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Effect of Lambda-Cyhalothrin as Nanopesticide on Cotton Leafworm, *Spodoptera littoralis* (Boisd.)

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THE cotton leafworm *Spodoptera littoralis* is the most devastating insect pests to many crop plants. The overuse of pesticides increased pastes resistance eliminate. This study has been devoted to develop a novel synthetic scheme to produce pesticide nanocomposite of very high efficiency compared to its original one. The method is based on using silver nanoparticles (AgNPS) as a pesticide carrier by loading the pyrethroid pesticide Lambda-cyhalothrin (L-CYN) into the surface of prepared AgNPS. The nature of binding has been investigated via Transmission electron microscopy (TEM) and Fourier-transform infrared spectroscopy (FTIR) techniques. The new formulation of the pesticide nanocomposite AgNPS@L-CYN has been tested for its larvicidal activity against second instar larvae of lab and field cotton leafworm. Our findings indicated that silver lambda-cyhalothrin nanocomposite was more effective (37 times) on cotton leafworm larvae than lambda-cyhalothrin alone. The required concentration for controlling cotton leafworm decreased more then. This approach might be successful ones for decreasing the resistance of this pest to pesticides and reduce environmental pollution.

Keywords: Cotton leafworm, *Spodoptera littoralis,* Lambda-cyhalothrin pesticide, Pyrethroid pesticide, Silver nanoparticles, Nanocomposite.

Introduction

Pests and weeds eliminate caused highly decreasing of agricultural crop production, which have direct impacts on food security. Insects damage crop plantations or wood structure and others vectors of many diseases, causing serious economic and health losses. Its estimated that 14–25% of total agricultural production is lost by insect pests [1]. The cotton grown in Egypt could be subjected to the insect damage during its growth cycle. The cotton leafworm, *S. littoralis* is the most destructive pest of cotton in Africa and Mediterranean Europe countries due to its a wide range of hosts, voracity and reproductive potential. An infestation frequently leads to complete defoliation and devouring of the leaves, the caterpillars interfere with plant development by destroying growth points and flowers. Besides the bolls will be hollowed out, which often causes them to wilt and drop [2].

The use of conventional pesticides is the available effective method up to date for controlling *S. littoralis*. Frequent use of insecticides causes a major problem to insect pest resistance eliminate. These insecticides inhibit acetylcholinesterase enzyme or affect the voltage gated of sodium

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channels of insects [3]. The development of insect resistance to one molecular target means that many insecticides are rendered ineffective for pest control, increase of costs, and many problems of environmental / personal exposure. The control of insects and other vectors with bulk form of insecticides led to the contamination of ground water, plants, soil, animals and damaging beneficial non-target organisms [4] . Otherwise, many pesticides are poorly or insoluble in water, so large amounts of organic solvents are required to solubilize them. Most of the organic solvents contaminated the environment [5] . Nanopesticides presented an attractive solution for this problem, due to their effective concentration that expected to be much lower compared to original materials and they could be formulated in water without organic solvents. Recently several approaches were reported on development of nanopesticide formulations [6] .

This field compromises broad research aspects including study of interaction between nanomaterials and insects, formulation of the active ingredients into nanoemulsions and dispersions of existing pesticides, development of new nanopesticide formulations using nanomaterials as active pesticide agents, or using these nanomaterials as nanocarriers for their delivery [7,8].

In the current paper, we reviewed the use of nanoparticles (NPs) in crop protection, emphasizing the control of pests in the agricultural and urban environment. At the same time, we provide the framework on which the technology of NPs is based and the various categories of NPs that are currently used for pest control. Apart from the use of NPs as carriers of a broad category of active ingredients, including insecticides and pheromones, some NPs can be used as insecticides alone. More- over, several types of NPs are produced by natural resource-based substances, which make them promising ‘‘green’’ alternatives to the use of traditional pest control agents. Finally, the potentials in the use of NPs are briefly illustrated and discussed [9].

A few researchers all over the world are working in nanopesticide applications metal nanoparticles, such as silver, are unique because for they offer the possibility of altering their surfaces in order to introduce specific functionalities for environmental applications. Silver nanoparticles (AgNPS) are most prevalent metallic nanoparticles in consumer products due to their effect on microbes [10]. The ultimate target of nanosilver composition for necessary world applications is to obtain nanoparticles with the following characteristics: (a) constant and lean size distribution, (b) well-known shape, (c) known chemical structure with no impurities, and (d) no aggregation or clot [11]. The capping agent acts as a colloidal stabilizer, which keeps water in nanomateral. These are very eligible characteristics can be carried out for silver nanoparticles because silver is an electron dense metal [12].

The present study aims to synthesize silver nanoparticles (AgNPS) and encapsulation nanopesticides (AgNPS@L-CYN). The toxic and histopathological effects of Lambda-cyhalothrin pesticide, AgNPS and AgNPS@L-CYN, L-CYN against lab and field S. littoralis larvae were determined.

Experimental

Laboratory experiments conducted to determine the toxicity of Pesticides were applied in Insect Population Toxicology Department, Central Agricultural Pesticides Laboratory (CAPL), Agriculture Research Centre, Ministry of Agriculture, Giza, Egypt.

All samples were characterized by Transmission Electron Microscopy (TEM) as a base tool for scaling the particles size, structure and shape, and the plasmonic effect were detected by UV-VIS spectroscopy. The nature of linkage between pesticide and AgNPS were investigated using IR-Spectroscopy.

Chemicals

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.), starch soluble ($(C_6 H_{10} O_5)_n$, 99% powdered solid), with average molecular weight =81.37, produced by Chemajet Pharmaceutical Co.) and lambda-cyhalothrin active ingredient ($C_{23}H_{19}ClF_3NO_3$, 99%, with average Molecular Weight = 449.85, produced by Dr. Ehrenstorfer GmbH, Empirical

Insects

Laboratory strain of the cotton leafworm larvae were reared on castor bean plant leaves for 30 generations without any exposure to insecticides. The larvae were kept in suitable temperature and

humidity (25±2ºC and 60±5% R.H) for a period of 24 hr (16L:8D) [13]. Larvae of field strain were collected as eggs from ELBeheira Governorate, Egypt and reared as mentioned before for one generation.

Synthesis and Characterization of AgNPS and AgNPS@ L-CYN.

Synthesis of silver nanoparticle and encapsulated nanolambda – cyhalothrin.

Synthesis of encapsulated nano- Lambda-cyhalothrin according to Nnemeka et al. [14] with the modification that encapsulation was completed italic during synthesis of the silver nanoparticles by direct physical gelation [15]. The synthesis was carried out via chemical reduction of silver nitrate by glucose as follows: to a mixture of 1% (0.06 M) $AgNO_3$ and 0.2 M glucose solution (1 : 3 volume ratio) in a loosely covered flask containing 1% starch dispersion (1g in 100ml distilled water), then adding 30 ml of formulation of Lambda-cyhalothrin(0.01g active ingredient in 100ml ethanol 95%). This mixture was stirred and heated at 40°C for 3 hours in a fume cupboard. The resultant complex was cooled and centrifuged at 11 000 rpm for 20 minutes using the Hettich-Mikro 22-R centrifuge. Subsequently, each ST–AgNP–L-CYN nanocomposite (AgNPS@ L-CYN) precipitated by addition of acetone (30 ml), re-centrifuged at 6000 rpm for 5 minutes then the centrifuge pellets were oven-dried at 40 ºC for 24 hours. The resulted 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@L-CYN

The prepared AgNPS, AgNPS@ L-CYN were characterized by Transmission Electron Microscopy (TEM) as a base tool for scaling the particles size, structure and shape, and the plasmonic effect were detect by UV-VIS spectroscopy.

UV-visible spectral analysis.

The statement of the composition of AgNPS, AgNPS@ L-CYN nanoparticles was detected using a UV-VIS spectrophotometer (Scan Software Version: 3 (182) Parameter List: Instrument Cary 5000, Instrument Version1.12, Start (nm) 800, Stop (nm) 200) in Mammalian Toxicology Department of (CAPL). Aliquots (3 ml) of the suspension were measured to determine the surface plasmon resonance absorption maxima with distilled water as reference.

Transmission Electron Microscopy (TEM imaging)

The transmission electron microscopy (TEM) images were carried out in National Research Centre, Dokki, Giza, Egypt. Dispersed AgNPS and AgNPS@ L-CYN in absolute ethanol were dropped onto coated copper grids and allowed ethanol to evaporate. Micrographs were obtained using a High Resolution Transmission Electron Microscope (HR-TEM) (FEI TECNAI 02) having software TECNAI G2. The HR-TEM is JOEL JEM-M2100 operating at 200 kV equipped with Gatan digital camera Erlangshen ES500.

Fourier transform-infrared (FTIR) spectral analysis.

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

Toxicity of AgNPS, Lambda - cyhalothrin and AgNPS@P against second larval instars of the cotton leafworm

Laboratory and field larvae of *S. littoralis* were treated as second instar by leaf dip technique bioassay with different concentrations of AgNPS, Lambda – cyhalothrin and AgNPS loaded lambda-cyhalothrin (AgNPS@L-CYN) against the second instar larvae of lab and field *S. littoralis*. Serial aqueous concentrations of tested compounds were prepared with distilled water [16]. Clean castor bean leaves were dipped for 15 second in each compound concentration, and left to dry at room temperature then put in petri-dishes. Five replicates were carried out for each concentration and others were dipped in distilled water for the same period as control. Ten second instar larvae of each lab and field cotton leafworm were added to each treated, control dishs and preserved at room temperature.; Mortalities were recorded after 24 hr [17]. The corrected mortality percentages were calculate according to Abbott's formula [18] and the LC_{50} of tested compounds statistically were computed with SPSS program according to [19].

Histopathological effects of tested compounds on midgut tissues of the cotton leafworm, Spodoptera littoralis larvae.

All tested compounds at their sublethal concentration (LC_{50}) were applied to second instar larvae of the lab cotton leafworm (*S. littoralis*),

ten vivid 6th instar larvae from each treatment and control were placed in a 10% formalin solution in a tube and kept in the refrigerator until the microscopic examination. The morphological alterations of the midgut tissue structure and organization of each specimen were analyzed by microscopic examination and compared to the tissues taken from the control group.

Results and Discussion

Synthesis and characterization of AgNPS and AgNPS@L-CN

In this procedure, glucose and starch served as the dual role of both a reducing agent and a stabilizer. Then the core particles; AgNPS were jointed with Lambda-Cyhalothrin and gave AgNPS@L-CN composite, which was also produce in ethanol as opposed to harsh nonpolar solvents. Characterization of AgNPS and AgNPS@L-CYN were determined.

Practical application of nanosilver composition aims to reproduce a mono-dispersed nanoparticles with a well-determine shape. The critical steps of accurate selection of the reducing and stabilizer agents can be more facilely controlled when the nanoparticles are synthesizing. So that, water-soluble, highly mono-dispersed and spherical AgNPS were synthesized. That is a one-pot, method economical of formulation. Many types of polymers have been evaluated for designing polymer NP formulations, which are similar to those used in the pharmaceutical or cosmetic sectors, consisting mainly of polyesters (*e.g.,* poly-e-caprolactone and polyethylene glycol (PEG)), polysaccharides (*e.g.,* chitosan, alginates, and starch), and recently biodegradable materials of biological origin such as beeswax, corn oil, or lecithin or cashew gum [20,21]. It is fully telling this the reduction of silver ions in watery solution to silver nanoparticles is accompanied by the color turn (yellowish-brown or greyish) due to the excitation of surface plasmon vibrations in silver nanoparticles [22-26]. The color change, as an effect of agglomeration (assembly of the particles), is a well-understood phenomenon [27], A safer and economical insecticide delivery system was developed by facile formulation of starch-silver nanoparticle encapsulated dichlorovos and chlorpyrifos [14].

The most attractive NPs that are considered as carriers for delivery of pesticides are based on polymers (soft NPs), synthetic silica, titania, alumina, Ag, Cu, and natural minerals/ clays with nanoscale dimensions (inorganic or solid NPs).

UV-visible absorption spectroscopy

The first confirmation of silver nanoparticles (AgNPS) and nanolambda-cyhalothrin (AgNPS @ L-CYN) shown in Fig. 1. The obtained (AgNPS) and (AgNPS@L-CYN) both showed a broad peak at 423 and 433 nm, respectively, in the spectra, which are due to the excitation of surface plasmon resonance (SPR) of silver atoms. This has been reported to describe the collective excitation of conduction electrons in a metal [27–28].

The obtained results from UV-Visible Absorption Spectroscopy showed appearance of one broad peak in each AgNPS and AgNPS@P, which are due to the excitation of surface plasmon resonance (SPR) of silver atoms. Plant-mediated synthesis of NPs was confirmed by UV-visualization spectrophotometry, followed by Fourier transform infrared spectroscopy (FTIR) [29].

Nnemeka, et al.[14], found that silver nano-insecticide both showed a broad peak at 418 nm and 422 nm, respectively, in the spectra which are due to the excitation of surface plasmon resonance (SPR) of silveratoms. This has been reported to describe the collective excitation of conduction electrons in a metal. During their synthesis, three processes occurred in the reaction mixture (equations 1–3):

(1) reduction of the silver ions to silver nanoparticles during exposure to the glucose solution (2) encapsulation of dichlorvos and chlorpyrifos insecticides into the nanoparticles so formed and (3) the inclusion/sorption of the particles in the starch matrix which had been gelatinized due to the heat applied.

$$Ag_{(aq)}^{+} + starch_{(aq)} \; [Ag\ (starch)]_{(aq)} \qquad (1)$$

$$[Ag\ (starch)]_{(aq)}^{+} + C_5H_{11}O_5CHO_{(aq)} \; [Ag^0\ (starch)]_{(gel)} + C_5H_{11}O_5COOH(aq) \qquad (2)$$

$$[Ag^0\ (starch)](gel) + a.i. \quad [Ag^0\ (starch).a.i.]_{(s)} \qquad (3)$$

The dispersion of the silver ions in the starch matrix [eqn (1)] forms a stable gelatinous complex [Ag(starch)]+ which goes on to react with glucose (aldehyde, reductant) to form Ag nanoparticles (embedded in the starch) and gluconic acid [eqn (2)]. In this milieu, the active ingredient (a.i. = chlorpyrifos or dichlorvos) got (included/sorbed) to the matrix surfaces. This is a one-pot, economical method of formulation. It is well reported that the reduction of silver ions in aqueous solution to silver nanoparticles is accompanied by the colour change (yellowish-

brown or greyish) due to the excitation of surface plasmon vibrations in silver nanoparticles [22, 24-26, 29]. Nnemeka et al. [30] found the spectra exhibited a strong peak at 419 nm, which has been reported to be the characteristic of the Surface Plasmon Resonance (SPR) of silver nanoparticles [27, 31]. In metal nanoparticles like silver, there is free movement of electrons because the valence and conduction band lie close to each other, giving rise to surface plasmon resonance. SPR is the collective oscillation of electron of silver nanoparticles in resonance to light waves [31]. The colour of the composite was grey-black at the end of the synthesis.

UV-VIS absorption spectra proved to be quite sensitive to the formation of silver colloids because silver nanoparticles exhibeted an intense absorption peak due to the surface plasmon (it described the collective excitation of conduction electrons in a metal) excitation. The Uv-vis absorption spectra of corn starch AgNps is presented in Fig.2. Formation of strong absorption band centered at 400nm clearly suggests formation of Ag nanoparticles embedded in the starch matrix. For the broadening observed, according to literature broad peaks in the beginning of formation of AgNps, is attributed to very small particles (seeds) [32].

Transmission Electron Microscopy (TEM) imaging

The size and shape of the silver colloid particles measured by TEM imaging. A representative TEM image of these particles given in Fig. 2 (A). The particles are mostly spherical. From the sizes of particles, measured on the TEM images, an average size (diameter) of the silver nanoparticles loaded lambda-cyhalothrin (AgNPS@L-CYN) was 3.24 –15.21nm. The color change, as an effect of agglomeration (assembly of the particles), is a well-understood phenomenon, as shown in Fig. 2 (B).

The same phenomenon was appeared with Mhoamed et al., 2012. TEM images revealed that silver nanoparticles loaded Profenofos (AgNPS@P) are mostly spherical with very small size. TEM micrographs were determine the morphology of nanoparticles and the obtained spheres [14, 25, 26]. Nnemeka et al. [30] found that the size and shape of the AgSRF within the nanoacomposite were recorded by the HR-TEM. The images depict a spherical and well dispersed nanoparticles with size range of 23-35 nm. Hamedi et al. [26] noted the properly magnified bar size of 20nm making the image visualization very clear [33]. HR-TEM has been reported to be the best method of morphology determination [25].

Studies with transmission electron microscopy (TEM) of NP effects on plants confirmed their penetration into the cell organelles and localization of the NPs at mitochondria or nucleolus in both plant and insect tissues, which suggests that they can be used for targeted delivery of pesticides or fertilizers [7, 34].

Fourier Transform Infrared Spectroscopy (FT-IR)

FTIR spectroscopy of the starch silver nanoparticles registered agree with the functional groups of glucose and starch used in the reduction and capping/stabilization as shown for AgNPS@L-CYN (A) and L-CYN(B) (Fig. 3) The broad and strong band at 3346.54 & 3442.66 cm^{-1} is due to the O–H stretching vibration. Peaks at (2960.04 & 2926.77) and (2959.84 & 2926.16) cm^{-1} for AgNPS@L-CYN and L-CYN,

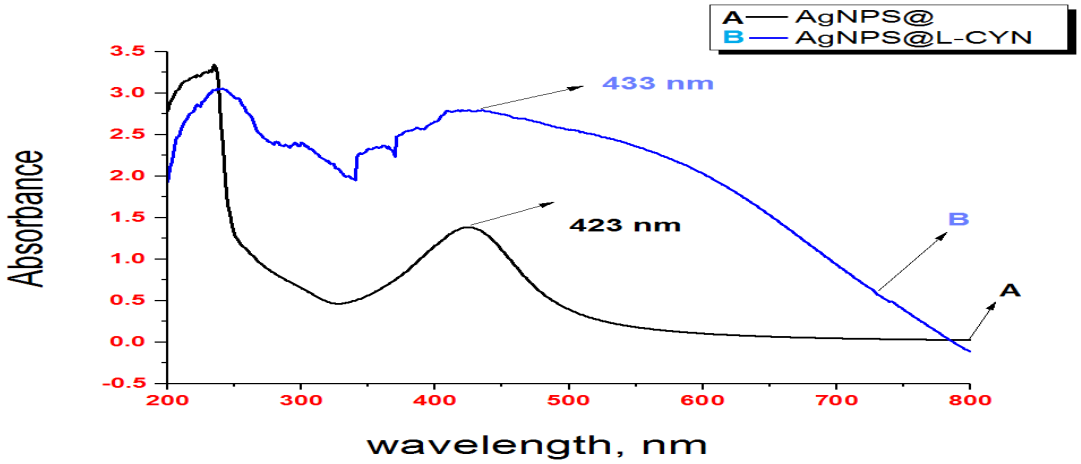

Fig. 1. The transmission electron micrographic image of AgNPS alone (A) and AgNPS@L-CYN (B).

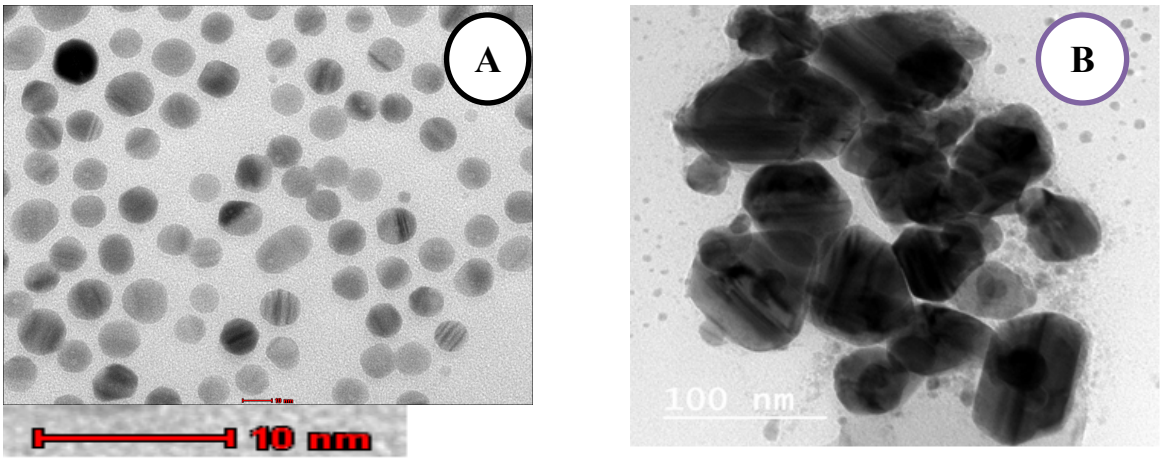

Fig. 2. The transmission electron micrographic image of AgNPS alone (A) and AgNPs @L-CYN (B)

resp., corresponded to the asymmetrical and symmetrical bending frequency of the methylene groups. Figure 3 upholds the existence of silver nanocomposite loaded films due to the existence of additional peaks at 1404 cm^{-1} and 842 cm^{-1} corresponding to insecticides. The shifting of the peak is due to the formation of the co-ordination evidence inter the silver atom and the electron loaded groups (oxygen/carbonyls) sitting in starch. This reason an increase in evidence length and frequency [35]. The L-CYN and (AgNPS@L-CYN) showed this band at (1607.71 and 1607.93) cm^{-1}, resp., found that 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 L-CYN showed these bands at (2959.84 and 2926.16) cm^{-1}. Also, AgNPS@L-CYN showed these bands at (2960.04 and 2926.77 sh) cm^{-1}. This change may be due to the effect of neighboring carboxylic group, which involved in linkage with AgNPS [10].

C-H deformation (δCH3) appeared as strong multiple bands of high intensity around 1380 cm^{-1} and 1465 cm^{-1} for symmetric and asymmetric deformation, respectively. The L-CYN and (AgNPS@CYN) under investigation showed asymmetric deformation vibration δCH3asymm bands at 1457.54 and 1459.59 cm^{-1}. This band may be also merge with C-F band that may be appear at 1000- 1400 cm^{-1} [35]. The unbounded ‘’free’’ hydroxyl group showed strong absorption band in the 3650-3580 cm^{-1} region. The L-CYN and (AgNPS@L-CYN) showed bands at 3442.66 and 3446.54cm^{-1}, resp.

υC=O of saturated aliphatic esters fall in the range 1750-1730 cm^{-1}. The L-CYN and (AgNPS@L-CYN) showed the C=O band at 1743.34 and 1742.34 cm^{-1} as very sharp band. The υC=O of AgNPS@L-CYN become stronger than free L-CYN which could be taken as an evidence for the participation of carbonyl ester group in linkage with AgNPS.

In this study, the IR spectra of C-O bond for L-CYN and AgNPS@L-CN changed -and weaken comparing with the free L-CYN, indicating the contribution of the C-O group in the chelation with AgNPS**.** The IR spectra for CN bond ascribed to be appeared in L-CYN free and AgNPS but disappeared in AgNPS@L-CYN. This might be indication for involving nitrile group in linkage with AgNPS .This change may be due to the effect of neighboring carboxylic group, which involved in linkage with AgNPS [10]. The L-CYN and (AgNPS@CYN) under investigation, the υC=O of AgNPS@L-CYN become very stronger than free L-CYN which could be taken as an evidence for the participation of carbonyl ester group in linkage with AgNPS. Esters of aromatic acids absorb strongly in the 1310-1250 cm^{-1} region [36].

Toxic effect of lambda-cyhalothrin, nanosilver and Nanolambda-cyhalothrin against second instar larvae

The larvicidal activities of synthetic pyrethroid Lambda-Cyhalothrin (L-CYN) active ingredient, silver nanoparticle (AgNPS) alone and silver nanoparticle loaded Lambda-Cyhalothrin (AgNPS@L-CYN) were studied against *S. littoralis* second instar larvae for lab and field. The results in Table 1 revealed that mortality rate increased with the increase in concentrations of tested compounds. The mortality percentages of AgNPS ranged between

5 to 50 % of lab larvae with concentrations of 124.9 to 999.0 ppm from AgNPS and 5 to 30 % of field larvae with concentrations 124.9 to 1498.5 ppm from AgNPS (Table 1 and Fig 3). The mortality percentages of lab larvae ranged between 10 to 95 % with AgNPS@L-CYN concentrations of 0.01 to 0.32 ppm (Fig 4), compared with those 5 to 90 % of Lambda-Cyhalothrin (L-CYN) with concentrations 0.29 to 9.38 ppm. In concerning to field strain, the percentages mortality recorded 10 to 95 % for AgNPS@L-CYN with concentrations 0.08 to 2.5 ppm, compared with those 5 to 90 % for lambda-cyhalothrin with concentrations 2.34 and 75 ppm (Fig 5,6). The results of the concentration dependent assay suggested that, Relative Resistance ratio (RRr) decreased more than 35 times for lab strain, where the value LC50 values were was 1.76 and 0.05 ppm for (L-CYN) and AgNPS@L-CYN, respectively. While RRr decreased more than 37 times for field strain, the value LC50 was 15.04 and 0.41ppm for (L-CYN) and AgNPS@L-CYN, respectively.

The results denoted that the field strain was more resistant for (AgNPS), (L-CYN) and (AgNPS@L-CYN) than lab strain. The results of the concentration dependent assay suggested that, Relative Resistance ratio (RRr) decreased more than 35 and 37 times for lab and field strains, respectively. This formulation would produce a synergetic effect to combat the adverse effect of the conventional insecticides to the environment that silver-cyhalothrin nanocomposite was more efficient in controlling mosquito larvae than free cyhalothrin [14, 36].

Sooresh et al. [11] highlighted the conjugation of monodispersed stable silver nanoparticles to the insecticide deltamethrin. It also explored the possibilities of using this newly created and effective pesticide encapsulated nanosilver (PENS) in the fight against disease carrying insects. Effective insect vector control is essential to prevention of vector-borne infectious diseases. The results from this mosquito bioassay showed that mosquitoes exposed to both DM and PENS at 9 X 10^{-3} ppm resulted in 100% death after 24 h. Four hours of mosquito, exposure to 9 X 10^{-4} ppm DM resulted in 5% knockdown and 95% death; while 4 hr of mosquito exposure to 9 X 10^{-4} ppm PENS resulted in 15% knockdown and 85% death. At this concentration, 100%mosquito

TABLE 1.Toxicity effect of silver nanoparticle (AgNPS) & lambda-cyhalothrin compared with nanolambda-cyhalothrin (AgNPS@L-CYN) against second instar larvae of the lab and field cotton leafworm, Spodoptera littoralis.

Strain	Silver (AgNPS)				lambda-cyhalothrin (LCYN)				nanolambda-cyhalothrin (AgNPS@L-CYN)				
	Con. (ppm)	M %	LC_{50} (ppm)	RR*	Con. (ppm)	M %	LC_{50} (ppm)	RR*	RRr**	Con. (ppm)	M %	LC_{50} (ppm)	RR*	** RRr
lab					0.1463	5				0.005	10			
					0.2925	20				0.01	20			
	124.88	6			0.585	40	0.88	---	35.2	0.02	40	0.027	----	1
	249.75	16	1000.94	----	1.17	60				0.04	60			
	499.5	30			2.34	80				0.08	85			
	999	50			4.69	90				0.16	95			
					2.34	5				0.08	10			
	124.88	2			4.69	20				0.16	40			
	249.75	6			9.375	40	15.04	17.1	50.13	0.32	60	0.3	11.11	1
Field	499.5	12	4271.83	4.27	18.75	50				0.63	70			
	999	20			37.5	80				1.25	80			
	1498.5	28			75	90				2.5	95			

RR* (Resistance Ratio) = LC_{50} of the field strain / LC_{50} of the lab strain

RRr** (Relative Resistance Ratio) = LC_{50} of the strain / The lowest LC_{50} values of the same strain LC_{50} values of the same strain

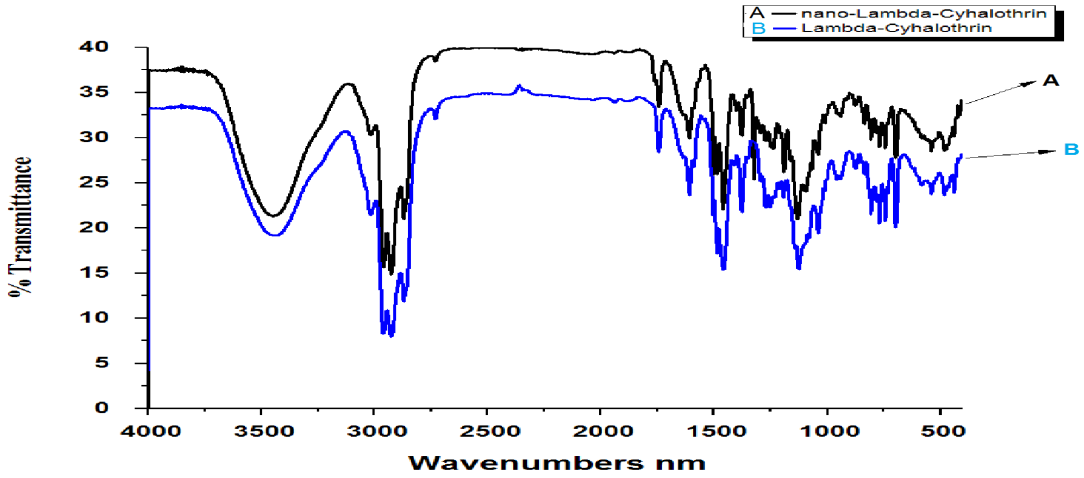

Fig. 3. The FT-IR spectra for AgNPS@L-CYN (A)and L-CYN (B).

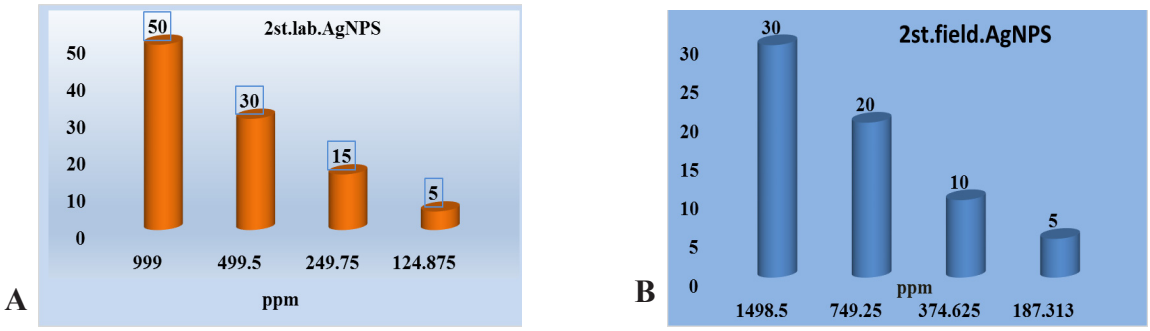

Fig. 4. The mortality percentage of second instar larvae for AgNPS to (A) lab and (B) field strains of S. *littoralis.*

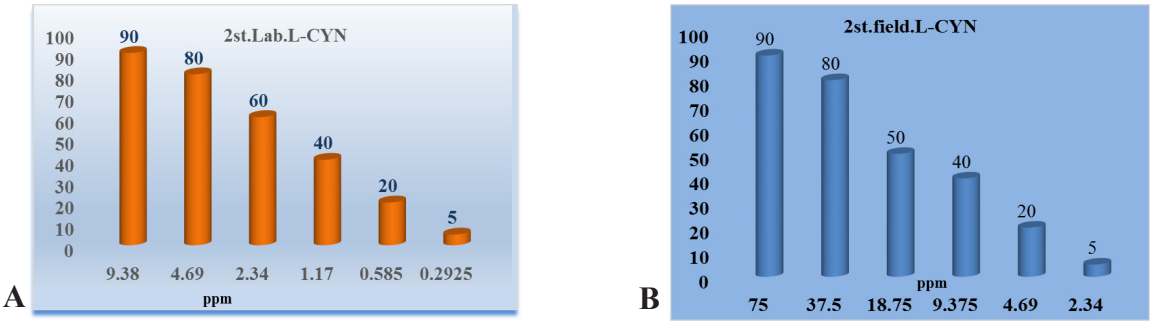

Fig. 5. The mortalities percentage of second instar larvae for L-CYN to (A) lab and (B) field strains of S. *littoralis.*

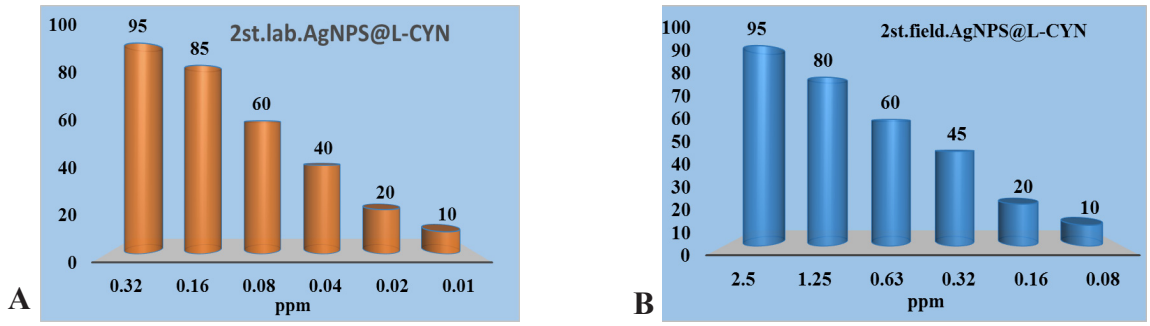

Fig. 6. The mortalities percentage of second instar larvae for AgNPS@L-CYN to (A) lab and (B) field strains of S. *littoralis.*

death was observed in DM vial while 95% death (with 5% knockdown) was observed after PENS exposure at the end of the 24 hr period. These results proved the effectiveness of the nanoconjugate in comparison to DM over time and dose. The remaining 5% were knocked down proving its effectiveness. These results showed that the newly developed nanoconjugate PENS did not inactivate the primary function of the pesticide and was able to kill mosquitoes even at low concentrations.

The most attractive NPs that are considered as carriers for delivery of pesticides based on polymers (soft NPs), synthetic silica, titania, alumina, Ag, Cu, and natural minerals/ clays with nanoscale dimensions (inorganic or solid NPs). Some common paradigms of insecticides explored using this nanotechnology approach were essential oils, including neem oil [37-39].

Many types of polymers have been evaluated for designing polymer NP formulations, which are similar to those used in the pharmaceutical or cosmetic sectors, consisting mainly of polyesters (*e.g.,* poly-e-caprolactone and polyethylene glycol (PEG)), polysaccharides (*e.g.,* chitosan, alginates, and starch), and recently biodegradable materials of biological origin such as beeswax, corn oil, or lecithin or cashew gum [20-21].

Histopathological effects of tested insecticides on midgut tissues the cotton leafworm, Spodoptera littoralis larvae

The midgut is the middle portion of the insect digestive system where food digested and absorbed. Some epithelium cells produce enzymes and other absorbs the digested food [40]. In this part of digestive tract the most nutrient absorbed. Examination of midgut tissues in the 6^{th} instar larvae of *S. littoralis* treated as second instar by light microscope was shown in figures 7 – 10. The photomicrograph of transverse section in untreated larvae midgut revealed that the gut wall have two layers of muscular tissue (Circular and longitudinal). These layers contained connective tissue between the muscle fibers. After the muscular tissues there was a single layer of epithelial cells which surrounded by thin basement membrane. The epithelial cells constituted of regenerative, goblet and columnar cells. The regenerative cells had oval and conspicuous nuclei near the center of them. The small goblet cells with reduced granular cytoplasm and spherical nuclei. These goblet cells were scattered between the regenerative cells. The columnar cells were closed to lumen of the gut and peritrophic membrane, also, these cells had a striated microvilli (brush border) (Fig. 7).

Effects of lambda – cyhalothrin treatments

LC_{50} treatment of lambda – cyhalothrin insecticide caused some changes in cells of treated 6^{th} instar larvae midgut, the epithelium cells detached from their basement membrane in some areas and some cells were destroyed and emptied their cytoplasmic contents in the space between the two membranes with destroyed brush border and partial disappearance of the peritrophic membrane (Fig. 8).

Effects of nanosilver treatment

The LC_{50} treatment of nanosilver particles produced thickness and deformation of midgut epithelial cells in treated larvae. Also, basement membrane, some columnar cells, microcell and peritrophic membrane were destroyed (Fig. 9).

Effects of nano-lambda – cyhalothrin treatments

The caused the most deformation in midgut tissues of larvae was observed the LC_{50} treatment in nano-lambda- cyhalothrin. This treatment caused highly destroying of all epithelial cells, their microvilli both basement and peritrophic membranes (Fig. 10).

The treatment of *S. littoralis* larvae with LC_{50} of chemical insecticides produced some deformations in midgut tissues of these larvae. The treatment of the cotton leafworm larvae with nanosilver particles caused high destroying of midgut tissue, while the chemical pesticides bounded to nanosilver particles (nano-lambda-cyhalothrin) produced a highly damage of midgut tissues in treated larvae. When the digestion process is intensified in the midgut, so it is the most regionwhich attacked with foreign substances. The midgut epithelial cells showed signs of apoptosis manifested as shrinkage of the cells and the presence of condensed chromatin with some vacuolization. This process is considered as the proper mechanism of cells against pathogens and toxic compounds. The effect of sublethal doses of neem oil on the midgut and peritrophic matrix

at concentrations of 0.006, 0.05 and 0.4 % was invastigated [41-43]. Methomyl treatments led to basement and peritrophic membrane detachment and destruction, appearance of numerous vacuoles and destruction of epithelial cells that emptied their cytoplasmic content in the midgut lumen of treated 4th instar larvae of *S. littoralis* [44]. A histological study on midgut from *S. littoralis* larvae fed on castor bean leaves treated with recommended field rate of spinsad showed some alterations occurred after 48 and 96 hrs. of treatment compared to the normal midgut of the control insects. The histological changes included degeneration in the epithelial lining of the midgut and in the peritrophic membrane. Such histological effects are presumed to be responsible for the reduction for the reduction in growth and food utilization caused by Spinosad [45].

Conclusions

Our findings denoted that the feasibility of using (AgNPS@L-CYN) in controlling the cotton leafworm, *Spodoptera littoralis*, which had very low doses than L-CYN insecticide and AgNPS without using any additives. These results probably extrapolated to suggest this AgNPS@L-CYN could avail selectively as a prospect insecticide.

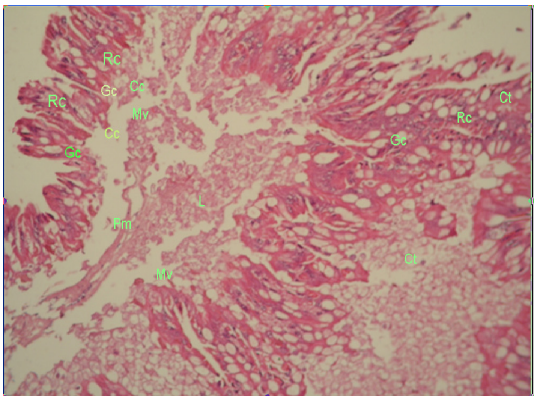

Fig. 7. Photomicrography of partial transverse section from untreated S. *littoralis* 6th instar larvae midgut (stained with Hematoxylin and Eosin, H + E 200x).

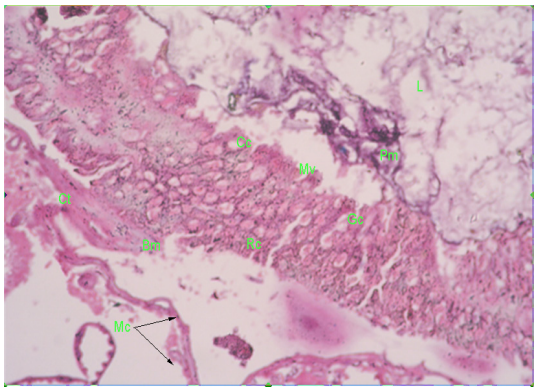

Fig. 8. Photomicrography of partial transverse section from midgut of S. *littoralis* 6th instar larvae treated with LC50 of Lambda-cyhalothrin insecticide (stained with Hematoxylin and Eosin, H + E 200x).

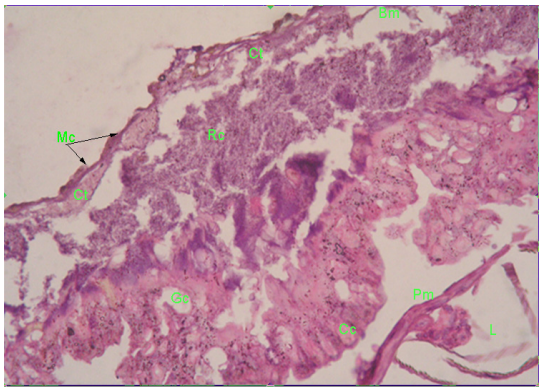

Fig. 9. Photomicrography of partial transverse section from midgut of S. *littoralis* 6th instar larvae treated with LC50 of nanosliver particles (stained with Hematoxylin and Eosin, H + E 200x).

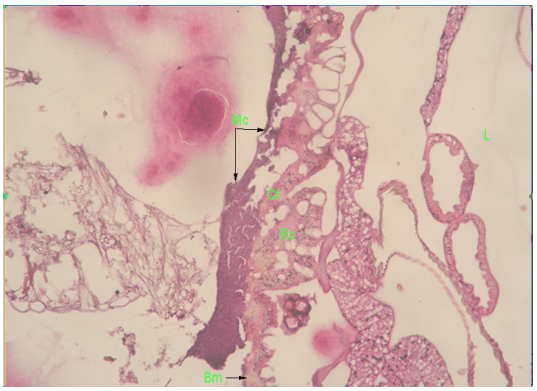

Fig. 10. Photomicrography of partial transverse section from midgut of S. *littoralis* 6th instar larvae treated with LC50 of nano – Lambda – cyhalothrin insecticide (stained with Hematoxylin and Eosin, H + E 200x).

* Basement membrane (Bm), Columnar cells (Cc), Connective tissue (Ct), Goblet cells (Gc), Lumen of gut (L), Muscular layers (Mc), Microvilli (Mv), Peritrophic membrane (Pm) and Regenerative cells (Rc).

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تأثير اللمبداسها لوثرين كمبيد نانو على دودة ورق القطن *Spodoptera littoralis* (Boisd).

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تعتبر دودة ورق القطن Spodoptera littoralis (Boisd.) اكثر الافات الضارة على كثير من المحاصيل النباتية. استخدام المبيدات بكثرة ادى الى زيادة المقاومة الافات لهذه المبيدات. هذه الدراسة محاولة لتصنيع مركبات نانومترية من مبيدات الآفات تتميزة بكفاءة عالية جداً مقارنةً بمنتجاتها الأصلية. هذه طريقة تعتمد على استخدام جزيئات السيلفر النانومترية محمل عليها مبيد بيروثرويد لمبداسهالوثرين. تم اجراء الفحوصات للمركب الناتج باستخدام جهاز(via TEM and FT-IR techniques). المركب الجديد الناتج تم اختباره على العمر اليرقى الثانى للسلالتين المعملية والحقلية لدودة ورق القطن. النتائج تدل على ان مركب النانولمبداسهالوثرين الناتج اكثر فاعلية على يرقات دودة ورق القطن من مبيد اللمبداسهالوثرين بمفرده. وجود ان التركيزات المستخدمة فى التحكم لدودة ورق القطن تقل اكثر من 37 ضعف. هذه النتيجة مفيدة لتقليل ظاهرة المقاومة الافات لهذه المبيدات وبالتالى يمكن الحد من التلوث البيئى بالمبيدات .