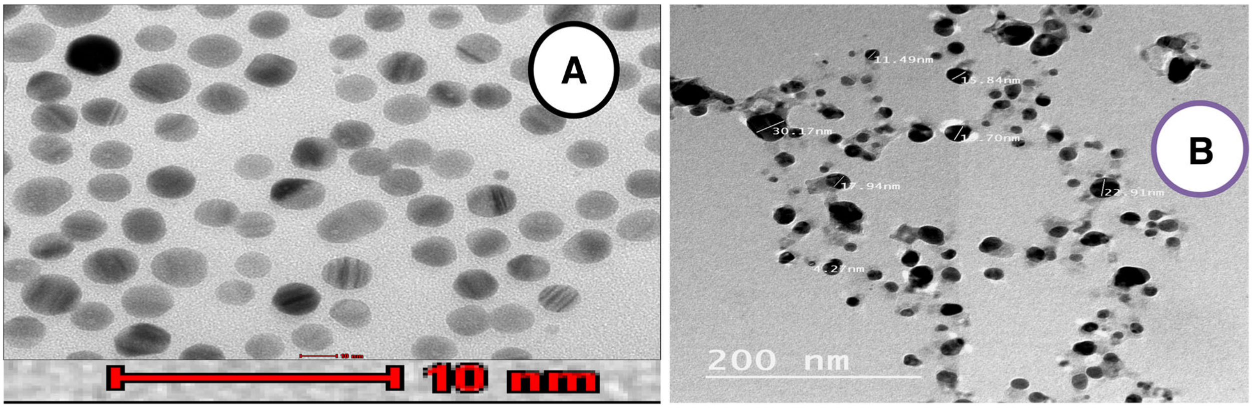

Fig. 2 The transmission electron micrographic image of **a** AgNPS only and **b** AgNPs@P

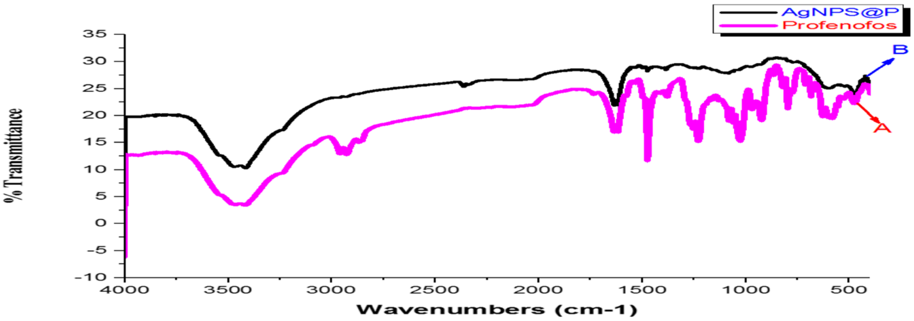

Fig. 3 The Fourier transform-infrared (FT-IR) spectra for (A) profenofos and (B) AgNPS@P

possess a methoxy or ethoxy group – underlie differences in their behavior as acetylcholinesterase enzyme inhibitors compared to the rest of the organophosphorus class (Eddleston et al. 2009).

Nanotechnology has been used over the last 10 years to potentially revolutionize agricultural practices. Some suggested applications, such as the development of precision farming devices or genetically modified crops, nanofungicides, nanoherbicides, nano-encapsulation and nanopesticides, can be of use (Zhang et al. 2013). Metal nanoparticles are unique because they show the potential to change their surfaces in order to introduce specific functionalities for environmental applications (Haick 2007). Nanopesticides present an appealing solution for pesticide problems because their effective concentrations are much lower compared to those of formulated pesticides and they are soluble in water without organic solvents. Recently, several publications reported on the evolution of nanopesticide formulations (Bhattacharyya et al. 2010). The target of nanopesticide composition for necessary world applications is to obtain nanoparticles with the following characteristics: (1) a constant and lean size distribution, (2) a well-known shape, (3) a chemical structure known to have no impurities and (4) no congregation or clotting properties (Sooresh et al. 2011). The use of a capping agent works as a colloidal stabilizer that enhances water suspension ability. These very eligible characteristics apply to silver nanoparticles because silver is an electron-dense metal (Rotello 2003).

This work is a trial to synthesize silver nanoparticles stabilized by starch as the form of encapsulation for the synthetic organophosphorus pesticide profenofos (using an inexpensive and reproducible method) and to characterize them. Also, the toxic effects of profenofos, silver nanoparticles (AgNPS) and nanocomposite profenofos (AgNPS@P) on larvae of the laboratory and field cotton leafworm can be evaluated.

Table 1 Toxicity effect of silver nanoparticles (AgNPS) and profenofos compared with nanoprofenofos (AgNPS@P) against second-instar larvae of laboratory and field cotton leafworm, *Spodoptera littoralis*

Strain	Silver (AgNPS)				Profenofos					Nanoprofenofos (AgNPS@P)				
	Conc. ppm	M (%)	LC_{50} in ppm	RR[a]	Conc. ppm	M (%)	LC_{50} in ppm	RR[a]	RRr[b]	Conc. ppm	M (%)	LC_{50} in ppm	RR[a]	RRr[b]
Laboratory			1000.9	----	0.71	20	4.49	----	44.9	0.011	10	0.1	----	1
					1.41	28				0.023	24			
	124.88	6			2.82	46				0.045	30			
	249.75	16			5.63	52				0.09	44			
	499.5	30			11.3	66				0.18	60			
	999	50			22.5	76				0.36	74			
					45	86				1.44	90			
Field			4109.6	4.11	22.5	14	79.52	17.7	84.6	0.18	10	0.94	9.4	1
	187.31	6			45	36				0.36	20			
	374.63	10			90	56				0.72	40			
	749.25	20			180	70				1.44	60			
	1498.5	30			270	84				2.88	86			

[a] RR (Resistance Ratio) = LC_{50} of the field strain/LC_{50} of the laboratory strain
[b] RRr (Relative Resistance ratio) = LC_{50} of the strain/lowest LC_{50} values of the same strain
M mortality

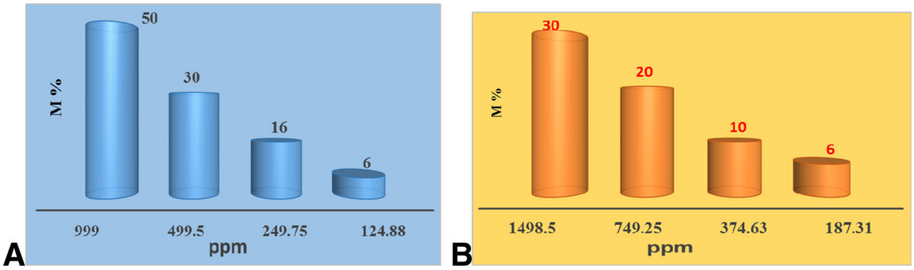

Fig. 4 The mortality percentages of second-instar larvae for AgNPS to **a** laboratory and **b** field strains of *Spodoptera. littoralis*

Materials and methods

Chemicals

These are silver nitrate ($AgNO_3$, 99.9%, with average molecular weight = 169.87, produced by Alpha Chemika Co.), glucose ($C_6\ H_{12}\ O_6$, 99% with average molecular weight = 180.2, produced by El-Nasr Pharmaceutical Chemicals Co.), soluble starch ($(C_6\ H_{10}\ O_5)_{n,}$ 99% powdered solid with average molecular weight = 81.37, produced by Chemajet Pharmaceutical Co.). Profenofos ($C_{11}H_{15}BrC_{lO}.3PS$, 98.9% with average molecular weight = 373.63) is produced by Dr. Ehrenstorfer GmbH, Empirical Formula (Hill Notation).

Insects

The laboratory insects are reared on castor bean plant leaves for 7 years, without any exposure to insecticides, in the Insect Population Toxicology Department, Central Agricultural Pesticides Laboratory (CAPL), Agriculture Research Centre, Ministry of Agriculture, Giza, Egypt under laboratory constant conditions (25 ± 2 °C and 60 ± 5% R. H for a period of 16 h. L: 8 h. D) (Hatem et al. 2011). Field insects were collected as eggs from EL-Beheira Governorate, Egypt and reared, as mentioned before, for one generation.

Synthesis and characterization of AgNPS and AgNPS@P

Synthesis of silver nanoparticles and encapsulated nanoprofenofos

Starch-silver nanoparticles encapsulate the profenofos, according to Nnemeka et al. (2016), with the modulation of encapsulation being completed in situ during synthesis of the silver nanoparticles by direct physical gelation (Nnemeka et al. 2016; Vimala et al. 2011 and Tali 2009). The synthesis was carried out via chemical reduction of silver nitrate by glucose as follows: a mixture of 0.06-M $AgNO_3$ and 0.2-M glucose solution (1: 3 volume ratio) in a loosely covered flask containing 1% starch dispersion (1 g in 100 ml distilled water), were added to 10 ml of profenofos. The mixture was stirred and heated for 3 h and the resultant complex was cooled and centrifuged at 11,000 rpm for 20 min using a Hettich-Mikro 22R centrifuge. Subsequently, each ST–AgNP–P nanocomposite (AgNPS@P) was precipitated the with addition of 30 ml acetone, re-centrifuged at 6000 rpm for 5 min and sediment oven-dried at 40°C for 24 h. The nanocomposite was finely ground, kept in a sample bottle, and stored in a vacuum desiccator in the dark for further use and characterization.

Characterization of AgNPS and AgNPS@P

All prepared samples were characterized by transmission electron microscopy (TEM) as a base tool for scaling the particle size, structure and style, and the plasmonic effects were detect by ultraviolet-visible (UV-VIS) spectroscopy. The nature of the linkage between pesticide and AgNPS was investigated using Fourier transform-infrared (FT-IR) spectroscopy.

UV-VIS spectral analysis

The composition of the nanoparticles was detected using a UV-VIS spectrophotometer (Scan Software Version: 3 (182) Parameter List: Instrument Cary 5000, Instrument

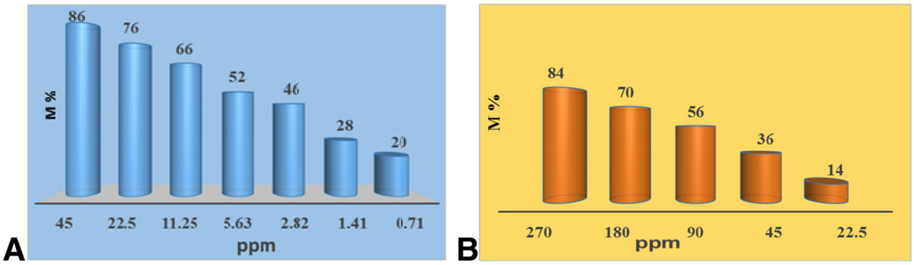

Fig. 5 The mortality percentages of second-instar larvae for profenofos to **a** laboratory and **b** field strains of *Spodoptera littoralis*

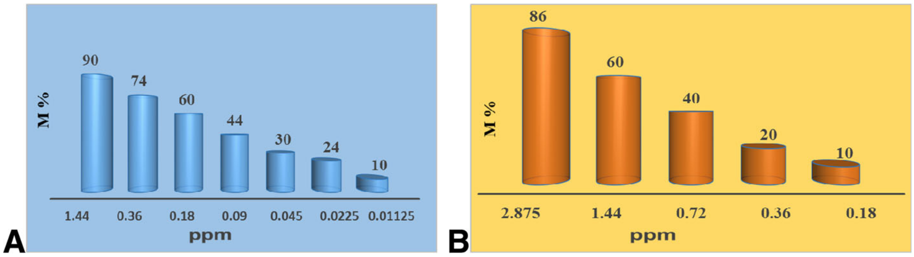

Fig. 6 The mortality percentages of second-instar larvae for AgNPS@P to **a** laboratory and **b** field strains of *Spodoptera littoralis*

Version 1.12, Start 800 (nm), Stop 200 (nm)) in the Mammalian Toxicology Department of CAPL. Aliquots (3 ml) of the suspension were measured to determine the surface plasmon resonance (SPR) absorption maxima with distilled water as a reference.

Transmission electron microscopy (TEM imaging)

The TEM images were carried out in the National Research Centre, Dokki, Giza, Egypt. Dispersed ST–AgNP–P samples in absolute ethanol were dropped on to coated copper grids and allowed ethanol to evaporate. Micrographs were obtained using a high-resolution transmission electron microscope (HR-TEM) (FEI TECNAI 02) with software TECNAI G2. The HR-TEM is a JOEL JEM-M2100 operating at 200 kV equipped with a Gatan Erlangshen ES500 digital camera.

Fourier transform-infrared (FT-IR) spectral analysis

The FT-IR spectra were recorded by an AVATAR 330 FT-IR Thermo Nicolet (Software EZOMNIC V 6.1A) in the Pesticide Analysis Department of CAPL. The samples were scanned within a range of 400–4000 cm^{-1}.

Toxicity of AgNPS, profenofos and AgNPS@P against second and fourth larval instars of the cotton leafworm

The leaf-dip technique bioassay was applied to examine the effects of profenofos, AgNPS alone, and AgNPS@P against the second- and fourth-instar larvae of laboratory and field *S. littoralis.* Serial concentrations were prepared by dilution of the tested compounds with distilled water (Vimala et al. 2011). Clean castor bean leaves were dipped for 15 s in each compound concentration, and left to dry at room temperature then put in petri-dishes. Others were dipped in distilled water for the same period as the control. Five replicates were carried out for each concentration and control. Ten larvae of the second or fourth instars from each laboratory and field cotton leafworm were added to each treated and control dish. All the treated and untreated (control) larvae were allowed to feed on the leaves and all the samples were preserved at room temperature. Mortalities were recorded after 24 h (Thomas and Ralf, 2015).

Statistical analysis

The corrected mortality percentages were calculated by using Abbott's formula (Abbott 1925) and the sublethal concentration of pesticides statistically computed according to Tali (2009). The median lethal concentration (LC_{50}) was determined at the 95% confidence level ($P < 0.05$) using the program Ldp Line, an application that calculates by probit analyses according to Finney (1971).

Results

Synthesis and characterization of AgNPS and AgNPS@P

In this procedure, glucose and starch served the dual role of both a reducing agent and a stabilizer. Then, the core particles, AgNPS were combined with profenofos to produce AgNPS@P, which was also produced in ethanol as opposed to using harsh non-polar solvents. The characterizations of AgNPS and AgNPS@P are shown in detail.

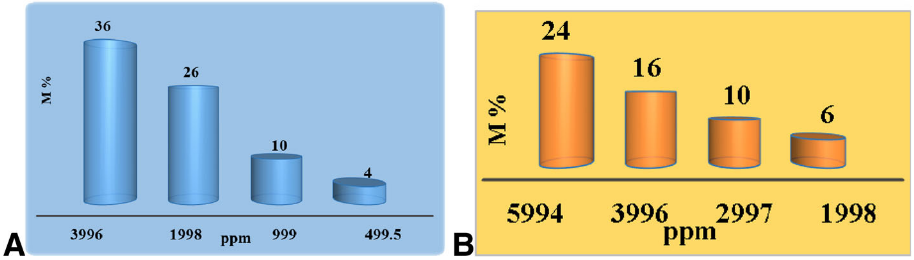

Fig. 7 The mortality percentage of fourth-instar larvae for AgNPS to **a** laboratory and **b** field strains of *Spodoptera littoralis*

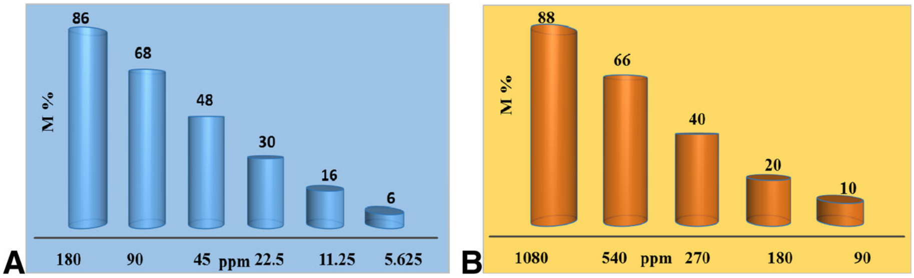

Fig. 8 The mortality percentages of fourth-instar larvae for profenofos to **a** laboratory and **b** field strains of *Spodoptera littoralis*

UV-VIS absorption spectroscopy

The first confirmation of the silver nanoparticles (AgNPS) and nanoprofenofos (AgNPS@P) is shown in Fig. 1. The obtained (AgNPS) and (AgNPS@P) both showed a broad spectral peak at 423 and 426 nm, respectively.

TEM imaging

The size and shape of the silver colloid particles have been measure by TEM imaging. A representative TEM image of these particles is given in Fig. 2a. The particles are mostly spherical. From the sizes of numerous particles, measured on the TEM images, the average size (diameter) of the silver nanoparticles loaded with profenofos (AgNPS@P) is 11.49–30.17 nm.

Fourier transform infrared (FT-IR) spectroscopy

FT-IR spectroscopy of the starch-silver nanoparticles was registered agree the functional groups of glucose and starch interested in the reduction and capping/stabilization as shown for profenofos (A) and AgNPS@P (B) (Fig. 3).

The broad, strong bands at 3416.40 and 3416.01 cm^{-1} are due to the O–H stretching vibration for profenofos and AgNPS@P, respectively. Also, it is clear that, C–H aliphatic vibrations appearing as peaks at 2960.26 and 2927.63 cm^{-1} correspond to the asymmetrical and symmetrical bending vibrations of the methylene groups for profenofos, while this peak disappeared at 2960.26 and 2927.63 cm^{-1} and appeared as a peak at 2360.98 for AgNPS@P. Aliphatic C–H appeared as two strong bands at 2962 + 10 cm^{-1} and 2872 + 10 cm^{-1} corresponding to asymmetrical and symmetrical stretching modes. The C=C stretching vibrations for hetero-aromatic compounds were observed in the region 1600–1400 cm^{-1}. Profenofos and AgNPS@P show this band at (1618.20 and 1637.58 cm^{-1}) and (1618.37 and 1637.58 cm^{-1}), respectively.

Toxic effects of nanosilver, profenofos and nanoprofenofos

Nanosilver, profenofos and nanoprofenofos toxicity against second-instar larvae

The larvicidal activities of silver nanoparticles (AgNPS) alone, profenofos, and silver nanoparticle-loaded profenofos (AgNPS@P) were studied against second-instar larvae of each of laboratory and field *S. littoralis.* The results shown in Table 1 revealed that the mortality rate increased with an increase in the concentrations of the tested pesticides for the laboratory insects, where the mortality percentages ranged between 6 and 50% with AgNPS concentrations of 124.88–999 ppm, and 6–30% with AgNPS concentrations of 187.31–1498.5 ppm for field insects (Table 1 and Fig 4). These percentages ranged between 10 and 90% with AgNPS@P concentrations of 0.01–1.44 ppm, compared with those of profenofos of 20–86% with AgNPS@P concentrations of 0.71–45 ppm for the laboratory strain (Table 1 and Fig. 5). Concerning the field strain, the percentages of mortality recorded were 10–86% with concentrations of 0.18–2.88 ppm for AgNPS@P and 14–84% for concentrations of 22.5–270 ppm for profenofos (Table 1 and Fig. 6). The results of the concentration-dependent assay suggest that the comparison between the LC$_{50}$ of

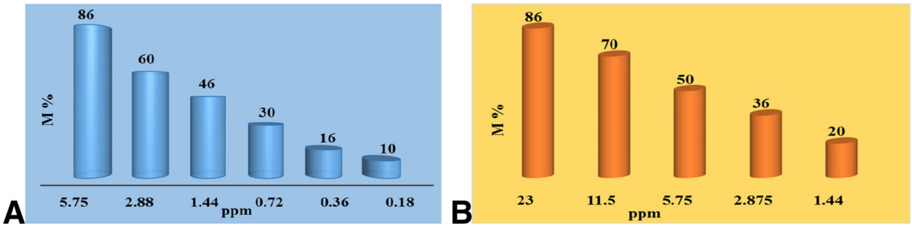

Fig. 9 The mortality percentages of fourth-instar larvae for AgNPS@P to **a** laboratory and **b** field strains of *S. littoralis*

profenofos (4.49 ppm) and AgNPS@P (0.1 ppm) for second instar larvae of the laboratory strain produced a Relative Resistance ratio (RRr) = 44.9 fold, i.e.

Nanosilver, profenofos and nanoprofenofos toxicity against fourth-instar larvae

The larvicidal activities of the silver nanoparticles (AgNPS), profenofos, and silver nanoparticle-loaded profenofos (AgNPS@P) against laboratory and field fourth-instar larvae of the cotton leafworm are shown in Table 2 and Figs. 7, 8 and 9. The mortality percentages of laboratory larvae ranged between 4 and 36% with AgNPS concentrations of 499.5 to 3996 ppm and the field strain ranged between 6 and 24% with concentrations of 1998 to 5994 ppm (Table 2 and Fig. 7). The mortality ranged between 10 and 86% for laboratory larvae with AgNPS@P concentrations of 0.18 to 5.75 ppm (Table 2 and Fig. 8) compared with those of profenofos 6 to 86% with concentrations of 5.625 to 180 ppm. In the case of field larvae, the percentages recorded were 20 to 86% with AgNPS@P concentrations of 1.44 to 23 ppm, compared to profenofos 10 and 88% with concentrations of 90 to 1080 ppm (Table 2 and Fig. 9).

Discussion

The practical application of the nanosilver composition aims to reproduce mono-dispersed nanoparticles with a well-determined style. The critical steps of accurate selection of the reducing agent and stabilizer can be more easily controlled when the nanoparticles are synthesizing. Hence, water-soluble, highly mono-dispersed and spherical AgNPS were synthesized. This is a one-pot method with an economical formulation. It is clear that this reduction of silver ions in a watery solution to silver nanoparticles is accompanied by a color change (to yellowish-brown or grayish) due to the excitation of surface plasmon vibrations in the silver nanoparticles (Gao et al. 2011; Theivasanthi and Alagar, 2011; Ramakrishna et al. 2012; Akbari et al. 2011 and Hamedi et al. 2012). The color change, as an effect of agglomeration (assembly of the particles), is a well-understood phenomenon (Mohamed et al. 2012). A safer and more economical insecticide delivery system was developed by the facile formulation of starch-silver nanoparticle-encapsulated dichlorovos and chlorpyrifos (Nnemeka et al. 2016).

The results obtained from UV-VIS spectroscopy showed the appearance of one broad peak in each AgNPS and AgNPS@P, which is due to the excitation of the SPR of silver atoms. This has been reported as describing the collective excitation of the conduction of electrons in a metal (Mohamed et al. 2012).

TEM images revealed that the silver nanoparticle-loaded profenofos complex (AgNPS@P) is mostly spherical and of a very small size. TEM micrographs were used to determine the morphology of nanoparticles and the obtained spheres (Akbari et al. 2011; Hamedi et al. 2012 and Nnemeka et al. 2016).

The FT-IR spectroscopy test detected a broad, strong band in profenofos and AgNPS@P due to the O–H stretching vibration. Asymmetrical and symmetrical bending vibrations of the methylene groups appeared in specific peaks for both AgNPS and AgNPS@P. This may be an indication for involving the nitrile group in linkage with AgNPS (Avram and Mateescu 1972 and Abouelkassem et al. 2016). Aliphatic C–H appeared as two strong

Table 2 Toxicity effect of silver nanoparticles (AgNPS) and profenofos compared with nanoprofenofos (AgNPS@P) against fourth-instar larvae of laboratory and field cotton leafworm, *Spodoptera littoralis*

Strain	Silver (AgNPS)				Profenofos					Nanoprofenofos (AgNPS@P)				
	Conc. ppm	M (%)	LC_{50} in ppm	RR^a	Conc. ppm	M (%)	LC_{50} in ppm	RR^a	RRr^b	Conc. ppm	M (%)	LC_{50} in ppm	RR^a	RRr^b
Laboratory			6202.8	---	5.63	6	45.67	---	29.28	0.18	10	1.56	----	1
	499.5	4			11.3	16				0.36	16			
	999	10			22.5	30				0.72	30			
	1998	26			45	48				1.44	46			
	3996	36			90	68				2.88	60			
					180	86				5.75	86			
Field			14,598	2.35	90	10	357	7.82	69.31	1.44	20	5.15	3.3	1
	1998	6			180	20				2.875	36			
	2997	10			270	40				5.75	50			
	3996	16			540	66				11.5	70			
	5994	24			1080	88				23	86			

[a] RR (Resistance Ratio) = LC_{50} of the field strain/LC_{50} of the laboratory strain

[b] RRr (Relative Resistance ratio) = LC_{50} of the strain/lowest LC_{50} values of the same strain

M mortality

bands corresponding to asymmetrical and symmetrical stretching modes. υC–H absorption bands arising from asymmetrical vibrations are stronger than symmetrical ones. The C=C stretching vibrations for hetero-aromatic compounds were observed in the same region in both profenofos and AgNPS@P. The above findings indicate that silver nanoprofenofos was attached to the functional groups present in starch. The shifting of the peak is due to the formation of the co-ordination bond between the silver atom and the electron-rich groups (oxygen/carbonyls) present in starch. This causes an increase in bond length and frequency (Akbari et al. 2011; Hamedi et al. 2012; Abouelkassem et al. 2016 and Nnemeka et al. 2016).

The toxicity of profenofos increased 45 and 85 times to the second-instar larvae of both laboratory and field cotton leafworm when it became nanoprofenofos. This increase in toxicity was more than 29 and 69 times for the fourth-instar larvae of laboratory and field larvae. In addition, the field fourth-instar larvae were more resistant to AgNPS, profenofos and AgNPS@P than the laboratory strain, where the Resistance Ratio (RR) was 2.35-fold, 7.82-fold and 3.3-fold, respectively. Other findings of Abouelkassem et al. (2016) indicated that silver-cyhalothrin nanocomposite is more efficient in controlling mosquito larvae than is free cyhalothrin. Therefore, this formulation should produce a synergetic effect to combat the adverse effects of the conventional insecticides on the environment (Nnemeka et al. 2016).

Conclusions

From this study, it can be conclude that nanoprofenofos (AgNPS@P) has a very toxic effect on the cotton leafworm, *Spodoptera littoralis* compared to the original pesticide (profenofos). Therefore, we can use small amounts of the novel synthetic nanocomposite pesticide in the management of pests to reduce their resistance to conventional pesticides and reduce environmental pollution.

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Authors' contributions
All authors contributed in the production and writing of the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate
The manuscript does not contain any studies involving human participants, human data or
human tissue.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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