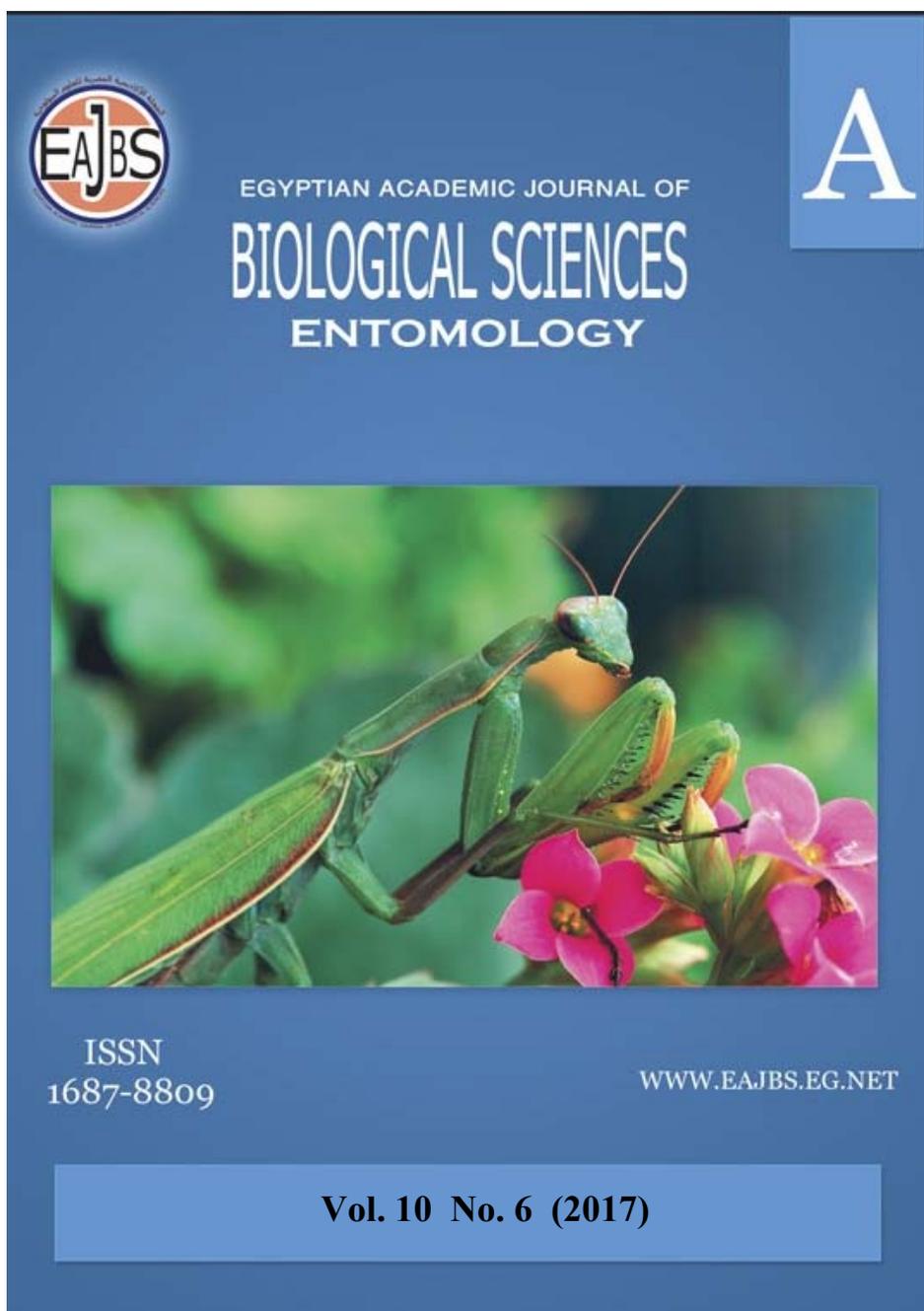


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Potency of Nano-Particle Compound And Two Traditional Insecticides Against The Spiny Bollworm, *Earias insulana* In Relation To Some Biological And Biochemical Aspects

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ABSTRACT

Laboratory trails were conducted to determine the effectiveness of zinc oxide nano-particles (ZnONPs) compared to the chitin synthesis, nomolt and bio- insecticide, emacte compounds against the first instar larvae of *Earias insulana*. Toxicity effect of the tested compounds on the first instar larvae with some biological aspects and chemical analysis were studied. The results revealed that LC_{50s} were 7.593, 29.32 and 0.536 ppm, when newly hatched larvae of *E. insulana* treated with (ZnONPs), nomolt and emacte, respectively. Obtained data also indicated an increase in larval duration to 20, 25, 23.3 and 18.0 days, for the aforementioned compounds, respectively, compared to 13.96 days for control. All treatments prolonged pupal periods compared to control, and caused highly reduction in the weight of larva and pupa stages, (ZnONPs) treatment was the most efficient in this matter as it resulted 91.1% and 93.73% reduction in larval and pupal weight, respectively, compared to control. The reduction in larval and pupal weight led to increase in the malformation and mortality percentages which recorded 77, 79 & 74 % for, ZnONPs, nomolt and emacte treatments, respectively.

According to biochemical analysis, all treatments reduced the level of the important total soluble protein, lipid and carbohydrate contents which play an important in the physiological, metamorphosis and developmental process of *E. insulana* during immature stages. In addition, (ZnONPs) and emacte treatments caused a high decrease in phenoloxidase which necessary in melanin production in cuticle formation, N-acetyl- glucosamine and chitinase activity. On contrast the chitinase activity was increased (1.5 times) approximately in case of nomolt, treatment as compared to control.

INTRODUCTION

The cotton bollworms, *Earias insulana* (Lepidoptera: Noctuidae) is considered one of the most important cotton pests in the world. It causes severe damage, resulting in great loss in both quality and quantity of cotton yield.

The disadvantage of the chemical control to cotton pests in the field is the appearance of toxic residues, pollution to the environment and the effect on the non-target organisms.

Nano technology has become one of the most promising new approaches for pest control in recent years, also, employs nano-particles (NPs) having one or more dimensions in the order of 100 nm or less (Auffan *et al.*, 2009). The trials for evaluating nanotechnology in controlling insects are based on their size-dependent qualities high surface-to-volume ratio and increasing chemical reactivity and penetration in the living cells (Medina *et al.* 2007)

A wide variety of nano-particles materials are used against some insects in the laboratory. Goswami *et al.* (2010) studied the applications of different kind of nano-particles such as silver nano-particles, aluminum oxide nano-particles, zinc oxide and titanium dioxide nano-particles in the control of rice weevil, *Sitophilus oryzae*. Also, Stadler *et al.* (2010) applied successfully alumina against stored grain pest *Callosobruchus maculatus*. Debnath *et al.* (2011) demonstrated that application of SNP could significantly increase mortality effect of NPs with increasing the time after application. Vinuthaet *et al.* (2013) recorded the potential of nano particles against *Helicoverpa armigera*. In addition, Salem *et al.* (2015) used aluminum and zinc oxides nano-particles as a new method in controlling the red flour Beetle, *Tribolium castaneum* (Herbest).

The benzoyl-urea groups of chitin synthesis inhibitors, such as Teflubenzuron are most active on the early life stages of insects that undergo complete metamorphosis in major lepidopteran and different order pests as it disrupts chitin deposition during ecdysis and caused malformation and prolonged duration in both larval and pupal stages (Kandil *et al.* 2013 and Said *et al.* 2017).

Emamectin benzoate is a novel macrocyclic lactone insecticide derived from natural occurring avermectin isolated by fermentation from the soil microorganism *Streptomyces avermitilis*. Biochemistry acts by stimulating the release of γ -aminobutyric acid, an inhibitory neuro- transmitter resulting in prevention of muscle contraction, cessation of feeding followed by death (Tomlin, 2003)

The aim of this study is to investigate the effect of zinc oxide nano-particles and two conventional insecticides manly, nomolt and emacte against the spiny bollworm *Earias insulana* in relation to some biological and biochemical aspects under laboratory conditions.

MATERIALS AND METHODS

Insect used:

Spiny bollworm (SBW), *Earias insulana*:

The laboratory strain of *Earias insulana*, used in this study was obtained from laboratory colony of Bollworms Research Department, Plant Protection Research Institute, Sharkia Branch; Agricultural Research Center (ARC), reared for several generations away from any contamination with insecticides on an artificial diet that previously described by Amer (2015).

Compounds used:

- 1- Common name: Teflubenzuron (benzoylurea)
Trade name: Nomolt® 15% EC
Rate of application: 50 cm³ / 200 L.
- 2- Common name: Emamectin benzoate
Trade name: Emacte 2.15 % EC.
Rate of application: 150 cm³ / 200 L.

- 3- Nano-material: Zinc-oxide nano-particles (ZnONPs) with purity of 99.99% obtained from Egypt Nanotech Company Limited El-Wahaat Road, 6th October, Cairo Egypt.

Bioassay:

To determine the toxicity of tested compounds against newly hatched larvae of *E. insulana*, different concentrations around the LC₅₀ values of the tested compounds were prepared as followed:

- 1- Nomolt: 3.75, 1.87, 0.97, 0.46 and 0.23 ppm.
- 2- Emacte: 0.19, 0.95, 0.47, 0.23 and 0.12 ppm.
- 3- Zinc-oxide nano-particles: 2000, 500, 250 and 125 ppm.

One ml of the different concentrations for each tested compound was sprayed on the surface of an artificial diet (5gm) putted in each petri-dish and allowed to dry. Newly hatched larvae of the SPW were allowed to feed on the treated diet for each compound (three replicates for each concentration / compound each 30 larvae) and kept under constant conditions at 26 ± 1°C and 75±5 %RH. After 24 hours for emacte and 3 days for both nomolt and ZnONPs treatments, the dead larvae were counted to represent acute toxicity of the three tested compounds.

The latent effect of LC₅₀ values for the tested compounds was studied on some biological aspects of *E. insulana*, 150 larvae used for each compound divided into three replicates each replicate 50 larvae, transferred on surface of an artificial diet previously treated with the LC₅₀ values for each tested compound. The survivors' larvae of LC₅₀ treatments were transferred individually after 24 hr for emacte and 3 days for nomolt and ZnONPs treatment to glass tubes ((2 X 7.5 cm) each containing about 3.0 gm of artificial diet by camel hair brush and (another group of tubes containing diet without any treatment) used as a control) by camel hair brush. The tubes were capped with cotton and kept under the previous conditions in an incubator and inspected daily until pupation. Some biological aspects included accumulated larval mortality, and % of malformation, durations of larvae, pupae and total immature stage were recorded.

Biochemical analyses:

Sample for biochemical assay:

Treated larvae of 14 days old were analyzed chemically for each compound with untreated check in Physiological Dept. of plant Protection Researches Institute, (P.P.R.I.). The larvae numbers of nomolt and emacte treatments were 35 & 28 larvae = 0.5 gm., respectively, while it was (56 larvae = 0.5gm) for (ZnONPs) treatment, compared to (9 larvae = 0.5gm) in control.

Biochemical studies:

Preparation of insects for analysis:

The treated larval samples were homogenized in distilled water. The homogenates were centrifuged at 8000 r. p. min. at 5C⁰ in refrigerated centrifuge. The supernatants were kept in deep freezer at 20C⁰ till use for biochemical assays.

Total protein content of whole homogenate was measured according the method described by Bradford, (1976). Total lipids were determined by colorimetric method described by Knight *et al.* (1972). Total carbohydrate were estimated colorimetric method described by Croupton and Birt (1967), However, determination of N-acetylglucosamine by the sensitive method of Waterhouse *et al.* (1961), Phenoloxidase activity was determined according to modification of Ishaaya, (1971) and chitins' activity was prepared according to Bade and Stinson, (1981).

Statistical Analysis:

Analysis was conducted to estimate LC₅₀ and LC₉₀ values with their fiducially limits by Probit (proban) analysis software according to Finny (1971). The potency levels in and the toxicity index were also calculated, Sun (1950).

RESULTS AND DISCUSSION**Toxicological effect of the tested compounds:**

The susceptibility of *Earis insulana* larvae to (ZnONPs), nomolt and emacte was showed in **Table (1)**. The obtained resulted revealed that emacte was the most toxic insecticides against the 1st instar larvae of *E. insulana*; whereas nomolt was the least toxic one on the other hand zinc oxid nouo-particles occupied the middle situation among the aforementioned compounds. The corresponding LC₅₀ values were 0.54, 7.59 and 29.32 ppm and these values recoded 4.66, 48.70 and 62.41 ppm at LC₉₀ levels; respectively. According Sun's (1950) toxicity index, emacte the highest toxic one was selected as stander compound and giving arbitrary 100units. Toxicity index of zinc oxide nano-particles and emacte at LC₅₀ values recorded 7.12 and 1.84% as toxic as the toxicity of emacte and at LC₉₀ level; the toxicity of a forementioned compounds attained 9.57 and 7.47% as the toxicity of emacte against the newly hatched larvae of *Earis insulana*. According to potency levels, nomolt the lowest effective one was chosen as standard insecticide. The toxicity of zinc oxide nano-particles and emacte recorded 3.86 and 54.30 times at LC₅₀ and 1.28 and 13.39 times at LC₉₀ as toxic as the toxicity of nomolts, respectively.

In the same trend, Raslan *et al.* (2009) found that LC₅₀ and LC₉₀ of Emamectin benzoate against newly hatched larvae of *Pectinophora gossypiella* were 1.65 and 11.19 ppm. Lopez *et al.* (2009) mentioned that Emamectin benzoate was highly toxic to *Helicoverpa zea* males with LC₅₀ values 0.718, 0.525 and 0.182 ppm for 24, 48 and 72 hr. respectively.

Table (1): Toxicological effect on the tested compounds against newly hatched larvae of *Earis insulana*.

Compounds	Slop	LC ₅₀ (ppm)	LC ₉₀ (ppm)	*Toxicity index based on		**Potency levels based on	
				LC ₅₀ (ppm)	LC ₉₀ (ppm)	LC ₅₀ (ppm)	LC ₉₀ (ppm)
ZnO (NPs)	0.6205	7.59	48.70	7.12	9.57	3.86	1.28
Nomolt	0.635	29.32	62.41	1.84	7.47	1	1
Emacte	1.341	0.54	4.66	100	100	54.30	13.39

LC₅₀ or LC₉₀ of the most toxic compound

$$*Sun's\ toxicity\ index = \frac{LC_{50}\ or\ LC_{90}\ of\ the\ tested\ compound}{LC_{50}\ or\ LC_{90}\ of\ least\ toxic\ compound} \times 100$$

$$**Potency\ levels = \frac{LC_{50}\ or\ LC_{90}\ of\ the\ tested\ compound}{LC_{50}\ or\ LC_{90}\ of\ the\ tested\ compound}$$

Biological aspects:**Accumulated mortality and morphological deformation in the treated larvae:**

Data presented in **Table (2)** indicated a gradual in larval mortality until the end of larval stage. Accumulated mortality rates from 1- 18 days were 65, 65 and 69 % for treated larvae with ZnONPs, nomolt and emacte, respectively, compared with 6 % in control. The highest percent of malformed larvae was achieved by nomolt treatment (14%) followed by ZnONPs treatment (12%) and then, with a wide range, emacte (5%) compared to 1% in control. Figures (1) and (2) revealed normal larva and pupa. While, most of morphological deformation was appeared during the molting period for nomolt treatment; the larvae were darkling in color with high reduction in their in comparison to control (Fig.3). The old and new cuticle can be seen at the same time as a result of nomolt treatment which caused incomplete larval molting and fail to pupate (Fig.4).The failure in pupation also takes another appearance as the pupa couldn't make its cocoon forming stripper pupa and so the pupation process couldn't complete (Fig.5).

Table (2): Effect of treatments on larval mortality and malformation rate of *Earis insulana*.

Comp.	Initial number of treated larvae	% Gradual larval mortality after treatments			% Accumulated larval mortality (up to 18 days)	% Malformed larvae	% Total mortality & malformation
		(1-5) days	(5-9) days	(9-18) days			
ZnO (NPs)	150	50	6	9	65	12	77
Nomolt	150	53	3	9	65	14	79
Emamectin benzoate	150	61	7	1	69	5	74
Control	60	2	1	3	6	1	7

Zinc oxide Nano particles, treatment caused dwarfed larvae with abnormal distribution of cuticle pigment leads to abnormal color besides inability for shedding out the old cuticle leads to failure molting and mortality, (Fig :6 to 10). This may be explained by (Medina *et. al.* 2007) who recorded that the toxic effects of NPs can be attributed to the small size and large surface area, thereby increasing chemical reactivity and penetration in the living cells.

These results are in agreement with Khedr (2015) indicated that, in continuity teflubenzuron, the signs of failure molting that led to mortality include the extrusion of body fluids from treated larvae during the molting process were detected; (López, 2010) recorded that emacte had high effect on mortality of *Helicoverpa zea* Boddie and Cotton leaf worm *Spodoptera littoralis* Bois.

Larval and pupal duration:

The effect of zinc oxide as nano-particles, nomolt and emacte on spiny bollworm larval duration was given in Table (3). Results indicate that LC₅₀ values caused a significant prolongation in larval duration, compared to control. The mean larval durations on ZnONPs, nomolt and emacte were 25.0, 23.3 and 18.0 days,



Fig. (1): *Earias insulana* normal larva.



Fig. (2): *Earias insulana* normal pupae.



Fig. (3): Very small sized nomolt treated larva with abnormal somewhat darkling in colour.



Fig. (4): Incomplete larval moulting (the larva cannot shed out the old cuticle, the old and new cuticle can be seen together, as a result of nomolt treatment).



Fig. (5): The pupa couldn't make its cocoon forming stripper pupa and so the pupation process couldn't complete as a result of nomolt treatment.



Fig. (6): Darker to black dead larvae with a very small size as a result of ZnONPs treatment.



Fig. (7): A very small sized 2nd instars larvae with lean textures and pale cuticle colour as a result of ZnONPs treatment.

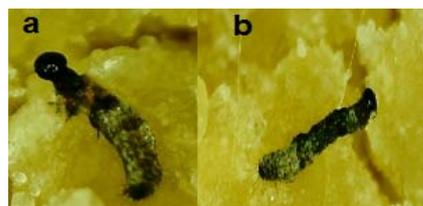


Fig. (8): Dwarfed larvae of ZnONPs treatment indicated:
(a) Sever malformed head capsule and thoraces.
(b) Reduction in cuticle pigment with abnormal distribution



Fig. (9): Dwarfed larvae of ZnONPs treatment indicated the absence of most cuticle colour and surrounded by Nano particles.



Fig. (10): Very small sized ZnONPs treated larva with abnormal distribution of cuticle pigment.

respectively, compared with 13.96 days for control. The average duration of *Earis insulana* pupae resulted from the treated larvae were 12.36, 12.5, 10.3 days/ pupae, for tested compounds respectively, with great elongation in pupal period compared with 8.56 days in control

Larval and pupal weight:

The average larval and pupal weights resulted from treated newly hatched larvae with the LC₅₀ values of the tested compounds of SBW were highly decreased to 0.006 0.0252 and 0.0434gm/ larva and 0.0042, 0.0142 and 0.0401 gm/pupae for ZnONPs, nomolt and emacte, respectively, while it recorded 0.0611 gm/ larva and 0.057gm/ pupa in control, Table (3). The reduction in weight for *Earis insulana* larvae was estimated by approximately 10 times less than control for ZnONPs treatment, besides the redaction in pupal weight recorded approximately 13.6time less than control

Table (3): Effect of tested compounds on duration and weight of immature stages of *Earis insulana*.

Comp.	larval duration (day)	Increase of larval duration	larval weight (mg)	% Weight reduction	Pre-pupal duration (day)	Pupal duration (in day)	increase of pupal duration	pupal weight (mg)	% Weight reduction	Total immature duration (day)
Zinc oxide (NPs)	25.00 ^d ±0.56	1.79	0.0063 ^a	91.1	3.0 ^c ±0.23	12.36 ±0.3	1.45	0.0042 ^{1a}	93.75	39.66 ^c ±1.23
Nomolt	23.30 ^c ±0.35	1.66	0.0252 ^a	64.55	1.83 ^c ±0.19	12.30 ^c ±0.2	1.44	0.0142 ^b	78.83	37.43 ^c ±0.93
Emacte	18.00 ^b ±0.66	0.30	0.0434 ^b	3 8.96	1.56 ^b ±0.14	10.30 ^b ±0.2	1.21	0.0304 ^c	54.6	29.86 ^b ±1.3
Control	13.16 ^a ±0.36	-----	0.0711 ^c	-----	1.36 ^a ±0.10	8.53 ^a ±0.29	-----	0.0671 ^d	-----	23.85 ^a ±0.9
LSD	2.651	0.325	---	2.158	0.311	1.665	---	0.0006	--	3.441

These results confirmed those previously obtained by Mouharib (2009) who showed that emacte benzoate at LC₅₀ value reduced the developed pupae along pupation period. Also, Lopez *et al.*, (2010 and 2011) found that treatment of *H. zea* with emamectin benzoate significantly reduced larval survival to the pupal stage. Kandil *et al.*, (2013 & Said *et al.*, (2017) they recorded that the LC₅₀ value of the IGR compounds hexafluomeron and Teflubenzeron increased developmental period of *P. gossypiella* larvae and pupae compared with the control.

Biochemical aspects:

The total soluble protein and the total lipids:

It is clear from the presented data in the **Table (4)** that newly hatched larvae of *Earis insulana* treated with ZnONPs and nomolt caused highly reduction in the

level of total soluble protein to reach 18.33 and 17.67mg/g.b.wt., respectively, in contrast, the larvae treated with emacte, increased the level of total soluble protein to 30.93 mg/g.b.wt. compared to 26.57 mg/g.b.wt. in control. On other hand all tested compounds reduced the total lipid content to 25.60, 17.52 and 27.30 (mg./gb.wt) in larvae treated with ZnONPs, nomolt, and emacte, respectively, compared to 32.41 mg./gb.wt in control, (Table 4).

Moreover, a high decrease in the total carbohydrates (approximately to half time) as estimated by 13.52, 15.35 mg./gb.wt./ larvae in ZnONPs and nomolt treated larvae, respectively, while it moderately decreased to 20.25 mg./gb.wt./ larvae in emacte treatment compared to 28.53 mg./gb. Wt./larvae in control. However, this reduction in the total protein, lipid and carbohydrate content which necessary for energy, development, or survivorship of larvae may reflect the decrease in activities, very small size, reduction in weight and death of treated larvae. Data represented in Table (4) revealed that the phenoloxidase which necessary in melanin production in cuticle formation, was highly decreased approximately to half time (10.96 units/g.b.wt) when the larvae treated with Zinc-oxide nano particles that leads to disappear of cuticle color in treated larvae (Figs 7 and 9). In contrast, nomolt treatment caused an increase in phenoloxidase to 27.69 units/g.b.wt./ larvae which may explain the dark color of treated larvae (Fig 3) compared to 21.63 units/g.b.wt. in control.

Table (4): Biochemical effects of the tested treatments on the tested newly hatched larvae of *Earis insulana*.

Biochemical aspects	Zinc Oxide NPs	Nomolt	Emacte	Control
Total protein (mg/g.b.wt)	18.33±0.36	17.67±0.84	30.93±0.80	26.57±0.26
Total lipid (mg/g.b.wt)	25.60±0.37	17.52±0.25	27.30±0.90	32.41±0.95
Total carbohydrate (mg/g.b.wt)	13.52±0.80	15.35±0.65	20.25±1.20	28.53±0.98
Free-amino acid (µg D,L-alanine/g.b.wt)	311±3.74	232.0±3.89	416.0±9.82	502.0±16.25
Phenoloxidase (O.D. units/g.b.wt)	10.96±1.10	27.69±0.89	19.80±0.77	21.63±0.40
N- acetyl-glucosamine (µg NAGA /g.b.wt)	132.5±5.82	111.00±3.54	160.80±8.20	187.33±3.60
Chitinase (µg NAGA x10 ³ /min/g.b.wt)	366±6.44	988.33±17.90	621±10.20	644.0±1.34

Results are going on line with that obtained by Kandil *et al.*, (2012 and 2013) who found that larvae of *P. gossypiella* treated with different IGRs caused an increase in total lipid activity by one-two times and decreased total protien than control. On other hand obtained results are partially in agreement with those of Assar *et al.*, (2016) who recorded that the used Teflubenzuron (IGR's) against the 4th instar larvae of *Spodoptera littoralis* cussed high significantly decreased in total carbohydrates, proteins, lipids, acetylcholinesterase, chitinase, phenoloxidase, carbohydrates hydrolyzing enzymes. Also, Said *et al.* (2017) who recorded that

Teflubenzuron (IGR's) caused high reduction in total protein, lipid and chitin enzyme for *P. gossypiella* larvae than control.

As shown in Table (4) all treatments reduced N- acetyl-glucosamine (μg NAGA /g.b.wt) which is necessary in chitin formation. The higher reduction recorded 111.00 μg NAGA /g.b.wt for nomolt treatment, while the lowest reduction was 160.80 μg NAGA /g.b.wt for emacte treatment compared to 187.33 μg NAGA /g.b.wt. in control.

Data in Table (4) showed that the chitinase activity of *E. insulana* larvae treated with ZnONPs and emacte, decreased to 366 and 621 μg NAGA x103/min/g.b.wt / larvae, respectively, on the other hand, chitinase activity was highly increased in nomolt treatment compared to control; approximately 1.5 times as it recorded 988.33 and 644.0 μg NAGA x103/min/g.b.wt. / larvae for nomolt and control respectively.

From the previous data it could be concluded that the increase in chitinase activity may cause inhibition of the synthesis of new cuticle specifically; chitin biosynthesis and so resulted in failure ecdyse or molt that may lead to death.

The results are confirmed with the findings of , El-Sheikh *et al.*, (2013) the stated that increase in chitinase activity and fluctuated changes were recorded when *S. littoralis* was exposed to Teflubenzuron. Also, Said *et al.* 92017 reported that the chitins activity of the larvae PBW treated with Teflubenzuron was 1101.67 compared to 1050 in control, while, the units (μg N- acetyl glucose amine liberated x 103/min/g. b.wt.) increased Two times approximately , compared to larvae untreated.

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ARABIC SUMMARY

فاعلية أحد مركبات النانو واثنين من المبيدات التقليدية ضد دودة اللوز الشوكية، و علاقتها ببعض الجوانب البيولوجية والبيوكيميائية

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أجريت تجارب معملية لدراسة تأثير جزيئات النانو لمركب أكسيد الزنك ومركب نومولت من مجموعة مانعات الانسلاخ بالاضافة الى احد المركبات الحيوية و هو الايمامكتين على العمر اليرقي الاول لحشرة دودة اللوز الشوكية تم دراسة التأثير السمي لكل من المركبات محل الدراسة على العمر اليرقي الاول الى جانب بعض المظاهر البيولوجية و النظم البيوكيميائية اوضحت النتائج ان قيم التركيز نصف المميت قد سجلت 7.593، 29.32 و 0.536 جزء في المليون لكل من جزيئات النانو لمركب أكسيد الزنك ومركب نومولت ومركب الايمامكتين على التوالي كما أثرت المعاملات بالمركبات السابقة الذكر بالاطاله المعنوية في أعمار اليرقات لتصل الى 20، 25، 30 يوم على التوالي مقارنة ب 96، 13 يوم للكنترول ايضا أظهرت المعاملات اطالة في أعمار العذارى الناتجة مقارنة بالكنترول كما أدت المعاملات الى نقص شديد في اوزان كل من اليرقات و العذارى الناتجة بفاعلية اقوى جزيئات النانو لمركب أكسيد الزنك و الذى تسبب في نسبة من النقص سجلت 91.1 و 93.73 % لكل من اليرقات و العذارى على التوالي والذي بدوره تسبب في تشوه وكذلك نسبة من الموت قدرت ب 77 و 79 و 74 % لكل من اوكسيد الزنك(النانو) ومركب نومولت ومركب الايمامكتين على التوالي، كما تسببت المعاملات في نقص كلي في المحتوى الكلي للبروتين والدهون و الكربوهيدرات والتي تلعب دورا هاما في التحورات الفسيولوجية وعملية التطور للاطوار الغير ناضجة. كذلك تسبب مركب اوكسيد الزنك(النانو) بالاخص في نقص شديد في كل من انزيم الفينول اوكسيديز، الضروري لانتاج صبغة الميلانين وتكوين الكيوتيكل، وكذلك نشاط انزيم الكيتينيز اللازم لعمليات الانسلاخ على العكس من ذلك فقد تسبب كل من مركب نومولت ومركب الايمامكتين في زيادة نشاط انزيم الكيتينيز وصلت الى 1.5 مره تقريبا مقارنة بالكنترول.