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Toxicological, biological and biochemical effects of two nanocomposites on cotton leaf worm, *Spodoptera littoralis* (Boisduval, 1833)

Soheir F. Abd El-Rahman[1], Sayeda S. Ahmed[2*], Mahmoud H. Abdel Kader[3]

[1] Plant Protection Research Institute, Agriculture Research Center, Dokki, Giza, Egypt.

[2] Department of Economic Entomology and Pesticides, Faculty of Agriculture, Cairo University, Giza, Egypt.

[3] Department of Photochemistry, National Institute of Laser Enhanced Science, Cairo University, Giza, Egypt.

* Corresponding author: sayeda01@hotmail.com

Abstract: Cotton leaf worm, *Spodoptera littoralis* (Boisduval, 1833), is one of the most dangerous pests in Egypt, causing economically significant losses of different crops. The present study was aimed to evaluate toxicological, biological and biochemical effects of two nanocomposites, Silver (Ag) and graphene oxide (GO) nanomaterials over magnesium chlorophillin (Mg-Chl/Ag and Mg-Chl/GO) at three concentrations (1, 10, 100 ml/L) against 2nd instar larvae of *S. littoralis*. The results showed that larval mortality rate was positively correlated with the increase of concentrations of the tested nanocomposites and time after exposure. The mortality rate in nanographene oxide composite were higher than nano silver. LC_{50} (lethal concentration of 50% of a group of test larvae) values were 10.27 and 16.14 mg/L at Mg-Chl/Ag and Mg-Chl/GO compound at 2 h exposure to light. Some biological aspects of *S. littoralis* resulted from the treated 2nd instars larvae with two nanocomposites were recorded. Larval, pupal durations, pupation, adult emergence rate and pupal weight were significantly lower in all concentrations compared to untreated larvae. All tested samples showed decrease in total carbohydrates, total proteins and total lipids. Obtained results suggest that using silver and graphene oxide nanomaterials over the magnesium chlorophillin would be a useful component for controlling *S. littoralis.*

Keywords: Cotton leaf worm, bio-pesticides, nanotechnology, biology

Introduction

The Egyptian cotton leaf worm, *Spodoptera littoralis* (Boisduval, 1833), a moth of the family Noctuidae (Lepidoptera) is considered one of the most serious, destructive pests infesting a wide range of cultivation. This insect is distributed in Egypt, in other Middle East countries as well as in temperate zones in Asia and Africa (Salama *et al.* 1990). Apart from cotton, the larvae of *S. littoralis* infest more than 90 important plant species belonging to 40 families including important field crops and various fruits and ornamental trees (Tiessen 2012). This insect causes great losses in quantity and quality of the attacked crops (Azab *et al.* 2001, Kandil *et al.* 2003). The larvae causes a variety of damage as a leaf feeder, sometimes as a cutworm on seedlings, and occasionally damaging the bolls (Darvishzadeh *et al.* 2014). Frequent use of conventional insecticides to control this insect has led to the development of a generation's resistance to them (Nkya *et al.* 2014). Therefore, different safe control methods and effective insecticides after the recent increases in environmental pollution and insect resistance are needed (Ahmed *et al.* 2019).

Nanotechnology has become one of the most promising new approaches for pest control (Owolade *et al.* 2008). In the past decade, nanomaterials (NMs) have provided

a wide range of novel pesticide formulations or pesticide metallic nanoparticles (NPs), such as nanoemulsion, nanocapsules, nanosuspension, and metallic oxide NPs. These materials had higher efficacy on pest control and lesser harmful effects on the environment compared to the traditional ones (Buffle 2006). The recent knowledge of nano-technology materials may result in developing toxic effects on various insect species (Tunçsoy 2018). Stadler *et al.* (2010) showed that nano alumina could be successfully used to control stored grain pests. Silver nanoparticles (AgNp) can be used as a valuable toll in pest management programs of cowpea seed beetle, *Callosobruchus maculates* (Fabriscus, 1775) (Rouhani *et al.* 2012). Chakravarthy *et al.* (2012) used Ag and TiO_2 nanoparticles against 2nd instar of *Spodoptera litura* (Fabriscus, 1775) larvae in the laboratory and found that the two tested nanoparticles proved effective against *S. litura* larvae and hence can be selectively used for suppression of the pest. Also, hydrophobic nano-silica was effective against *S. littoralis* and could be useful component of an integrated pest management strategy on tomato plants (El-Bendary *et al.* 2013). El-Helaly *et al.* (2006) evaluated the effect of nano-silica in comparison with Silica+Diazinon as a recommended insecticide on newly hatched larvae of *S. littoralis* as foliar spray on squash plants in the greenhouse. Sabbour & Hussein (2016) reported that both silica gel and nano silica gel decrease the infestation by *Tuta absoluta* (Meyrick, 1917) under laboratory, green house and field conditions. Zaki *et al.* (2017) examined that the titanate nanotubes and its composites with *Bacillus thuringiensis* (Bt-TNTs) as a novel nanopesticides to resist cotton leaf-worm *S. littoralis*. Silver (Ag) and graphene oxide (GO) nanomaterials over copper chlorophillin (Cu-Chl/Ag and Cu-Chl/GO) were most effective for controlling *Thrips tabaci* Lindeman, 1889 on onion field (Merghany *et al.* 2019) and also used to control *S. littoralis* in cotton fields (Ahmed *et al.* 2018). Hashem *et al.* (2019) evaluated three synthesized silica nanoparticles against the second instar larvae of *S. littoralis*. Results showed that, SiNP hydrophobic, SiNP hydrophilic, SSiNP hydrophilic had high effects on biochemical parameters.

Therefore, the present study was planned to investigate the efficacy of selected nanocomposites on some toxicological, biological and biochemical parameters of *S. littoralis* under laboratory conditions.

Materials and methods

Insects culture

A laboratory strain of *S. littoralis* has been reared in the Plant Protection Research Institute, Agricultural Research Centre, Dokki, Giza. Insects were reared on castor-oil leaves, *Ricinus communis*, under controlled conditions in an incubator at 25 ± 2°C, 65 ± 5% R.H. and 8 h light: 16 h darkness. Larvae were supplied with fresh castor bean leaves as a source of food which was provided daily until pupation. Pupae were kept in clean jars (500 g) till adult emergence. Adults were kept in chimney glass cages, fed on 10% honey solution and freshgreen leaves of *Nerium oleander* were provided for egg laying (Osman *et al.* 2015).

Nanocomposites

Both silver (Ag) and graphene oxide (GO) nanomaterials were selected to be used in the formation of the natural extract porphyrin-based photosensitizer magnesium chlorophillin (Mg-Chl). Electrostatic deposition method was used for grafting the Mg-Chl over the two nanomaterials to form the required photosensitizer nanocomposites (Mg-Chl/Ag and Mg-Chl/GO) according to the methods described by Farghali *et al.* 2015. Three concentrations of all nanocomposites are used [10^{-3} (100 ml/L), 10^{-4} (10 ml/L) and 10^{-5} (1 ml/L)].

Characterization of the nanocomposites

The size, morphology and composition of the two tested nanocomposites were determined by high resolution transmission electron micrograph (JEOL 20100) (HR-TEM) as shown in Fig. 1. The the size of the Mg-Chl/Ag nanocomposite was found to be 30 nm compared to 10 nm of the silver nanoparticles alone. On the other hand, the graphene nanocomposite was charactrized by nanosheets form. The particle size distribution is most suitable for spherical or cubic nanoparticles and not for sheets.

Bioassay

Three concentrations of nanocomposites was used against 2nd instar larvae of *S. littoralis* under light and dark conditions by leaf dipping technique. Leaf discs of castor were dipped in each concentration for 2 minutes and placed after completing the dry in glass jars (250 ml capacity). Each concentration had three replicates with 50 larvae for each. The treated leaves were replaced by untreated ones after 24 hours of treatment and the larvae were directly exposed to sunlight. The sunlight period was two hours for each concentration (Light treatments), similar treatments were left without exposing to light (Dark treatments). Using two controls (larvae fed on untreated leaves), one has been exposed to light and the other is left without exposing. All treatments were kept at 25 ± 2°C and 65 ± 5% R.H. Numbers of dead larvae were recorded at 1, 3, 5, 7, 10 and 15 days after exposure to calculate mortality percentages which were corrected according to Abbott's formula (Abbott 1925). The LC_{50} and LC_{90} of all treatments were determined.

Biological aspects

To determine the duration of larvae alive after treatments, they were introduced separately into glass jars containing untreated fresh castor leaves and kept at 25°C and 65 ± 5% R.H. All the larvae received a daily check until pupation. The pupae were carefully weighted individually and kept under previously specified conditions, with checking daily until adult emergence to record the pupal duration, pupation rate and adult emergence rate.

Biochemical effects

Preparation of samples for biochemical studies: The biochemical essay was done after treated 2nd instar larvae with LC_{50} values of two tested nanocomposite and exposed to 0 and 2 h to sunlight. After 48 h from treatments, 0.5 g weight of alive treated and untreated larvae were frozen until need. Frozen larvae were homogenized in distilled water using a Teflon homogenizer. Homogenates were centrifuged at 8 000 r.p.m. for 15 minutes at 2°C in a refrigerated centrifuge. The deposits were homogenates and supernatants, which are referred to as total proteins, total lipids and total carbohydrates.

Determination of total proteins: Changes in the level of total proteins in the tissue of larvae were determined by the method of Bradford (1976). Sample solutions 50 µl were pipetted into a test tube and the volume was adjusted to 0.1 ml with phosphate buffer (pH 6.6). Five ml of protein reagent were added to the test tube and the contents were mixed (inversion or vortexing). The absorbance at 595 nm was measured after 2 min and before 1 h against blank prepared from 0.1 ml of phosphate buffer (pH 6.6) and 5 ml of protein reagent. The weight of protein was plotted against the corresponding absorbance resulting in standard curve used to determine the protein in unknown samples.

Determination of total carbohydrates: Total carbohydrates were determined by the method described by Singh & Sinha (1977). Sample solution 100 µl was diluted to one ml with H_2O, then 5 ml anthrone reagent. A blank containing 1.1 ml of H_2O and 5 ml of anthrone reagent was placed. All tubes were placed in

a boiling water bath for 10 min then were left to cool for 15 min at room temperature.

Determination of Total Soluble Lipids (TSL): Total lipids were estimated according to Knight *et al.* (1972) using phosphovanillin reagent**.** Sample solution 250 µl was added to concentrated sulfuric acid (5 ml) in a test tube and heated in a boiling water bath for 10 min. After cooling to room temperature, the digest (500 µl) was added to phosphovanillin reagent (6.0 ml). After 45 min color was measured at 525 nm against reagent blank prepared from 500 µl distilled water and 6.0 ml phosphovanillin reagent. The result is expressed as mg lipid/insect.

Statistical analysis

Data were statistically analyzed using an analysis of variance (ANOVA), with the means separated using Duncan's Multiple Range criterion ($P<0.05$) between all treatments. F value and Duncan tests using SPSS computing program were adopted to determine the significant between treatments as described by Snedecor & Cochran (1967). Bioassay data were pooled and analyses (LC_{50} and LC_{90} confidence limit values) according to the methods described by Noack & Reichmuth (1978).

Results and discussion

Toxic effects of nanocomposites on S. littoralis larvae

The obtained results in Tables 1 and 2 summarize the effect of nanocomposites at different concentrations against the 2nd larval instar of *S. littoralis*. Data clearly showed that, the larval mortality rate was positively correlated with the concentrations of the tested nanocomposites and time after exposure. Generally, mortality rate was higher as concentrations and time after treatment increased. The percentages of mortalities were significantly affected in all the treatments in comparison with control. Also, the mortality rate in nanographene oxide composite was higher than nano silver. At dark treatment, mortality reached 58.3% after 10 days of treatment at the highest concentration of Mg-Chl/GO while it reached 55% at the same concentration of Mg-Chl/Ag (Table 1). Sunlight exposures had also higher effect than the darkness treatments. In two hour exposure treatment, the highest concentration (10^{-3}) of Mg-Chl/GO and Mg-Chl/Ag were 96.67% and 91.60%, respectively after 15 days of treatment (Table 2).

In previous studies Ahmed *et al.* (2019) studied the larvicidal activities of silver nanoparticles (AgNPS) alone and silver nanoparticle-loaded profenofos (AgNPS@P) against 2nd larvae of *S. littoralis*. The results shown that, the mortality percentages ranged between 6% and 50% with AgNPS concentrations of 124.88–999 ppm. While these percentages ranged between 10% and 90% with AgNPS@P concentrations of 0.01–1.44 ppm. These results were consistent with current study that cleared the mortality percentages reached 91.6% when treated 2nd instar larvae with 10^{-3} concentration of silver nanoparticle-loaded magnesium chloro-phylline. Other findings of Abou-Elkassem *et al.* (2016) indicated that silver-cyhalothrin

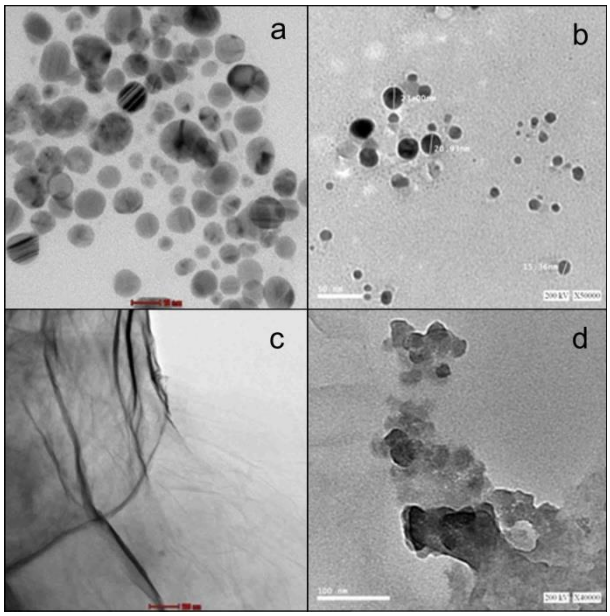

Fig. 1. TEM image of: a) Ag alone; b) Mg-Chl/Ag; c) Graphene oxide alone; d) Mg-Chl/GO.

Table 1. Mortality percentages of *S. littoralis* 2nd instar larvae treated with Mg-Chl/Ag and Mg-Chl/GO nanocomposite at dark. Values with the same letters are insignificantly different.

Treatment	Mortality rate [%] [days]					
	1	3	5	7	10	15
Mg-Chl/Ag 10^{-3}	26.70 ± 3.33 cd	31.67 ± 1.67 c	36.67 ± 1.67 bc	41.67 ± 1.67 bc	55.00 ± 2.89 cd	55.00 ± 2.88 cd
Mg-Chl/Ag 10^{-4}	20.00 ± 2.89 b	25.00 ± 2.89 c	33.37 ± 3.33 bc	40.00 ± 5.00bc	46.67 ± 1.67 bc	51.67 ± 1.66 bcd
Mg-Chl/Ag 10^{-5}	11.70 ± 1.67 b	15.00 ± 2.89 b	26.67 ± 4.41 b	35.00 ± 2.89 b	38.30 ± 1.67 b	45.00 ± 0.00 b
Mg-Chl/GO 10^{-3}	31.70 ± 1.67 d	33.30 ± 4.41 c	38.33 ± 6.01 c	46.67 ± 4.40c	58.30 ± 4.41 d	58.30 ± 4.40 d
Mg-Chl/GO 10^{-4}	26.70 ± 1.67 cd	31.67 ± 1.67 c	36.67 ± 1.67 bc	45.00 ± 2.89 bc	51.60 ± 4.40 cd	55.00 ± 2.80 cd
Mg-Chl/GO 10^{-5}	20.00 ± 2.89 c	25.00 ± 2.89 c	30.00 ± 2.89 bc	38.30 ± 3.30bc	40.00 ± 2.89 b	48.30 ± 1.60 bc
Control	0.00 ± 0.00 a	3.33 ± 1.67 a	5.00 ± 0.00 a	8.30 ± 1.67 a	11.67 ± 1.67 a	11.67 ± 1.60 a
F value	22.385	15.772	11.644	15.202	27.029	40.167
P	0.0	0.0	0.0	0.0	0.0	0.0

Table 2. Mortality percentages of *S. littoralis* 2nd instar larvae treated with Mg-Chl/Ag and Mg-Chl/GO nanocomposite at 2 h exposure. Values with the same letters are insignificantly different.

Treatment	Mortality rate [%] [days]					
	1	3	5	7	10	15
Mg-Chl/Ag 10^{-3}	45.00 ± 2.89 bc	51.67 ± 1.67 c	70.00 ± 2.89 de	81.60 ± 1.67 d	91.67 ± 1.67 e	91.60 ± 1.67 de
Mg-Chl/Ag 10^{-4}	40.00 ± 2.89 bc	48.30 ± 1.67 bc	53.30 ± 3.30 c	63.30 ± 1.67 c	66.67 ± 1.67 bc	81.67 ± 1.67 bc
Mg-Chl/Ag 10^{-5}	33.30 ± 8.30 b	36.67 ± 6.67 b	42.60 ± 5.36 b	46.67 ± 4.40 b	61.60 ± 4.40 b	75.00 ± 2.80 b
Mg-Chl/GO 10^{-3}	56.67 ± 3.30 c	65.00 ± 2.80 d	76.60 ± 1.60 e	95.00 ± 2.80 e	95.00 ± 2.80 e	96.67 ± 1.67 e
Mg-Chl/GO 10^{-4}	40.00 ± 10.00 bc	56.60 ± 6.00 cd	66.60 ± 3.30 de	76.67 ± 3.30 d	80.00 ± 5.77 d	85.00 ± 5.00 cd
Mg-Chl/GO 10^{-5}	38.30 ± 4.40 bc	55.00 ± 2.80 cd	60.00 ± 2.80 cd	73.30 ± 1.67 d	76.60 ± 1.67 cd	80.00 ± 0.00 bc
Control	3.30 ± 1.67 a	7.60 ± 2.67 a	11.00 ± 1.00 a	11.00 ± 1.00 a	15.00 ± 2.89 a	15.00 ± 2.80 a
F value	8.595	22.823	48.064	112.029	65.143	105.389
P	0.0	0.0	0.0	0.0	0.0	0.0

Table 3. Toxicity of the tested compounds against the 2nd instar larvae of *S. littoralis*.

Exposure time to light [h]	Treatments	LC_{50}			LC_{90}			Slope
		[mg/L]	Confidence limits [mg/L]		[mg/L]	Confidence limits [mg/L]		
			Lower	Upper		Lower	Upper	
0	Mg-Chl/Ag	20.206	5.213	253.8	$1\,820.5*10^{3}$	12 328.3	$3\,162.9*10^{13}$	$0.23 \pm 8.98*10^{-2}$
	Mg-Chl/GO	33.983	10.904	418.7	$841.7*10^{3}$	10 735.8	$3\,672.1*10^{10}$	$0.29 \pm 9.00*10^{-2}$
2	Mg-Chl/Ag	10.270	4.016	26.6	18 073.8	1 576.0	$9\,869.9*10^{3}$	$0.39 \pm 9.11*10^{-5}$
	Mg-Chl/GO	16.136	4.575	100.8	508 015.2	7 342.2	$2\,472.0*10^{9}$	$0.28 \pm 9.00*10^{-2}$

Table 4. Some biological aspects for *S. littoralis* larvae treated with nanosilver and graphene over magnesium chlorophylline (Mg-Chl) at dark condition. Mean values with the same letters are insignificantly different.

Treatment	Larval duration	Pupal duration	Pupation rate [%] Mean* ± SE	Weight of pupa	Adult emergence [%]
Mg-Chl/Ag 10^{-3}	19.12 ± 0.51 a	7.45 ± 0.16 a	33.30 ± 1.92 b	0.19 ± 0.010 a	36.90 ± 2.47 ab
Mg-Chl/Ag 10^{-4}	19.96 ± 0.57 a	7.95 ± 0.18 a	58.89 ± 2.00 c	0.21 ± 0.004 a	50.81 ± 3.87 c
Mg-Chl/Ag 10^{-5}	21.42 ± 0.55 b	9.90 ± 0.44 b	73.89 ± 1.46 de	0.21 ± 0.006 a	55.80 ± 4.34 c
Mg-Chl/GO 10^{-3}	18.65 ± 0.25 a	7.21 ± 0.11 a	23.33 ± 0.96 a	0.19 ± 0.010 a	28.82 ± 4.63 a
Mg-Chl/GO 10^{-4}	19.35 ± 0.45 a	7.80 ± 0.20 a	53.89 ± 2.00 c	0.21 ± 0.005 a	46.26 ±1.96 bc
Mg-Chl/GO 10^{-5}	19.88 ± 0.32 a	9.00 ± 0.39 b	71.11 ± 2.42 d	0.23 ± 0.001 ab	48.65 ± 3.22 c
Control	24.09 ± 0.15 c	12.76 ± 0.37 c	77.22 ± 2.22 e	0.27 ± 0.015 b	77.82 ± 2.52 d
F value	16.633	35.281	118.064	5.363	20.435
P value	0.0	0.0	0.0	0.0	0.0

Table 5. Some biological aspects for *S. littoralis* larvae treated with nanosilver and graphene over magnesium chlorophylline (Mg-Chl) after 2 hour exposure to sunlight. Mean values with the same letters are insignificantly different.

Treatments	Larval duration	Pupal duration	Pupation rate [%] Mean ± SE	Weight of pupa	Adult emergence [%]
Mg-Chl/Ag 10^{-3}	17.37 ± 0.38 ab	6.00 ± 0.52 a	22.78 ± 1.46 ab	0.1613 ± 0.003 a	55.03 ± 0.29 ab
Mg-Chl/Ag 10^{-4}	18.17 ± 1.56 abc	9.08 ± 0.49 c	35.56 ± 2.00 c	0.1700 ± 0.006 a	62.38 ± 5.18 bc
Mg-Chl/Ag 10^{-5}	21.38 ± 0.78 d	9.60 ± 1.40 c	42.78 ± 1.46 d	0.1800 ± 0.008 a	64.64 ± 4.80 bc
Mg-Chl/GO 10^{-3}	16.58 ± 0.23 a	5.45 ± 0.21 a	20.00 ± 0.96 a	0.1800 ± 0.014 a	41.39 ± 2.82 a
Mg-Chl/GO 10^{-4}	19.08 ± 1.56 bcd	6.73 ± 0.14 ab	26.67 ± 1.92 b	0.1800 ± 0.009 a	50.76 ± 5.94 ab
Mg-Chl/GO 10^{-5}	20.10 ± 1.03 cd	7.72 ± 0.36 b	41.11 ± 1.46 d	0.1900 ± 0.010 a	54.40 ± 5.43 ab
Control	24.17 ± 0.21 e	12.76 ± 0.37 d	73.33 ± 0.96 e	0.2460 ± 0.010 b	75.73 ± 1.03 c
F value	16.695	39.701	143.135	10.292	6.843
P value	0.0	0.0	0.0	0.0	0.001

Table 6. Total carbohydrates, total protein and total lipid content of *S. littoralis* treated with LC_{50} of nanosliver and graphene oxide over magnesium chlorophyllin exposed to sunlight; b.wt. – body weight.

Exposure period [h]	Treatments	Total carbohydrates [mg glucose/g b.wt.]			Total protein [mg glucose/g b.wt.]			Total lipid [mg glucose/g b.wt.]		
		Mean ±SE	Change %	Activity ratio	Mean ±SE	Change %	Activity ratio	Mean ±SE	Change %	Activity ratio
0	Mg-Chl/Ag 10^{-3}	35.05 ± 0.82	6.86	1.07	52.26 ± 1.18	22.590	1.23	27.91 ± 0.63	- 9.47	0.91
	Mg-Chl/GO 10^{-3}	21.56 ± 0.65	- 34.27	0.66	42.46 ± 0.62	- 0.399	0.99	25.87 ± 0.89	- 16.01	0.84
2	Mg-Chl/Ag 10^{-3}	18.89 ± 0.61	- 42.41	0.58	31.89 ± 0.79	- 25.190	0.75	15.97 ± 0.73	- 48.19	0.52
	Mg-Chl/GO 10^{-3}	25.26 ± 0.61	- 22.99	0.77	38.79 ± 0.80	- 9.010	0.91	26.03 ± 0.38	- 15.57	0.84
Control		32.80 ± 0.95	–	–	42.63 ± 1.00	–	–	30.83 ± 1.67	–	–

nanocomposite is more efficient in controlling mosquito larvae than free cyhalothrin. Osman *et al.* (2015) evaluated the effect of silica and zinc oxide nanoparticles on the 2nd larval instar of *S. littoralis* under laboratory conditions. Result indicated that the highest cumulative larval mortality was 86.67% and 83.33% with 2000 ppm concentration for nanozinc oxide (ZNp) and nanosilica (SNp), respectively after 12 days post exposure. Borie *et al.* (2014) studied the effect of six concentrations of hydrophobic nanosilica against neonates of *S. littoralis* on soybean plants under laboratory conditions. The data showed that, the high concentration (425 ppm) recorded 95.33% after 15 days post application. In addition, Derbalah *et al.* (2014) found that silica nanoparticles had low mortality against newly hatched larval of the pink bollworm, *Pectinophora gossypiella* (Saunders, 1844). On the other hand, studies showed that insecticides on *S. littoralis* larvae caused the same mortality after few days from treatments. Sabri *et al.* (2016) examined the lethal effects of biorational insecticides; methoxyfenozide, spinosad, emamectin benzoate, indoxacarb and lufenuron against different life stages of *S. litura*. They stated that the order of insecticides toxicity on the basis of mortality was methoxyfenozide > spinosad > indoxacarb > emamectin > lufenuron.

Lethal concentrations

The calculated LC_{50} and LC_{90} values with their confidence limits for the treated 2nd instar larvae of *S. littoralis* are shown in Table 3 and Fig. 2. According to LC_{50} values, Mg-Chl/Ag nanocomposites was higher effective than Mg-Chl/GO compound. Two nanocomposites were more toxic at 2 h exposure to light (10.27 and 16.14 mg/L) than in dark treatments (20.21 and 33.98 mg/L). These results agree with Ahmed *et al.* (2019) who determined the LC_{50} of silver nanoparticles against 4th instar larvae of *S. littoralis* and which were 6 202.8 ppm. Osman *et al.* (2015) found that, according to LC_{50} values, nanosilica was the most effective compound against the 2nd larvae of *S. littoralis* followed by nanozinc oxide. The corresponding LC_{50} values were 74.4 and 146.79 ppm, respectively. Ahmed *et al.* (2015) detected the

toxicity of Spinosad insecticide against 4th instar larvae of *S. littoralis* after 48 h, and found that the LC_{50} and LC_{90} values were 5 and 60 ppm respectively.

Biological effects

Some biological aspects of *S. littoralis* resulted from the treated 2nd instars larvae with two nanocomposites at two exposure period to sunlight as shown in tables 4 and 5. Generally, all the treatments cause highly significant effect than the control.

Larval and pupal durations in the nanocomposite treatment were significantly lower than those in the control group. At zero exposure (dark), the durations of larvae and pupae ranged between 18.65–21.42 days and 7.21–9.90 days, respectively in all treatments comparing with control (24.09 and 12.76 days) (Table 4). At 2 h exposure to light, the larval duration in all treatments ranged 16.58–21.38 days comparing with 24.17 days in the control. However, the pupal duration in all treatments ranged between 5.45 to 9.60 days, while in the control it was 12.76 days (Table 5).

The pupation and adult emergence rates were highly reduced in all the concentrations compared to untreated larvae. The highest reduction was observed at the highest concentration of Mg-Chl/GO. The pupation and adult emergence percentages were 23.33% and 28.82% at dark and 20.0% and 41.39% at 2 h exposure period, respectively. In addition, a high decrease in pupal weight was noticed at all the tested concentrations. All nanocomposite treatments were significantly reduced pupal weights comparing with those in control group. However, there was not significant differences between the weights of pupae developed from the treated larvae. The highest reduction in pupal weight was recorded in Mg-Chl/Ag (10^{-3}) at 2 hour exposure (0.16 mg), compared to the weight of pupa of 0.24 mg in the control.

These results were similar to those obtained by El-Bendary & El-Helaly (2013) who reported that the larval duration, pupal period and adult longevity of *S. littorali*s larvae treated with silica nanoparticles were not affected, as compared to that of the control. Shaker *et al.* (2017) studied the effects of the TiO_2 nanoparticle on 2nd instar larvae of *S. littoralis*. His results indicated that, the treated 2nd instar larvae at its LC_{50} values, larval duration were 13.5 days as compared to 10.2 days that of control. Also, the treatment caused a very significant decrease in the pupal duration compared to the control. Zaki *et al.* (2017) found that sodium titanate nanotubes (TNTs) led to 4% decrease in larval duration for 2nd instar larvae of *S. littoralis* and 7% increase in pupal duration. While, sodium titanate-*Bacillus thuringiensis* nanocomposite (Bt-TNTs) caused an increase of 11% in larval duration and 10% in pupal duration compared to the control. Osman et al. (2015) recorded that the 2nd instars larvae of *S. littoralis* treated with nano zinc oxide and nano silica compounds caused high reduction rates in the pupation, pupal weight and adult emergence.

Amer *et al.* 2015 evaluated toxic efficiency of four bio-agent compounds: *B. thuringiensis* (Bt), *Metarhizium anisopliae*, *Heterorabditis bacteriophora*, *Steinernema carpocapsae*, and chitosan (biopolymer), on 4th instar larvae and biological parameters of the cotton leaf worm, *S. littoralis*. They found that Bt was the most efficient bio agent compound as compared to other treatments on most biological parameters of *S. littoralis*, in addition to increasing larval mortality rate. Also, the same treatment decreased pupation, adult emergence and fecundity percentages, ovipositional period, egg laying rate. While, *M. anisopliae* and chitosan treatments shortened the larval duration one day (19 days) comparing 20 days in the control.

Pupation percentages decreased from 31% to 42% at different compound treatments. Also, adult moth emergency percentage decreased by 17% to 34% compared to the control (97%).

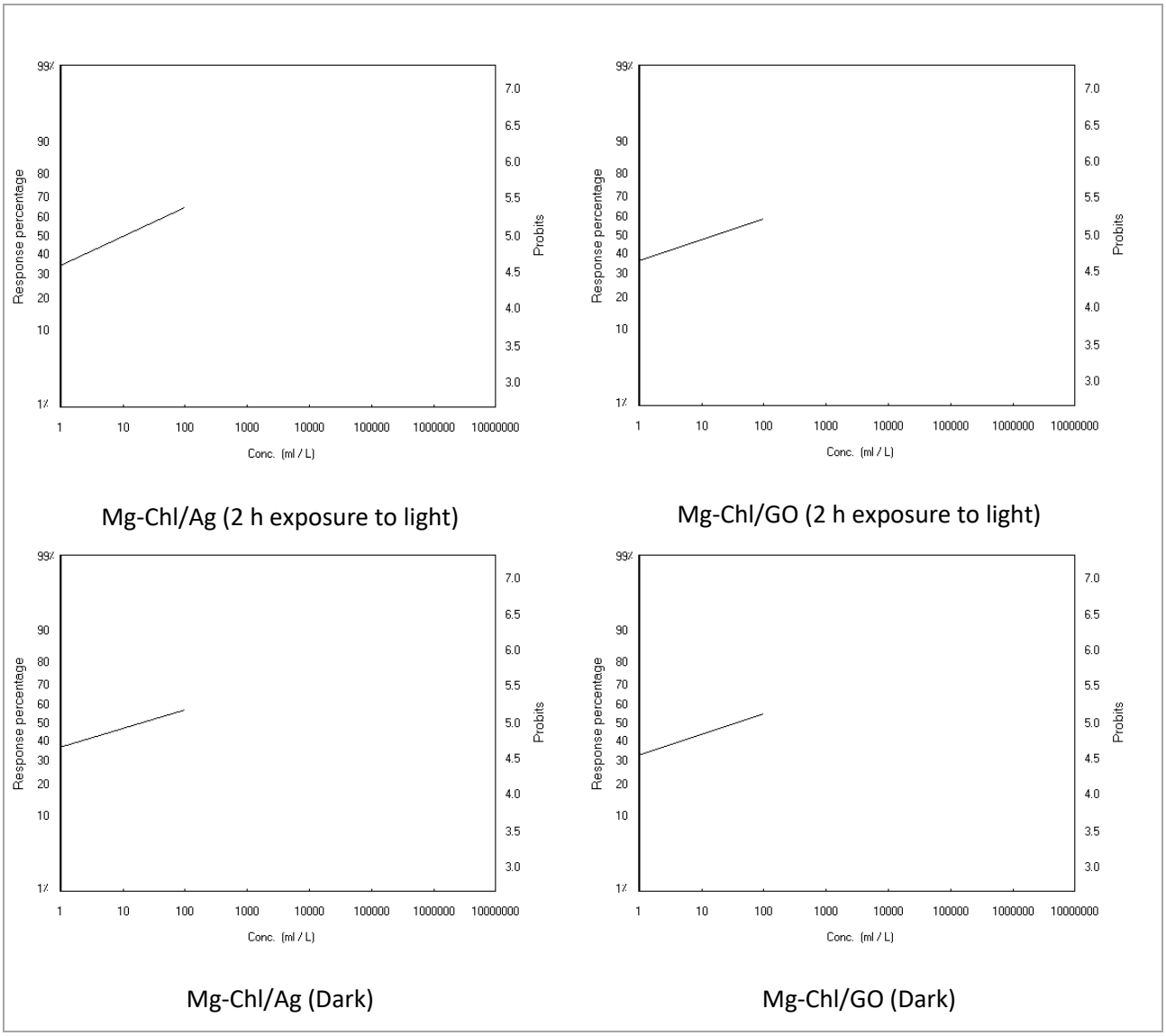

Fig. 2. Toxicity lines for the 2nd instar larvae of *S. littorals* treated with Nano sliver and graphene oxide over Magnesium Chlorophyllin.

Biochemical Study

The data summarized in Table 6 represent the changes in total carbohydrates, total protein and total lipid content of 2nd instars *S. littoralis* treated with LC_{50} of nanosilver and graphene oxide over magnesium chlorophyllin exposed to sunlight.

All nanocomposite treatments cuased significant decrease in total carbohydrates content of *S. littoralis* in light and dark conditions except Mg-Chl/Ag caused higher increase in total carbohydrates (35.0 mg/g b.wt.) than control (32.8 mg/g b.wt.). Also, the highest reduction of total protein was recorded in the Mg-Chl/Ag (31.89 mg/g b.wt.) at 2 h exposed to sunlight compared to the control (42.63 mg/g b.wt.). On the other hand, the same treatment at dark led to the increase in total protein (52.26 mg/g b.wt.). Finally, nano sliver and graphene oxide over magnesium chlorophyllin at light and dark conditions caused a significant reduction in total lipid for treated larvae of *S. littoralis.* The highest reduction occurred with the treatment by Mg-Chl/Ag at 2 h exposure to sunlight (48.19%).

It is well known that the metamorphic changes in larva under stress affects total soluble carbohydrate content. The carbohydrate content supplies the body with glucose which provides an energy source for the synthesis of larval and adult tissues. This may explain the high decrease in total carbohydrates content in 2nd instar larvae of *S. littoralis* treated with nanocomposite. These results are congreunt with Osman *et al.* (2015) who found SNp and ZNp caused decrease in both total carbohydrates and proteins in treated 2nd larval instar of *S. littoralis*. Dziewiecka *et al.* (2016) measured the activity of catalase (CAT) and gluthiathione peroxidases (GSTPx) as well as heat shock protein (HSP 70) and total antioxidant capacity (TAC) levels on *Acheta domesticus* (Linnaeus, 1758) when exposed to graphene oxide. The results proved the intensification of oxidative stress after GO injection, which was confirmed by increased enzyme activity. Moreover, in *S. litura*, amylase, protease, lipase and invertase activities decreased when exposed to AgNPs (Bharani & Namasivayam 2017). Yasur & Usha-Rani (2015) studied the impact of silver nanoparticles (AgNPs) on growth and feeding responses of Asian armyworm, *S. litura* and castor semilooper, *Achaea janata* (Linnaeus, 1758). The result indicate that AgNPs induces oxidative stress, which is countered by antioxidant enzymes. Induction of these enzymes may be an effective detoxification mechanism by which the herbivorous insect defends itself against nanoparticle treatment. As recently pointed out by Fouad *et al.* (2018) founded that total protein levels decreased in larvae of *Aedes albopictus* (Sukse, 1894) when exposed to Ag nanoparticles.

Abdel-Mageed *et al.* (2018) found the flufenoxuron and chlorfluazuron increased the activity of both alpha and beta esterases in *S. littoralis* and can facilitate the development of truly selective insecticides that can be employed in integrated pest management strategies. Abd El Rahman *et al.* (2019) investigated the impact of some pesticides belonging to different groups in the laboratory on the 4th instar larvae of *S. littoralis* to evaluate the effect of the tested compounds on the total protein, total carbohydrate, total lipids and acetylcholinesterase. They recorded that the tested insecticides decreased significantly the total carbohydrate, total protein, total lipids and the activity of acetylcholinesterase. Owner 5% was the most insecticide effective especially on the total lipids recording 45.12% reduction. On the other hand, dimilin 48% SC caused the highest change in the percentage of total protein and total carbohydrate (33.07% and 54.6%) respectively. Dimilin was the most effective insecticide on the activity of acetylcho-linesterase recording 28.06% inhibition. Anwar & Abdel-Mageed (2005) found that all castor oil, gossypol, diflubenzuron, tebufenozide, hexaflunuron, flufenoxuron, chlorfuazuro and lufenuron cause reduction in carbohydrate content, total protein and total lipid of *S. littoralis*. Abdel-Salam *et al.* (2018) found the protecto, viruset, cascade and ataborn caused significant decrease in the amount of total protein, total carbohydrate and total lipids in 4th instar of *S. littoralis* larvae.

Conclusion

Two nanocomposites (Mg-Chl/Ag and Mg-Chl/GO) against 2nd instar larvae of *S. littoralis* were more effect in the larval mortality rate at 2 h exposure to sunlight. However, results suggest that using these nanocomposites as a useful component for controlling *S. littoralis.*

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