Zagazig J. Agric. Res., Vol. 46 No. (1) 2019 27-36

Plant Protection and Pathology Research

http:/www.journals.zu.edu.eg/journalDisplay.aspx?Journalld=1&queryType=Master

TOXICITY OF GRAPHENE NANOPARTICLES WITH SODIUM COPPER CHLOROPHYLLIN OR MAGNESIUM CHLOROPHYLLIN AND EFFECT ON LEVELS OF ACID AND ALKALINE PHOSPHATASE ENZYME OF PEACH FLY, *Bactrocera zonata* (DIPTERA: TEPHRITIDAE)

Sameh M. Abd El-Naby*

Plant Prot. Res. Inst. (PPRI), ARC, Dokki, Giza, Egypt

Received: 28/11/2018 ; Accepted: 15/01/2019

ABSTRACT: Graphene nanoparticles are one of the most attractive nanomaterials for commercialization applications. They have been widely used for antimicrobial, electronic and biomedical products. In this review, The exposure time to light plays an important role in mortality percentage, the percentage mortality increase when the exposure time increase and there an inverse relationship between concentration with the exposure time. Photoactive compound magnesium chlorophyllin had more significant effect on adult *Bactrocera zonata* after exposure to sunlight than magnesium chlorophyllin with graphene nanoparticles so that there was no benfit to use the mixture of magnesium chlorophyllin with graphene nanoparticles, magnesium chlorophyllin was more effeciant than Copper chlorophyllin with graphene nanoparticles or Copper chlorophyllin. Level of acid phosphatases in the homogenate of *Bactrocera zonata*, After exposure to sunlight for two hours after treated by compound Copper chlorophyllin or Magnesium chlorophyllin, results showed high increase (52.44%, 40.71%). After treated by compound Copper chlorophyllin with graphene nanoparticle or Magnesium chlorophyllin with graphene nanoparticle, the changes in alkaline phosphatases after treated by compound Copper chlorophyllin show limited rduction (-16.28%), on the other hand Magnesium chlorophyllin showed high incrase (77.14%) relative to control.

Key wards: *Bactrocera zonata*, photoinsecticide, photosensitizer, chlorophyllin, illumination with sunlight, graphene nanoparticles, exposed, acid phosphatase (AcP), alkaline phosphatase (AlP).

INTRODUCTION

Nanotechnology is rapidly growing with nanoparticles produced and utilized in a wide range of applications in aerospace engineering, nanoelectronics, environmental remediation, medical healthcare and consumer products (**Singh *et al.,* 2009**). Nanoparticles (NPs), which are defined as particles having at least one dimension of 100 nm or less, are used to produce novel materials with unique physicochemical properties (**Maynard and Kuempel, 2005**). The broad usage of NPs will result in the contamination of the environment through atmospheric emissions, wastewater, agriculture and accidental release during manufacture/ transport (**Colvin, 2003**). For the past several years, the toxicology of NPs has been investigated in a wide range of species, including bacteria, algae, invertebrates (nematodes and crustaceans), fish, and vertebrates (rats and humans) (**Stampoulis *et al.,* 2009**). NPs can affect living organisms and are absorbed by bacterial, plant, animal and human cells (**Khodakovskaya *et al.,* 2011**). Graphene materials have been widely used in various fields, including energy storage; nanoelectronic devices; batteries (**Wang *et al.,* 2015**); and biomedical applications, such as antibacterials (**Gurunathan *et al.,* 2012**), drug delivery (**Huiyun *et al.,* 2012**), biosensors (**Gao *et al.,* 2014**), and tissue engineering (**Chaenyung *et al.,* 2014**), cell imaging (**Wang *et al.,* 2013**).

* **Corresponding author: Tel.** : +2001001818521
E-mail address: Dr.samehmostafa@yahoo.com

Nanotechnology also has the potential to improve the environment both through direct application of nanomaterial to detect, prevent and remove pollutant, as well as indirectly by using nanotechnology to design cleaner industrial processes and create environmentally responsible product (**Chinnamuthu and Murugesa Boopathi, 2009**). Nanotechnology is about to emerge in the world of pesticides and pest control. Nano particles help to oroduse new pesticides, insecticides and insect repellants (**Owolade *et al.,* 2008**). Although there have been numerous studies on the toxicity effects of nanoparticles on bacteria, fungi, and animal pathogens (**Elchiguerra *et al.*, 2005**), little research has been carried out to investigate the toxicity effect of nanoparticles on insects, specially storage pest. (**Wan and Zhong-Nian, 2005)** studied the effect of mixture of two nanoparticles with two insecticides on the pest mite (*Epitrimerus pyri* (Nalepa). According to the authors, cypermethrin and alpha terhienyl mixed with nano-particled zinc oxide and copper oxide was effective on the tested mite. Chlorophyll is one of the most important pigments for the transfer of light energy into a carbon resource. Indeed, the inhibition of chlorophyll affects photosynthesis and causes surplus electrons to combine with molecular oxygen, forming harmful reactive oxygen species (**Foyer *et al.,* 1994**). Several photosensitizing agents, which are activated by illumination with sunlight or artificial light sources (**Ben Amor *et al.,* 2000**), have been shown to be accumulated in significant amounts by a variety of insects when they are administered in association with suitable baits. The subsequent exposure of such insects to UV/visible light leads to a significant drop in survival. Of the photosensitizes tested so far, xanthenes (*e.g.* phloxin B) and porphyrins (*e.g.* haemato-porphyrin) appear to be endowed with the highest photo-insecticidal activity. In particular, porphyrins absorb essentially all the UV/visible light wavelengths in the emission spectrum of the sun; hence they are active at very low doses. Present evidence suggests that such photosensitizes act on the membranes of the midgut with consequent feeding inhibition, as well as on the neuromuscular sheath. No apparent onset of photoresistance has been observed. The rapid photobleaching of xanthenes and porphyrins when illuminated by visible light, as well as the lack of significant toxicity of such compounds in the dark, minimizes the risk of an important environmental impact of such photoinsecticidal agents.Detoxification enzyme, acid phosphatase (ACP) in insects is generally demonstrated as the enzymatic defense against foreign compounds and play significant roles in maintaining their normal physiological functions (**Li and Liu, 2007**). Phosphatases are capable of transphosphorylation in addition to hydrolysis. Phosphatases play an important role in the metabolism of carbohydrates, phospholipids and nucleotides. Acid phosphatase is important in biological processes that need high level of energy, such as development, growth, gamete's maturation and histolysis (**Ray *et al.*, 1984**).

The purpose of this paper, is to study the toxicity of mixture between chlorophyllin with graphene nanoparticles on acid and alkaline Phosphatases enzyme in *Bactrocera zonata.*

MATERIALS AND METHODS

Biological Study

Insects

The laboratory susceptible colony of the peach fruit fly, *Bactrocera zonata* was established from a strain continuously reared in the laboratory of Horticultural Insects Department, Plant Protection Research Institute, Agricultural Research, Center, Dokki, Giza, Egypt under conditions of (25±3°C, 60±5% R.H and photoperiod of 14 L: 10 D). Adults of *B. zonata* were reared in a cage (30 x 30 x 30 cm) with wooden frames and covered from each side with metal screen. Flies were fed with sugar and fortified protein hydrolysate at ratio of 3:1 respectively. Adults were provided with a small plastic bottle filled with water as drinking sites, until mating took place then females started oviposition. The cage was supplied with false plastic fruits that had many fine pores (as oviposition receptacles). These plastic fruits are filled with 3ml water to receive and prevent drying of the eggs. Also, at the top of these plastic fruits, small plastic vials containing cotton wicks saturated with guava juice were put to enhance egg lying within these false fruits. Larvae were reared on an artificial diet consisted of 500 ml distilled water, 3.00 g citric acid, 3.00 g sodium benzoate, 84.50 g sugar, 84.50 g brewer’s yeast and 330 g wheat bran. These ingredients were carefully mixed in large plastic container scattered on the surface of the diet which was placed in plastic trays of 20 x 10 x 8 cm that were tightly covered with muslin clothes using rubber bands. The trays placed in a wooden cage with sand at the bottom to allow the jumping larvae to pupate. All pupae were sieving the sand.

Laboratory Bioassay

The bioassay tests were performed on full grown larvae and 1-days pupae of *B. zonata*. All concentrations of photosensitizer were prepared by dissolving in water with sugar and fortified protein hydrolysate at ratio of 3 : 1 respectively. The treated Adults put in dark for 24 hr., and exposure to sunlight to different time (30 min, 60 min. and 120 min.). The average of mortality percentage was corrected using Abbott's formula (**Abbott, 1925**). The corrected mortality percentage of each compound was statistically computed according to **Finney (1971)**.

Synthesis of Graphene Nanoparticles

Graphene oxide compound was synthesized through the oxidation of graphite powder. The oxidation was performed using a modification of Hummer experimental procedure. After synthesis, the powder was fabricated into graphene oxide films through vacuum filtration and transfer to desired substrates. A mixture of concentrated H_2SO_4/H_3PO_4 (90:10 ml) was added to a mixture of graphite flakes (1.0 g) and $KMnO_4$ (6.0 g) producing an exothermic reaction. This mixture was then heated to 50°C and stirred for 12 hr. The mixture was then cooled overnight and poured onto ice (around 200 ml) with 30% H_2O_2 (1 ml). The resulting mixture was then centrifuged (4000 rpm for 1 hour) and the supernatant decanted away. The remaining solid material was then washed with 100 ml of HCl followed by centrifugation (4000 rpm for 1 hour) and the supernatant decanted away. Finally, the solid material was washed 3-6 times with 100 ml H_2O, then centrifuged at 4000 rpm for 30 minutes each time, and the supernatant decanted away. The material from the centrifuge tubes remaining after the multiple wash process was then dissolved into water, collected by vacuum filtration, and ground with a mortar and pestle. Both the powder and the films were then characterized using the spectral methods of analyses.

Direct sunlight

The treated larvae were exposed to the sunlight for 30 min, 60 and 120 mins. The fluency rate measured by the dosimeter taken as the average of intensities during exposure time.

Dark experiment

The larvae treated with Copper chlorophyllin only or with graphene nanoparticles left in the dark until the end of larval life. Magnesium chlorophyllin only or with graphene nanoparticles left in the dark until the end of larval life.

Biochemical Study

Preparation of samples for biochemical studies

The biochemical assay was done after *Bactrocera zonata* treated and exposued for two hour to sunlight. The larvae homogenates before centrifugation and supernatant was used directly for enzyme assay.

Determination of acid and alkaline phosphatase activities

Acid phosphatase (AcP) and alkaline phosphatase (AlkP) activities were determined according to the method described by **Powell and Smith (1954)**. In this method, the phenol released by enzymatic hydrolysis of disodium phenylphosphate, reacts with 4-aminoantipyrine, and by the addition of potassium ferricyanide, the characteristic brown colour is produced.

Assay of phosphatase enzyme activities

The reaction mixture consists of 1 ml carbonate buffer (pH 10.4) for AlkP, or 1 ml of citric buffer (pH 4.9) for AcP, 1 ml of 0.01 M disodium phenyl phosphate (substrate), and 0.1 ml pupal tissues homogenate .The mixture was mixed gently and incubated for exactly 30 minutes at 37°C. At the end of incubation period, 0.8 ml of 0.5 N NaOH was added to stop the reaction. Then 1.2 ml of 0.5 N $NaHCO_3$ was added, followed by 1 ml of 4-aminoantipyrine solution and 1 ml of potassium ferricyanide. The produced colour was measured immediately by spectrophotometer at 510 nm. The enzymatic activity is expressed as μg phenol released/ min/ g body weight.

RESULTS AND DISCUSSION

Toxicological Study

Table 1 and Fig. 1 indicate that the tested adult *Bactrocera zonata* to the Magnesium chlorophyllin (Mg-CH) exposure to sunlight for 2 hr., was the most susceptible (LC_{50}= 0.00002 M) compared to the other treated and has resistance ratio (RR=1), Magnesium chlorophyllin with graphene nanoparticles (Mg CH-Go) exposure to sunlight for 2hr (LC_{50} = 0.0002M) and has resistance ratio (RR = 10) compared with light Mg-CH. On the other hand, the treated *Bactrocera zonata* not exposed to sunlight, the value of LC_{50} was 0.417 M when treated by Magnesium chlorophyllin (Mg-CH) while the value of LC_{50} was 9.358 M when treated by Magnesium chlorophyllin with graphene. From this information it could be conclude that there is a direct correlation with the light. These agent become active against pest insects in sunlight or artificial light while potocompound are quickly activated by light. **Abd El-Naby (2002)** reported that the efficiency of photodynamic sensitizers as insecticidal agents is affected by a variety of experimental parameters; The first factor being photosensitizer concentration. The photoinsecticidal effect steadily increased with increasing the concentration of the photosensitizing, The second factor is the duration of the post treatment light exposure period. There an inverse relationship with the concentration and exposure time when increased the concentration this led to decrease exposure time to light.

Table 2 and Fig. 2 indicate that the tested adult *Bactrocera zonata* to the Copper chlorophyllin with graphene nanoparticles (COCH-Go) exposure to sunlight for 2hr. was the most susceptible (LC_{50} = 0.0002 M) than the other treated and had resistance ratio (RR = 1), Copper chlorophyllin (Co CH) exposure to sunlight for 2 hr., (LC_{50} = 0.008M) and had resistance ratio (RR = 40) compared with light Co CH- Go, on the other hand the treated *Bactrocera zonata* not exposed to sunlight, the (LC_{50} = 1908.947 M) treated by Copper chlorophyllin (Co CH) and (LC_{50} = 2.02E + 25 M) treated by Magnesium chlorophyllin with graphene nanoparticles.

Photosensitization involving light, a photo-sensitizer and oxygen is a potentially damaging event in biological systems. This generates several reactive oxygen species (ROS) such as singlet oxygen, hydrogen peroxide, superoxide and hydroperoxyl or hydroxyl radicals that are capable of damaging various sub-cellular structures and molecules (**Paillous and Fery-Forgues, 1994**). **Ben Amor *et al.* (2000)** reported that, several photosensitizing agents, which are activated by illumination with sunlight or artificial light sources, have been shown to be accumulated in significant amounts by a variety of insects when they are administered in association with suitable baits. The subsequent exposure of such insects to UV/visible light leads to a significant drop in survival.

The results summarized in Table 3 reveale that the tested adult *Bactrocera zonata* to the Magnesium chlorophyllin (MgCH) exposure to sunlight for 2hr. was the most susceptible (LC_{50} = 0.00002M) compared to the other treated, and second effective compound was Magnesium chlorophyllin with graphene nanoparticles (Mg CH-Go) exposure to sunlight for 2 hr., (LC_{50} = 0.0002 M) and Copper chlorophyllin with graphene nanoparticles (CuCH-Go) (LC_{50} = 0.0002 M) and chlorophyllin (Cu CH) (LC_{50} = 0.008M) had low effect on adult *Bactrocera zonata* Copper compared with Magnesium chlorophyllin after exposure to light.

The results summarized in Table 4 and Fig. 3 revealed the changes in acid phosphatases in the homogenate of *Bactrocera zonata*, after exposure to sunlight for two hours after treated by compound Copper chlorophyllin or Magnesium chlorophyllin which showed high increase (52.44%, 40.71%), respectively relative to control, *Bactrocera zonata*, after treated by compound Copper chlorophyllin with graphene nanoparticle or Magnesium chlorophyllin with graphene nanoparticle and exposure to sunlight for two hours showed limited incrase (9.77%, 17.91%), respectively relative to control.

The reults summarized in Table 5 and Fig. 4, indicat that, changes in alkaline phosphatase in the homogenate of *Bactrocera zonata*, after exposure to sunlight for two hours after treated by compound Copper chlorophyllin show limited rduction (-16.28%), on the other hand Magnesium chlorophyllin showed high incrase (77.14) relative to control. *Bactrocera zonata*,

Table 1. Toxicity data of Magnesium chlorophyllin with graphene nanoparticles against adult *Bactrocera zonata* after exposure of sunlight (355 w/m^2)

No.	Line name	LC_{50}	LC_{90}	Slope	RR	Index	Upper limit	Lower limit
1	light Mg CH	0.00002	0.0028	0.564	1	100	0.00003	4.32E-06
2	light Mg CH-Go	0.0002	0.0032	1.066	10	10	---	---
3	dark Mg CH	0.417	34204.36	0.261	20850	0.0048	---	---
4	dark Mg CH-Go	9.358	4.12E+06	0.227	4.68E+05	0.0002	---	---

*Mg CH light : (*Bactrocera zonata* treated by Magnesium chlorophyllin after exposure for sunlight)
*Mg CH Dark : (*Bactrocera zonata* treated by Magnesium chlorophyllin, dark)
*Mg CH-Go Dark : (*Bactrocera zonata* treated by Magnesium chlorophyllin with graphene nanoparticles dark)
*Mg CH-Go light : (*Bactrocera zonata* treated by Magnesium chlorophyllin with graphene nanoparticles light)

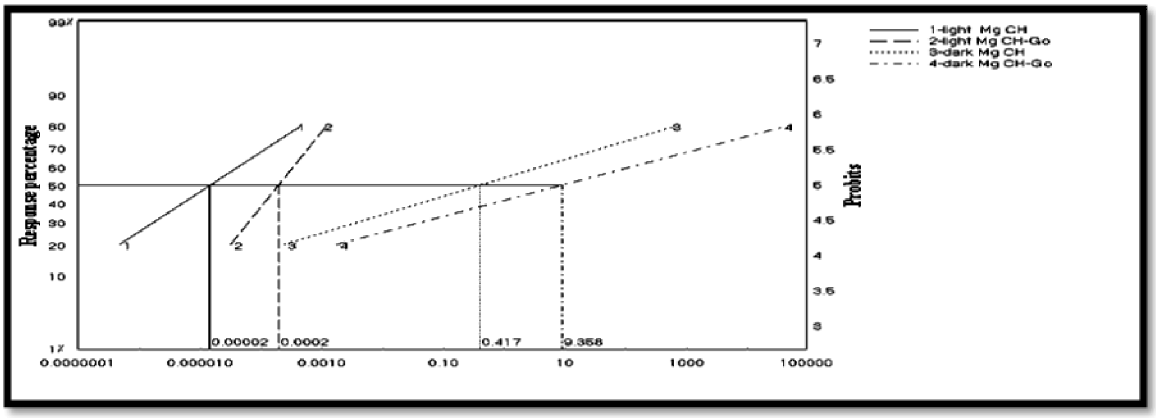

Fig. 1. Log-probit concentration lines of treated Magnesium chlorophyllin on adult *Bactrocera zonata* after exposure of sunlight (355 w/m^2)

Index compared with light Mg CH Resistance Ratio (RR) compared with light Mg CH

Table 2. Toxicity data of Sodium copper chlorophyllin with graphene nanoparticles against adult *Bactrocera zonata* after exposure to sunlight (355 w/m^2)

No.	Line name	LC_{50}	LC_{90}	Slope	RR	Index	Upper limit	Lower limit
1	light Cu CH- Go	0.0002	0.125	0.457	1	100	0.0006	0.00009
2	light Cu CH	0.008	18.446	0.381	40	2.5	1.848	0.0015
3	Dark Cu CH	1908.947	2.16E+13	0.127	9.54E+06	0.00001	---	---
4	Dark Cu CH- Go	2.02E+25	2.54E+62	0.035	1.01E+29	9.89E-28	---	---

*Cu light : (*Bactrocera zonata* treated by Sodium copper chlorophyllin after exposure for sunlight)
*Cu Dark : (*Bactrocera zonata* treated by Sodium copper chlorophyllin dark)
*Cu CH-Go Dark : (*Bactrocera zonata* treated by Sodium copper chlorophyllin with graphene nanoparticles dark)
*Cu CH-Go light : (*Bactrocera zonata* treated by Sodium copper chlorophyllin with graphene nanoparticles light)

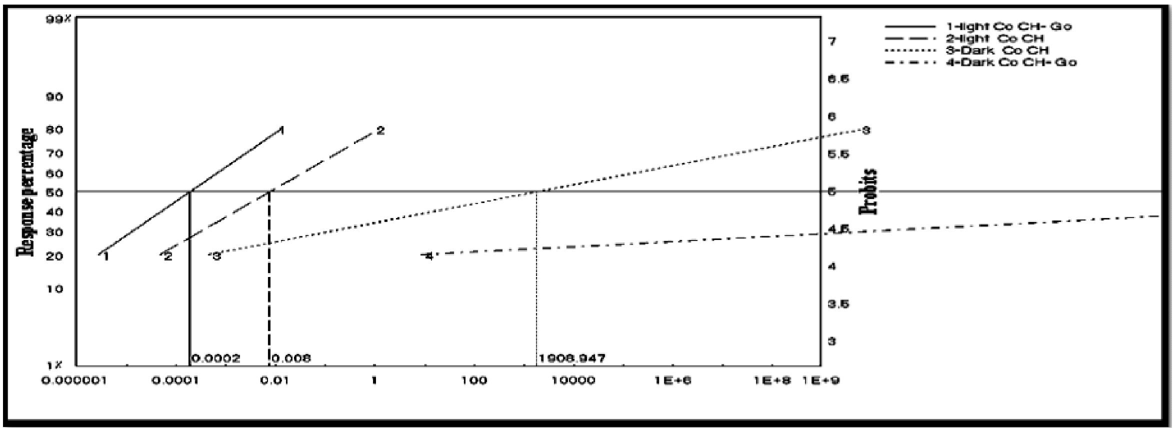

Fig. 2. Log-probit concentration lines of treated Sodium copper chlorophyllin with graphene nanoparticles on adult *Bactrocera zonata* after exposure to sunlight (355 w/m^2).

Index compared with light Cu CH- Go Resistance Ratio (RR) compared with light Cu CH- Go

Table 3. Toxicity data of chlorophyllin with graphene nanoparticles against adult *Bactrocera zonata* after exposure to sunlight (355 w/m^2)

No.	Line name	LC_{50}	LC_{90}	Slope	RR	Index	Upper limit	Lower limit
1	light Mg CH	0.00002	0.0028	0.564	1	100	0.00003	4.32E-06
2	light Mg CH-Go	0.0002	0.0032	1.066	10	10	---	---
3	light Cu CH- Go	0.0002	0.125	0.457	10	10	0.0006	0.00009
4	light Cu CH	0.008	18.446	0.381	400	0.25	1.848	0.0015
5	dark Mg CH	0.417	34204.36	0.261	20850	0.0048	---	---
6	dark Mg CH-Go	9.358	4.12E+06	0.227	4.68E+05	0.0002	---	---
7	Dark Cu CH	1908.947	2.16E+13	0.127	9.54E+07	1.05E-06	---	---
8	Dark Cu CH- Go	2.02E+25	2.54E+62	0.035	1.01E+30	9.89E-29	---	---

Index compared with light Mg CH Resistance Ratio (RR) compared with light Mg CH

Table 4. Level of acid phosphatase activities on adult *Bactrocera zonata* treated with LC_{50} of photosensitizer Sodium copper chlorophyllin with graphene nanoparticle after exposed to sunlight

	Acid phosphatase (µg phenol released/g b.wt./min.)							
	Compound	Adult	(%)	*	Compound	Adult	(%)	*
1	Cu-CH	156.00 ± 0.94	152.44	52.44	Mg-CH	144.00 ± 0.82	140.71	40.71
2	Cu Go	112.33 ± 0.92	109.77	9.77	Mg Go	120.67 ± 0.91	117.91	17.91
3	Cu-CH Dark	124.67 ± 0.63	121.82	21.82	Mg-CH Dark	138.67 ± 0.63	135.51	35.51
4	Cu Go Dark	145.67 ± 0.91	142.34	42.34	Mg Go Dark	113.67 ± 0.94	111.08	11.08
5	Control	102.33 ± 0.89	100.00	00.00	Control	102.33 ± 0.89	100.00	00.00

* (%) = percentage relative to control.

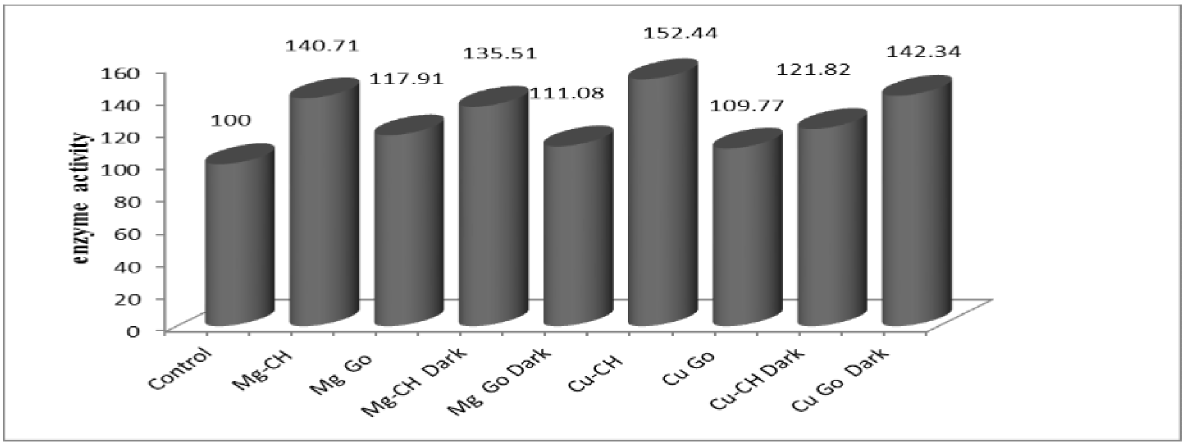

Fig. 3. Enzyme activity of acid phosphatase on *Bactrocera zonata* treated by photosensitizer Sodium copper chlorophyllin with graphene nanoparticle after exposed to sunlight at (355 w/m^2)

Table 5. Level of alkaline phosphatases activities on adult *Bactrocera zonata* treatment with LC$_{50}$ of photosensitizer Sodium copper chlorophyllin with graphene nanoparticle after exposed to sunlight

Alkalin phosphatase (µg phenol released/g b.wt./min.)								
	Compound	Adult	(%)	*	Compound	Adult	(%)	*
1	Cu-CH	5.49 ± 0.27	83.72	-16.28	Mg-CH	11.63 ± 0.30	177.14	77.14
2	Cu Go	12.17 ± 0.39	185.28	85.28	Mg Go	6.57 ± 0.29	100.00	00.00
3	Cu-CH Dark	9.83 ± 0.27	149.35	49.35	Mg-CH Dark	10.53 ± 0.33	160.39	60.39
4	Cu Go Dark	14.29 ± 0.47	217.69	117.69	Mg Go Dark	5.87 ± 0.24	89.32	-10.68
5	Control	6.57 ± 0.30	100.00	00.00	Control	6.57 ± 0.30	100.00	100.00

* (%) = percentage relative to control

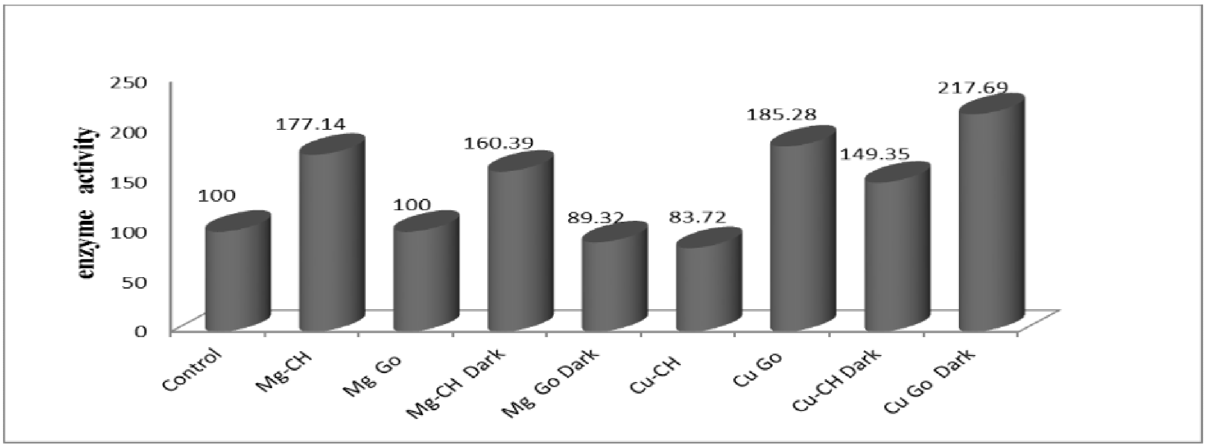

Fig. 4. Enzyme activity of alkaline phosphatase on *Bactrocera zonata* after treated by photosensitizer Sodium copper chlorophyllin with graphene nanoparticle after exposed to sunlight at (355 w/m^2)

after treated by compound Copper chlorophyllin with graphene nanoparticle exposure to sunlight for two hours showed high incrase (85.28%) relative to control, but treated by Magnesium Chlorophyllin with graphene nanoparticle found that, no chang in level of alkaline phosphatases activities. *Bactrocera zonata* treated by compound Copper chlorophyllin with graphene nanoparticle showed high incrase more than Copper chlorophyllin in dark experimental (117.69%, -10.68%), respectively relative to control. On the other hand after treated by compound magnesium Chlorophyllin showed high incrase in dark experimental than compound Copper chlorophyllin with graphene (60.39%, 49.35%), respectively relative to control.

Bactrocera zonata, treated by compound Copper chlorophyllin with graphene nanoparticle showed high incrase more than Magnesium chlorophyllin in dark experimental (42.34%, 11.08%), respectively relative to control. On the other hand after treated by compound Magnesium chlorophyllin showed high increase in dark experimental than compound Copper chlorophyllin with graphene (35,51%, 21.82%), respectively relative to control

DISCUSSION

It is great importance to develop new ecologically safe technologies to control insect pest populations. Photoactive compound Magnesium chlorophyllin was more effect on adult *Bactrocera zonata* after exposure to sunlight than Magnesium chlorophyllin with graphene nanoparticles so that no benfit to use the mixture of Magnesium chlorophyllin with graphene nanoparticles, Magnesium chlorophyllin was more effecint than Copper chlorophyllin with graphene nanoparticles or Copper chlorophyllin. From bioassays result it was found that, there was significant correlation between the results of bioassays and biochemical assays in this study. The highest increase of acid phosphatases activities level in total and on the other hand, the level of alkaline phosphatase activities show variouan in most treatments. Photosensitization involving light, a photo-sensitizer and oxygen is a potentially damaging event in biological systems; singlet oxygen has been identified as one of the major species responsible for biological damage caused by photosensitization (**Weishaput *et al.,* 1976**). In fact, UV/ visible light can penetrate to a depth of about 1 cm in most biological tissues; as the extent of penetration is wavelength dependent (**Svaasand *et al.*, 1990**), it is possible to modulate the volume of the photoinduced tissue damage by selecting a photosensitizing agent with specific absorption properties. That mean, light play an important role to complete photosensitization reaction of *Bactrocera zonata* after treated by Magnesium chlorophyllin, the increase of exposure time to sunlight may be more effect in enzyme level activity. Photoactive compounds usually used for photosensitization might be effective as pesticide agents, with low impact on the environment, being non-toxic and not mutagenic. Phosensitizer accumulates within the insect body and, following exposure to visible light, induces lethal photochemical reactions and death. Porphyrin inactivated catalase through the production of singlet oxygen, which caused amino acid damage (**Hirakawa *et al.,* 2013**).

REFERENCES

Abbott, W.S. (1925). A method of computing the effectiveness of an insecticide. J. Econ. Entomol., 18: 265-267.

Abd El-Naby, S.M. (2002). Study of photosensitization processes for control of *Spodoptera littoralis*, Master Dissertation, Nat. Inst. Laser Enhanced Sci., Cairo Univ., Egypt.

Ben Amor, T., L. Bortolotto and G. Jori (2000). Porphyrins and related compounds as photoactivatable insecticides. 3. laboratory and field studies. Photochem. and Photobiol., 71 (2): 124-128.

Chaenyung, C., S.S. Ryon, G. Xiguang, A. Nasim, M.R. Dokmeci and T. Xiaowu Shirley (2014). Controlling mechanical properties of cell-laden hydrogels by covalent incorporation of graphene oxide. J. Nano Small Micro, 10 (3): 514–23.

Chinnamuthu, C.R. and P. Murugesa Boopathi (2009). Nanotechnology and Agroecosystem. Madras Agric. J., 96: 17-31.

Colvin, V.L. (2003). The potential environmental impact of engineered nanomaterials. Nat. Biotechnol., 21(10): 1166–1170.

Elchiguerra, J.L., J.L. Burt, J.R. Morones, A. Camacho-Bragado, X. Gao, H.H. Lara and M.J. Yacaman (2005). Interaction of silver nanoparticles with HIV-1. J. Nanobiotechnol., 3 (6): 1-10.

Finney, D.J. (1971). Probit Analysis. Camb. Univ. Press, London.

Foyer, C.H., P. Descourvi`eres and K.J. Kunert (1994). Protection against oxygen radicals: an important defence mechanism studied in transgenic plants. Plant, Cell and Environ., 17 (5): 507–523.

Gao, L., C. Lian, Y. Zhou, L. Yan, Q. Li and C. Zhang (2014). Graphene oxide-DNA based sensors. Biosens Bioelectron., 60 (1873–4235 (Electronic)): 22–9.

Gurunathan, S., J.W. Han, A.A. Dayem, V. Eppakayala and J.H. Kim (2012). Oxidative stressmediated antibacterial activity of graphene oxide and reduced graphene oxide in *Pseudomonas aeruginosa*. Int. J. Nanomed., 7 (1178–2013) (Electronic)): 14.

Hirakawa, K., T. Yamanaka, J. Matsumoto and M. Yasuda (2013). Examination of protein-damaging activity of phosphorus(v) porphyrin photosensitizer for photodynamic therapy. J. Anal. Bioanal. Technol., S1: 003. doi:10.4172/2155-9872.S1-003:1-5.

Huiyun, W., D. Chunyan, D. Haiqing, S. Aijun, X. Wenjuan and C. Xiaojun (2012). Engineered redox-responsive PEG detachment mechanism in PEGylated nano-graphene oxide for intracellular drug delivery. J. Nano Small Micro, 8 (5): 760–769.

Khodakovskaya, M.V., K. de Silva, D.A. Nedosekin, E. Dervishi, A.S. Biris and E.V. Shashkov (2011). Complex genetic, photothermal, and photoacoustic analysis of nanoparticle plant interactions. Proc. Nat. Acad. Sci. United States of Ame., 108 (3): 1028–1033.

Li, X.Z. and Y.H. Liu (2007). Diet influences the detoxification enzyme activity of Bactrocera tau (Walker) (Diptera:Tephritidae). Acta Entomol. Sinica., 50 (10): 989-995.

Maynard, A.D. and E.D. Kuempel (2005). Airborne nanostructured particles and occupational health. J. Nanoparticle Res., 7: 587–614.

Owolade, O.F., D.O. Ogunleti and M.O. Adenekan (2008). Titanium Dioxide affects disease development and yield of edible cowpea. Electronic J. Environ., Agric. and Food Chem., 7 (50): 2942-2947.

Paillous, N. and S. Fery-Forgues (1994). Interest of photochemical methods for induction of lipid peroxidation. Biochim., 76: 355–368.

Powell, M.E.A. and M.J.H. Smith (1954). The determination of serum acid and alkaline phosphatases activity with 4-amino antipyrine. J. Clin. Pathol., 7: 245-248.

Ray, A., C.G.P. Rao, R. Srivevi and P.S. Ramamurty (1984). Changes in acid phosphatase activity in *Spodoptera litura* (Lepi: Noctuidae) during the post embryonic and adult development. Entomol., 9 (3): 161-167.

Singh, N., B. Manshian and G.J.S.L. Jenkins (2009). NanoGenotoxicology: the DNA damaging potential of engineered nanomaterials. Biomat., 30: 3891–3914.

Stampoulis, D., S.K. Sinha and J.C. White (2009). Assay-dependent phytotoxicity of nanoparticles to plants. Environ. Sci. and Technol., 43 (24): 9473–9479.

Svaasand, L.O., E. Martinelli, G.J. Gomer and A.E. Profio (1990). Optical characteristics of tumours in the visible and near-infrared. Proc. SPIE., 1203: 2–21.

Wan, S.Q. and G. Zhong-Nian (2005). Effect of action of mixture of two nanoparticles with two insecticides to pest mite (*Epitrimerus pyri*). Chinese J. Pest., 44 (12): 570-572.

Wang, D., L. Zhu, J.F. Chen and L. Dai (2015). Mn_3O_4-graphene hybrid as a high-capacity anode material for lithium ion batteries. J. Ame. Chem. Soc., 132 (1520–5126 (Electronic)): 13978–13980.

Wang, Y., H. Wang, D. Liu, S. Song, X. Wang and H. Zhang (2013). Graphene oxide covalently grafted upconversion nanoparticles for combined NIR mediated imaging and photothermal/photodynamic cancer therapy. Biomaterials., 34 (1878–5905 (Electronic)): 7715–7724.

Weishaput, K.R., C.J. Goomer and T.J. Dougherty (1976). Identification of singlet oxygen as the cytotoxic agent in photo-inactivation of a murine tumour. Cancer Res., 36: 2326–2329.

سمية النانوجرافين مع كلوروفيللين النحاس الصوديوم أو كلوروفيلين المغنيسيوم وتأثيره على مستوي إنزيمات الفوسفاتيز الحمضية والقلوية لذبابة الخوخ *Bactrocera zonata (***Diptera: Tephritidae)**

سامح مصطفى عبد النبى

معهد بحوث وقاية النباتات – مـركز البحوث الزراعية – الدقى – جيزة – مصر

جسيمات النانوجرافين هي واحدة من المواد النانوية الأكثر جاذبية لتطبيقات التسويق، وقد استخدمت على نطاق واسع كمضادات للميكروبات، والمنتجات الإلكترونية الطبية الحيوية، من نتائج الدراسة اتضح ما يلي: يلعب وقت التعرض للضوء دورًا مهمًا في نسبة الوفيات، تزداد النسبة المئوية للوفيات عندما يزداد زمن التعرض وتوجد علاقة عكسية بين التركيز ووقت التعرض، تأثير مركب الكلوروفيللين المغنيسيوم على الحشرة الكاملة لذبابة الخوخ بعد التعرض لأشعة الشمس أكثر فاعلية من تأثير كلوروفيللين المغنسيوم مع النانوجرافين ولذلك نوصى بعدم استعمال المزيج من الكلوروفيللين المغنسيوم مع الجرافين النانوية، وكلوروفيللين المغنيسيوم أكثر فعالية من كلوروفيللين النحاس مع النانوجرافين أو كلوروفيللين النحاس، مستوى إنزيم الفوسفاتيز الحامضي في رائق الحشرة المطحونة لذبابة الخوخ بعد التعرض لأشعة الشمس لمدة ساعتين بعد معاملتها بواسطة مركب كلوروفيللين النحاس أو كلوروفيللين المغنيسيوم أظهر نسبة عالية فى نشاط إنزيم الفوسفاتيز الحامضي (٥٢,٤٤% و ٤٠,٧١%)على التوالى، وبعد معالجته بواسطة مركب كلوروفيللين نحاس مع جسيمات النانوجرافين أو مغنيسيوم الكلوروفيللين مع النانوجرافين، التغيرات في إنزيم الفوسفاتيز القلوى بعد معالجتها بواسطة مركب كلوروفيللين النحاس أظهرت انخفاضاً محدوداً (-١٦,٢٨%) من ناحية أخرى أظهر كلوروفيللين المغنيسيوم نسبة عالية فى نشاط انزيم الفوسفاتيز القلوى (٧٧,١٤) مقارنة بالكنترول.

المحكمـــــون:

١ـ أ.د. طارق عفيفي عبد الحميد أستاذ فسيولوجيا الآفات – معهد بحوث وقاية النبات بالجيزة.

٢ـ أ.د. عطا علي مرسي شـلبي أستاذ المبيدات المتفرغ – كلية الزراعة – جامعة الزقازيق.