

Comparative effect of Ginger extract nanoparticles with some pesticides on Pink bollworm, *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae)

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ABSTRACT

Plant extracts are very cost-effective and eco-friendly and thus can be an economic and efficient alternative compounds for controlling insect pests. Laboratory trials were conducted to determine the effectiveness of Emamactien, ginger aqueous extract and ginger Ag NPs on one and three-days old eggs of *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae) and some biological effects such as larval and pupal durations, and adult emergence and generation time resulted from treated eggs. The results demonstrated that the hatchability for eggs were highly significantly affected compared with control. The percentages of eggs hatchability were 51,57 and 48 for one-day old eggs, respectively, compared with 97% in control and 53, 54 and 50 for three days old eggs dipping in LC_{50} values of Emamactien, ginger aqueous extract and ginger Ag NPs, respectively compared with 98% in control. The obtained results show a prolongation in larval and pupal developments resulted from treated one or three-days old eggs, the total immature stages (larval + pupal stage) periods were 30.4, 27.3 and 32.2 days resulted from treated one-day eggs, also these periods were 32.2, 30.6 and 34.4 days resulted from three-days old eggs treated with the three compounds, respectivily, at the tested level LC_{50} , compared with 21.8 days in control for both egg ages. The generation times from different egg ages treated was elongated was 39.1, 41.7 and 35.2 days on one days old eggs and increased to 40.5, 38.5 and 42.9 days when resulted from treated three days eggs treated with Emamactien, ginger aqueous extract and ginger Ag NPs at the tested level LC50, respectively, compared with 27.9 days for control.

Key world: Ginger Ag NPs, *Pectinophora gossypiella*, Emamactien, biological aspects,





Introduction

The bollworms like pink bollworm (*Pectinophora gossypiella*) (Lepidoptera: Gelechiidae)is a significant pest attacked the cotton in Egypt.The adults lays the eggs on different parts of cotton, squares, flowers or green bolls, PBW eggs are oval, about 0.55 mm long and 0.25mm wide, with a rough surface. When first laid they are whitish yellow, then orange and finally pink before hatching and set on plant from 3 -4 days before hatching larvae; Bio- insecticide composed of a naturally occurring compound, avermectin, extracted from the soil microorganism Streptomyces avermitilis (soil bacterium *Streptomyces avermitilis*) (Birah*et al.*, 2008; Temple *et al.*, 2009).

Natural plant extracts play an increasing role as alternatives to synthetic pesticides due to the increase of health hazards, negative effects on non-target organisms, and environmental pollution (Sharma et al., 2006). There are more than 2400 plant species belonging to 189 plant families, which are rich sources of organic compounds that have some biological and physiological effects on different insect pests (Rao et al., 2005). Different species from over 60 plant families have been identified as insecticidal (Prakesh&Rao, 1997).

Nanotechnology is growing very rapidly, and the nanoparticles technology is widely applied in various fields, especially agricultural and industrial activities (Said 2017) . In agriculture, nanoparticle technology can be applied in preparing new biopesticides or new bioagents using natural products and these compounds are effective against different insect pests (**Worrall** et al 2018) . several attempts have made to synthesize the green silver nanoparticles (AgNPs) using plant extracts such as lantana camara (Hikmat et al., 2018) and ginger extracs help to produce a new insecticides or insect repellents (Owolade et al., 2008 and Chinnamuthu& Murugesa 2009).

Many researchers appeared there have been numerous studies on the toxicity and repellants effects of nanoparticles on some insect; (Elchiguerra et al., 2005; Bhattacharyya et al., 2010 and Sangeetha et al. 2017). Although there have been numerous studies with little research has been carried out to investigate the toxicity effect of nanoparticles on insects , and they agreed that this method of using nano-particles was effective in insect management (Said 2017). Many researchers showed that the most nanotechnology used will





revolutionize agriculture including pest management in the near future such as Ag and alumina NPs which could be successfully used to control stored grain pests and many insect pest such *Erias insulan*, *Blattella* germanica (Yang et al. 2009, Bhattacharyya et al., 2010, Stadler et al. 2010, Said 2017 and El-Shanawy and Kandil (2017).

Due to the increased use of NPs in many fields of science has led for the need to know that one of the most common mechanisms of toxic action of NPs is oxidative stress or oxidative damage, this damage resulting in case of antioxidant defense system failure, the resulting oxidative damages, such as lipid per-oxidation and DNA damage. So it is important to study the impact of NPs on the environment in general and insect life in particular. The objective of the present study was to investigate the effect of Emamactien, ginger aqueous extract and ginger NPs, on pink bollworm eggs and the effect of these compounds on biological characteristics of the first generation of *P. gossypiella* which produced from treated different age's eggs.

Material and Methods Insect used:

The eggs used in this experiment were collected from laboratory strain of pink bollworm, *P. gossypiella* adults' culture which resulted from reared for several generations without any insecticides, as describe by Rashad and Ammar (1985). It was obtained from the Plant Protection Research Institute, Agricultural Research Center, and The experiments were done in biology laboratory ,Faculty of Agriculture , Menofia University, Egypt.

Reared adults for collected eggs

The newly emerged adults of *P. gossypiella* resulted from the reared under the laboratory conditions at 26 ± 1^{0} C; 10 pairs(33 X 99) were confined in a glass chimney cage (17 cm height and 7.12 cm in diameter), inside which a piece of cotton wool previously soaked in 20% sugar solution was suspended to be renewed 48 hrs. for moths' nutrition. The top and bottom of each cage were covered with screening mesh kept in position by rubber bands for stimulating eggs laying response in the females. Eggs were deposited thought the screening mesh, one piece of paper placed upper and lower the cages in open petri-dish that served as an ovipostion site, eggs were collected daily and kept in glass jars (1/2 kg). These eggs were used.





The pesticides used:

1-Emamectin:

Common name benzoate Trade name: (Emacte 2.15 %EC). Suspension Concentrate (SC) .Rate of application: $150 \text{ cm}^3/100 \text{ L}$.

2- Ginger leaves aqueous extract :

Rhizomes of Ginger (Scientific name: Zingiber Officinale, Family: Zingiberaceae, English name: Ginger) were obtained from griculture Research Center in El-qnater El-khairia, Kaliobia, Egypt in September 2019. Rhizome were identified in Horticulture department, Faculty of Agriculture, Menofia University. Plant samples) leaves) were washed and air-dried at 50 °C for 12- 24 hours until dried; the dried leaves were grinded into fine powder (Kulkarni et al 2012).

Preparation of aqueous extract:

500 grams of leaves plant sample (*Zingiber Officinale*) powders were steeped in 3000 ml of distilled water and the mixture was then kept in shaker incubator for 24 hrs at room temperature then filtered through filter paper and centrifuged at 3000 rpm. The filtrate was placed in Freez Drier to evaporate the water from it. The dried powder was transferred to a sterile universal flask in the refrigerator for later usage (Dorman et al. 2003)

3- Ginger Nanoparticles :

Preparation of 1 ml of silver nitrate solution:

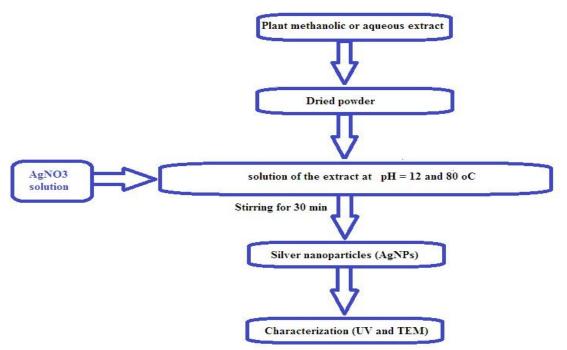
Seventeen milligrams (17 mg) of silver nitrate (Blulx laboratories (P) Ltd. 99.9% AgNO3, MW = 169.87 g/mol) were weighed using electronic balance and transferred into 500 ml Erlenmeyer flask. The silver nitrate was slowly dissolved by gently swirling the flask containing distilled deionized water. After all the solid has dissolved, more water was slowly added to bring the level of solution exactly to a volume mark of 1000 ml. The prepared 1 mM silver nitrate solution was stored at 4°C in amber colored bottle (Rajkumar and Malathi, 2015).





Synthesis of ginger aqueous extract AgNPs :

Take 1.0 gram from dray aqueous extract prepared in the preceding step, in conical flask and dissolved in 100 ml distilled water, adjust pH at 12 using 0.01N sodium hydroxide then keep all this system under magnetic stirring, when the temperature reaches 80°C add 1ml 0.1N silver nitrate ,and keep under magnetic stirring for 30 min (EL-Bisi,et al 2013)



Characterization technique of silver nanoparticles Ultraviolet-visible (UV-vis) spectra:

UV-vis spectra have been proved to be quite sensitive to the formation of silver colloids because AgNPs exhibit an intense absorption peak due to the surface Plasmon excitation which describes the collective excitation of conductive electrons in a metal.

Transmission Electron Microscopy (TEM) :

Shape and size of AgNPs were practically obtained using TEM; JEOL-JEM-1200. Specimens for TEM measurements were prepared by placing a drop of colloidal solution on 400 mesh copper grid coated by an amorphous carbon film and evaporating the solvent in air at room temperature. The average diameter of the prepared AgNPs was determined from the diameter of 100 nanoparticles found in several arbitrarily chosen areas in enlarged microphotographs.





Prepared concentrations form three tested compounds:

To study the activity of Emamectin, Ginger extracts and Ginger Ag NPs against *P.gossypiella* eggs. Serial concentrations in water were prepared as followed ;5 concentrations (40, 20, 10, 5, 2.5 and 1.25 gm/ 100 ml water) for ginger aqueous extract and 6 concentrations (80,40,20, 10, 5, 2.5 and 1.25%) for Ginger Ag NPs and five concentrations (2.936, 1.468, 0.739, 0.369 and 0.134 p) for Emamactien benzoate . these concentrations were freshly prepared for the stock solution of each compound.

Eggs treatment for toxicity study:

To estimate the toxicity compound; treatment of eggs was done by dipping a piece of paper containing eggs in the different tested concentrations of the three compounds. Three replicates (each replicate 100 to 150 eggs on paper) from one or three days old were use, other three replicates of similar eggs were dipping in water as a control. After that all papers treated or untreated left until dried. The treated and untreated eggs were kept in laboratory conditions $26\pm1^{\circ}$ C and $75\pm5^{\circ}$ R.H. The percentages hatchability and the LC_{50s} of three compounds were calculated by using propane software.

Eggs Treatments with LC50 values of three tested compounds:

After estimated the LC_{50} for each compound, three replicates for a piece of paper containing eggs from one day or Three days old for each replicate 150 to 200 eggs on paper were dipping in LC_{50} for each compound. For some biological aspects studies. Newly hatched larvae resulted from treated eggs (one or three days eggs) with LC_{50} for each compound were transferred individually to the diet tubes by camel hair brush, three replicates of 40 tubes, each tube (2 X 7.5 cm) containing 4 gm of diet were used. The same was done with the newly hatched larvae resulted from untreated eggs. The tubes were capped with cotton and kept in laboratory under 26±1°C and 75±5% R.H and inspected daily until pupation. Pupae resulted from each treatment were removed from all tubes and placed in clean tubes until adults emergence. Some biological aspects such as percentage of larval mortality, larval duration, pupal duration, percentage of adult emergence, sex ratio and generation time (eggs+ larvae+ pupae+ pre-oviposition period required for development) were determined.





-Statistical analysis

Each treatment was replicate 3 times. Statistically analyzed values were expressed as mean \pm SE of replication and Student's t-test was applied to locate significant (P, 0.05) differences between treated and control groups, (Snedecor, 1952, and Duncans (1957).





Results and Discussions

Synthesis of ginger aqueous extract (AgNPs) :

The successive formation of AgNPs was indicated by the appearance of brown colour, this is because of excitation of surface Plasmon vibrations in nano-silver) It was a quick interaction as demonstrated by the immediate colour change on blending the solution of silver nitrate and aqueous extract. This colour change demonstrates performing of redox reaction, whereby ions of Ag⁺ are reduced to Ag⁰ by the extract components, which are oxidized to different species (Halawani E. M. 2017).

Formation of Ag nanoparticles was investigated by UV-Vis spectroscopy to ensure the successful synthesis using Lambda 25 UV -Visble spectrometer, Perkin Elmer, Inc. Characteristic absorption peak of Ag-NPs in the UV-Vis spectra around 450 nm which was generated due to the surface Plasmon resonance (SPR) of Ag-NPs (Fig 1) (Liz-Marzan,2006).

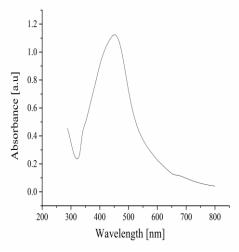




Fig 1. UV-Visible spectrum of the prepared Ag NPs ginger (aqueous extract)

Fig.2: TEM image of the prepared Ag NPs particles in the polymer matrix

Transmission electron microscopy (TEM) was utilized to lucidate shape and size of the prepared Ag nanoparticles (Ag, this investigation was made by using JEOL JEM 10x10 (Electron microscope-Japan). **It** was noted from TEM images in fig.2. That the prepared Ag nanoparticles





have a spherical shape and well dispersed in the polymer matrix with average particle size of-13.5 nm.

Toxicological evaluations of tested compounds against eggs of pink bollworm:

Toxicity levels for three tested compounds against eggs of *P*. *gossypiella* were estimate in the presented study at 4-7days passed from all treatment. Data summarized in Table (1) recorded that the LC₅₀ value of Fmamectin benzoate compound is considered to be the most toxic for tow age eggs than anther compounds, it estimated by0.958 ppm for one day old eggs and decreased to 0.68 ppm with three days old eggs, on the other hand, it reached to 16.341 and 14.22% with Ginger that considered the least efficacy comparing with the two tested compounds, the LC50 value of ginger extract AgNPs estimated by10.37 and 8.6%.

Treatment	Ages of eggs (days)	Toxicity			
		LC ₅₀ (ppm)	LC ₂₅ (ppm)	Slope	
	Emamactien	0.958	0.516	1.72 ± 0.15	
One day old	Ginger aqueous extract	16.341	6.53	1.53± 0.15	
	Ginger Nano	10.37	3.62	1.47 ±0.13	
	Emamactien	0.68	0.36		
Three days old	Ginger aqueous extract	14.22	5.91	1.6 ±0.17	
	Ginger Nano	8.6	2.11	1.36 ±0.14	

Table (1): Toxicological evaluation of three compounds against eggs of pink bollworm.

Effect on hatchability and incubation period of eggs:

Date in Table (2) showed the effect of three compounds on hatchability and incubation period of pink bollworm one and three days old eggs. It is obvious that the Emamactien, Ginger aqueous extract and Ginger AgNPs at the tested level LC50 reduced the percent of hatchability to 51,57 and 48 for one days old eggs, respectively, than 97% in control (chick). While when treated the three days old eggs with the LC50 level of three compounds the hatchability percent were53, 54 and 50 when dipping in three treatments Emamactien, Ginger and Ginger Nano, respectively, compared with 98% in control. These data showed that no difference was recorded for the hatchability between one and three days old eggs treated with LC50 of Emamactien, Ginger and Ginger



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Nano. However, the two age eggs were the most sensitive to Ginger Nano than Emamactien, Ginger extract. Temerak (2003) found no significant difference was recorded for the hatchability between 1&3 days old eggs of pink bollworm treated by spinosad.

Data in Table (2) show that the Ginger Nano, extracts was proved to be more effective in controlling PBW eggs, it was observed from nonhatchability and mortality rate of hatchability that the given nano extract was 11 to 28% higher in 24 h than Emamactien and Ginger extract. Also, (Fig 1-4) show that the delayed larval development or when contain fully developed, embryos that failed to hatch and apparently died just before hatching with the 3days old treated, delayed larval completed the development and non-hatched observed Fig (5&6) the present study are agreement with the findings of Abd El-Zaher. (2017) showed that mortality of S. litturalis was increased to reach after 7 days of treatment 91.1, 66.7, 82.2 and 91.1% for Flax, Ginger, Garlic and Jojoba oils respectively. Also, El- Shanawy and Kandil (2017) studied the effect of action of nanoparticles with bio-insecticide on E. insulana, they recorded that the zinc oxide and bio- insecticide was high toxicity and elongated all duration of pest. Hikmat et al. (2018) showed that Lantan camara plant extract nanoparticles has a toxicity effect against S. litura.

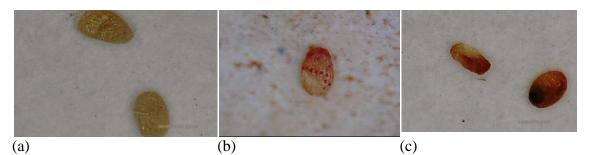


Fig .1: Control eggs (a) one day old eggs , (b)2 days old eggs and (c) 3 days old eggs

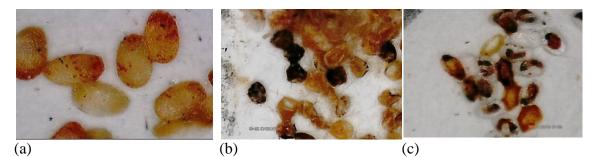
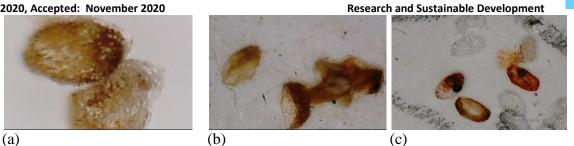


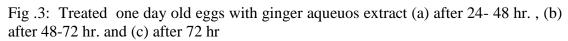
Fig .2 : Treated one day old eggs with Emamictien (a) after 24- 48 hr., (b) after 48-72 hr. and (c) after 72 hr







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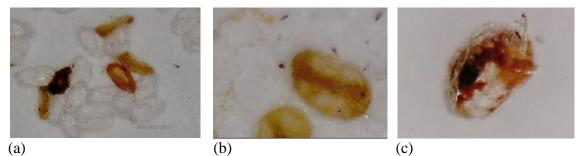


Fig .4: Treated one day eggs with ginger Ag NPs (a) after 24- 48 hr., (b) after 48-72 hr. and (c) after 72 hr(first instar larva died just before hatching)

The incubation period:

The incubation period of 1and3 days' ages of pink bollworm was significant high affected by LC50s treatments of three compounds (Table 2). The incubation periods estimated by 6.3, 5.6 and 6.6 days when one-day-old eggs treated with Emamactien, Ginger and Ginger Nano at the tested level LC50, respectively, compared with 3.3 days for control, but, when the three days old eggs treatment, these periods were 5.3, 4.5 and 5.6 days compared to 3.4 days for control. This data indicated that when one-day-old eggs treated by the three tested compounds, the incubation period of eggs increased approximately from 1.5 to 2.5 times than control.





Table (2) effect of three compounds on hatchability and incubation period of *P. gossypiella* eggs.

	One day old			Three days old		
Compound used	% of hatchability	Incubation times	%Mortality Larvae*	% of hatchability	Incubation times	%Mortality Larvae*
Emamactien	51.0	6.3±0.3a	8	53.0	5.3±0.3a	22.0
Ginger aqueous extract	57.0	5.6±0.8b	7	54.0	4.5±0.2b	13.0
Ginger Nano	48.0	6.6±0.4a	11	50.0	5.6±0.3a	28.0
Control	97.0	3.3±0.2c	3.0	98.0	3.2±0.1c	2.0
LSD		0.054			0.211	

*Mortality larvae after hatched by 24 hr.

Larval and pupal stage:

It is clear that the three tested compounds significantly prolonged the duration of the larval stage than that of the untreated check. Table (3) revealed that larval duration were 18.4,17.3 and 19.6 days resulted from treated eggs (one day old eggs), respectively, and increased to 20.6, 18.9 and 22.3 days when three days old eggs treated with Emamactien, Ginger and Ginger Ag NPs the tested level LC50, respectively, compared with 14.5 days in control. In addition, the data illustrated significant increase in pupal duration of P. gossypiella resulted from treated one or three days old eggs with all treatments. The averages ranged between 12.6 and 12.0 days with the tested level LC50 values of Ginger Nano and Emamactien at, respectively, it appeared the pupal stage required nearly equal in time 12.0 and 12.6 days, respectively, compared with 7.3 days in control. Mouharib (2009) showed that the Emamactien benzoate at LC50 value cussed along of pink bollworm pupation period, Lopezet al., (2010) found that treatment of Helicoverpa zea and Macrolophus pygmaeus with Emamactien benzoate was significantly reduced larval survival to the pupal stage, also, Gaaboub et, al. (2012) The pupal duration was 12.8 days with the higher concentrations of jojoba oil, while it was 6.8 days in control.





Total immature stage:

The times required for completed the total immature stages of pink bollworm resulted from one and three day old eggs was high significant and affected by LC50values for three compounds (Table, 3). This period estimated by 30.4, 27.3 and 32.2 days resulted from one day old eggs treated and 32.2, 30.6 and 34.4 days resulted from three days old eggs treated, at the tested level LC50, of Emamactien, Ginger and Ginger Nano compared with 21.8 days in control.

Table (3) effect of three compounds on immature stage *P. gossypiella* resulted from treated eggs.

	Different stages for one day old			Different stages for three days old			
Compound used	larva stage	Pupal stage	Total immature stage	larva stage	Pupal stage	Total immature stage	
Emamactien	18.4±0.3c	12.0±0.3b	30.4 ±2.8 c	20.6±1.3c	11.6±0.5	32.2 ±2.7	
Ginger aqueous extract	17.3 ±0.3 b	10.0 ±0.3 c	27.3±1.8b	18.9±1.4b	11.7 ±0.7	30.6±1.9	
Ginger Nano	19.6 ±0.3 d	12.6 ±0.3 b	32.2 ±1.9 d	22.3 ±1.6 d	12.1 ±0.9	34.4 ±2.4	
Control	14.5±0.3a	7.3 ±0.3 a	21.8±1.3a	14.5 ±0 .6a	7.6±0.3	21.8±1.8	
LSD	0.351	0.715	0.332	0.214	0.318	0.542	

Values are mean \pm SE of three replicates.

Values within the same column having the same letters are not significant different (ANOVA, Duncan's multiple range tests, P < 0.05)

Adult stage

The percentages of adult emergence were 71, 63 and 66% adults resulted from treated one day old eggs and 64, 67 and 60% adults resulted from tow age eggs treated with LC50 treatment of Emamactien Ginger and Ginger Nano, respectively, compared with 96 in control (Table 4). **Generation times:**

Analysis variance of the generations times results arranged in Table (4) proved that the generation time = estimated from eggs to pre-oviposition period of emerged females from two eggs ages treated with LC50 treatment of Emamactien ,Ginger and Ginger Nano were significant affected, as it was elongated to 40.9, 36.2 and 43.1 days resulted from one days old eggs and it were 40.5, 37.5and 42.9 days when resulted from three days eggs treated with Emamactien, Ginger and Ginger Nano, respectively, compared with 27.9 days in control.





Compound used	Generation	times resulted f	rom one day old	Generation times resulted from three days old			
	% Adult emergence	Pre- oviposition	Generation	% Adult emergenc e	Pre- oviposition	Generation time	
Emamactien	71	4.5±0.2a	40.9±4.2 a	64	3.1±0.3b	40.3±3.5a	
Ginger aqueous extract	63	3.3±0.40b	36.2±6.1c	67	3.6±0.3a	38.7±3.4c	
Ginger Nano	56	4.6±0.3a	43.9±5.1b	60	2.9±0.1c	42.9±2.9b	
Control	96	2.3±0.3c	27.9±2.4d	96	2.6±0.2d	27.9±1.2d	
LSD		0.217	1.811		0.113	1.045	

Table (4) effect of three compounds on Generation, pre oviposition period and %adult emergence *of P. gossyiella* treated eggs.

Generation times = times required from eggs+(larvae +pupae{immature stage}) + pre-oviposition period

Reproductive potential:

Statistical analysis of data summarized in Table (5) demonstrated that highly significant differences between oviposition period, reproductive and fertility for adults resulted from eggs treated with three compounds Emamactien, Ginger and Ginger Nano comparative with control. The respective, average number of eggs deposited (fecundity) by females were 122.0 eggs (at 7.3 days oviposition), 165 eggs(at 10.5 days oviposition), and 89.0eggs laid (at 6.6 days oviposition), /female resulted from one day old eggs treated, compared with 242.0 eggs/ female in control deposited at 12.6 days. On the other hand the number of eggs deposited by females resulted from three days old eggs treated estimated by 103.0 eggs (at 9.0 days oviposition), 143.0 (at 11.3 days oviposition), and 112.9 eggs(at 5.2 days oviposition), /females resulted from Emamactien, Ginger and Ginger Nano, respectively, compared with 236.0 eggs/ female in control. Reddy et al., 2007, Debnath et al., 2011and Rouhani et al. 2011 have studied the effect of nanoparticles, against some pests. they reported that the ZnO, TiO2 and Ag nanoparticles has a high activity on Frankliniella occidentalis (Pergande) and recorded the most





mortality were 28% on ZnO, 70% on TiO2and the lowest was 2% on Ag at $LC_{50}=195.27$ mg/L..

Tested Compound	Adults	resulted from one	e day old	Adults resulted from three days old			
	Oviposition Days ±SE	Fecundity total eggs laid / female	Fertility total eggs hatching ±SE	Oviposition Days ±SE	Fecundity total eggs laid / female	Fertility total eggs hatching. ±SE	
Emamactien	7.3±0.5 c	122.0±10.5c	78.0 ±4.20b	9.0±0.7c	103.0±5.3d	52.0±2.9c	
Ginger extract	10.5±0.7 b	165.0±8.3b	102.0 ±9.2a	11.3±0.6b	143.0±6.3b	77.0±3.5 b	
Ginger Nano	6.6±0.4 d	89.0±4.5d	51.0 ±5.1c	5.2±0.4d	112.9±7.1c	27.9±1.2d	
Control	12.6±1.2 a	242.30±0.3a	27.9±2.4d	12.9±0.9a	236.0±10.2a	114.0±8.4a	
LSD	0.43	12.24	2.23	1.22	6.54	11.35	

Table (5) Latent effect of three compounds on oviposition times and fecundity of *P. gossypiella* resulted from treated eggs.

Conclusion

Eggs hatchability effect of **Ginger** nanoparticles was slightly more than that of **Emamactien** or **Ginger** extractnormal, these results also showed that **Emamactie** was more than that of **Ginger aqueous extract**. In addition, there was high significant difference between effects of LC_{50} values of **Ginger** nanoparticles and **Emamactien** or **Ginger aqueous** extract against tow age from eggs treated. According to these findings, it is well obvious from our results that **Ginger** nanoparticles could be selected as a good agent to control *P. gossypiella* pest.

Acknowledgment

The authors thank Prof. Dr. Mervat Kandil form Plant Protection Research Institute, Agricultural Research Center for her assistance in performing some experiments.





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