TiO_2 nanoparticles as an effective nanopesticide for cotton leaf worm

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Abstract: The Egyptian cotton leaf worm, *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae) is an economically important pest with a wide range of host plants. It attacks certain vegetable and field crops such as cotton, tomatoes, cabbage and squash in Egypt, for causing severe injuries to the plants in all phonological crop stages. In addition, this species has acquired resistance to many insecticides. The overall objective of this investigation was to look for new control strategy through evaluate the effects of application of TiO_2 nanoparticles on the survival and biology of this insect. The experiment were carried out under laboratory conditions. The experiment was achieved with two treatments, consisting of TiO_2 nanoparticles application, and a negative control (distilled water) against the second and fourth instar larvae of *S. littoralis*. The LC_{50} value of the 2nd instar larvae treated with TiO_2 nanoparticles found to be 62.5 ppm, with slope 1.58 and LC_{50} value was 125 ppm with slope 2 for the 4th instar. The treatment was applied at six concentrations, 1000, 500, 250, 125, 62.5 and 31.25 ppm. The 2nd and 4th instar larvae were fed for 48 h on treated leaves with TiO_2 nanoparticles, the mortality was detected after 15 days post application. The following evaluations were performed: a) mortality (%), b) biological parameters of the insect were studied at the LC_{50} values. Results of the treatments of TiO_2 nanoparticles in larval test indicated higher toxic action at all concentrations used for the 2nd instar parallel with concentrations than the 4th one.

Keywords: TiO_2; nanoparticles; cotton; leaf worm; nanopesticide; agriculture

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1 Introduction

The cotton leaf worm, *Spodoptera littoralis* (Boisd.) is considered as one of the most destructive insect pests because they attack certain vegetable and field crops such as cotton, tomatoes, cabbage and. The use of huge quantities of chemical insecticides to control the insect has led to the development of resistance and pollution of the environment (EPPO, 2008; Bulmer et al., 2009; Yadav, 2010; Ditta, 2012). Currently, there is a growing need to use environmentally friendly nanoparticles in the field of plant protection to reduce dependency on chemical pesticides. Nanotechnology when applied in the right way has a strong potential of being used in agricultural pest control (Biswal et al., 2012; Brennan, 2012; El-bendary and El-Helaly, 2013). Nanotechnology opens up a wide array of opportunities in various fields like insecticides, pharmaceuticals, electronics and agriculture. The potential uses and benefits of nanomaterials in agriculture are enormous, one of them is the management of insect pests through the formulations of nanomaterials-based insecticides. Conventional strategies such as integrated pest management used in agriculture are insufficient, and also the application of chemical pesticides have adverse effects on animals and human. Therefore, using nanomaterials would provide green and efficient alternatives for the traditional management of insect pests in agriculture without harming the nature. The term nanopesticide covers a wide variety of products and cannot be considered to represent

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a single category. Nanopesticides can consist of organic ingredients (e.g., a. i. (active ingredient), polymers) and/or inorganic ingredients (e.g., metal oxides) in various forms (e.g., particles and micelles). Several novel inventions of different nanoparticles and nanomaterials are capable of diminishing the environmental problems. Nanotechnology based insecticides are devised by a number of companies. These formulations embrace nanoparticles of size 120-250 nm size range being more efficiently water soluble as compared to existing pesticides.

The principal aim of the present study was to evaluate entomotoxic nanocides of against *S. littoralis*.

2 Materials and methods

2.1 Material used

TiO_2 nanoparticles was purchased from El Nasr Company for Chemicals.

2.2 Insect rearing

The cotton leaf worm, *Spodoptera littoralis* was reared in the laboratory for several generations at room temperature ranged between 25-28°C and relative humidity (R.H.) between 60%-65%. Larvae were fed on castor bean leaves, *Ricinus communis* (L.) in a wide glass jars until pupation period and adults emergence. The newly emerged adults were mated inside glass jars supplied with a piece of cotton wetted 10% sugar solution as feeding source for the emerged moths and branches of Tafla (*Nerium oleander* L.) or castor bean leaves as an oviposition site (El-Defrawi et al., 1964). Egg masses were kept in plastic jars until hatching. The obtained second and fourth instar larvae were used for bioassay tests. The bioassay evaluations were performed under the same laboratory condition, at temperature 25-28°C and 60%-65% R.H., and 12 h photophase.

2.3 Characterization of TiO_2 nanoparticles

The samples were characterized by X-ray diffraction (XRD), field emission scanning electron microscope (FESEM) and Fourier transform (FT)-Raman spectroscopy.

2.4 Bioassay

A weighted amount of powder of the used TiO_2 nanoparticles was prepared based on active in gradient (ppm). A series of six different concentrations of the nanoparticle of TiO_2 (1000, 500, 250, 125, 62.5 and 31.25 ppm) were prepared by dilution with distilled water. Distilled water free from nanoTitanium particles was used as control treatment. Effect of LC_{50} on some biological activities such as larval and pupal duration, percent of pupation and adult emergence and larval and pupal malformations, fecundity, eggs hatchability and adult longevity and sex ratios of *S. littoralis* were carried out by using feeding application for 48 h on treated leaves with the Titanium particles. After 48 h, the treated leaves were replaced by another untreated one and the larvae fed on it until the pupation. Two replicates consists of 40 larvae for each concentration of the six used concentrations of both tested second and fourth instar larvae and control were utilized. Also, the observed malformations were recorded and photographed.

2.5 Statistical analysis

The total percent of the larval mortality until pupation was recorded and corrected according to the check by using Abbott formula (Abbott, 1925). The data were then analyzed using the probit analysis (Finney, 1971) and the LC_{50} values of both treated larvae instars were estimated. The different biological effects such larval and pupal duration, pupation and adults emergence percent and adult fecundity, eggs hatching, adult longevity and sex ratio were evaluated at the LC_{50} values. The obtained data of the biology were statically calculated through Excel for windows computer program to determine the *F*-value, *P*-value and least significant difference (LSD) at 0.05 or 0.01 freedom degrees.

3 Results and discussion

3.1 Characterization of the samples

XRD pattern of the investigated TiO_2 sample is illustrated in Figure 1. The sample of TiO_2 revealed anatase form with excellent crystallinity as obvious from the peak intensities. It crystallized in the well-known tetragonal symmetry with 4 molecules per unit cell. The data were compared and indexed with the ICDD (International Centre for Diffraction Data) card no 21-1272. The crystal size was calculated using Scherer’s formula and was found to be 95 nm. The FESEM image of the spherical TiO_2 nanoparticles is shown in Figure 2. The TiO_2 grains appeared to be with homogenous distribution with a small degree of coalescence. The phase of TiO_2 was confirmed from FT-Raman analysis and the spectra is shown in Figure 3, where active peaks near 142, 305, 510, 624 cm^{-1} are prominent for the anatase phase TiO_2 Nanoparticles because of Eg, B1g, A1g and Eg vibrational changes respectively. Neither signal characteristics of brookite nor rutile phases of TiO_2 having Raman shifts in the range of 249-826 cm^{-1} respectively, appear in the spectra.

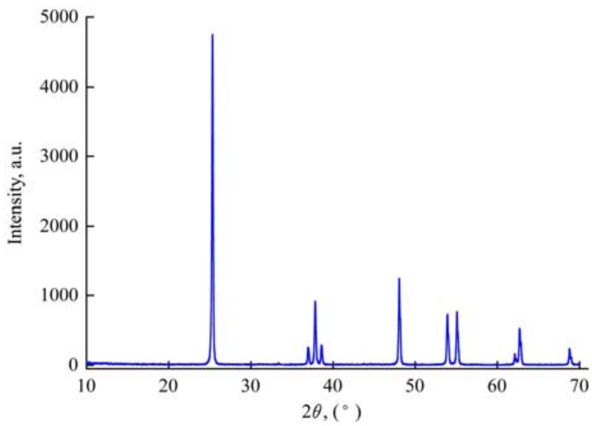

Figure 1 XRD patterns of Spherical TiO_2 nanoparticles

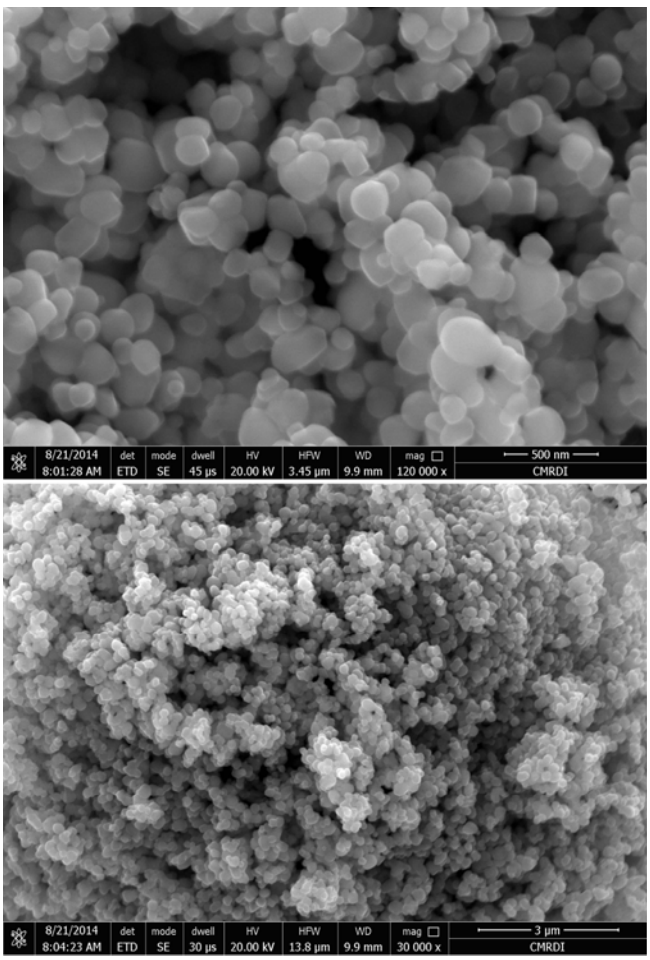

Figure 2 FESEM images of TiO_2 nanoparticles at different magnifications

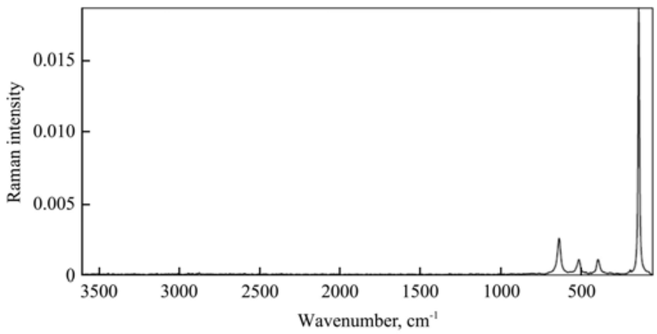

Figure 3 FT-Raman spectra of TiO_2 nanoparticles

3.2 Toxic effect

Data presented in Table 1 demonstrated that the mortality percentages for the 2nd and 4th instar larvae of *S. littoralis* was detected after 15 days post application. The mortality of larvae treated with TiO_2 nanoparticles were 100%, 100%, 100%, 90%, 50%, 40% and 100%, 100%, 80%, 50%, 40%, 30% for the two instars, respectively, at the tested concentrations. TiO_2 was more effective against the second instar larvae, where the LC_{50} value recorded was 62.5 ppm, with slope 1.58 and was less effective on the fourth instar, where the LC_{50} value was 125 ppm with slope 2.1 (as shown in Table 1 and Figure 4). These results were similar to those obtained by Goswami et al. (2010) who studied the effects of oxides nanoparticles like aluminium oxide, zinc oxide, titanium dioxide and silver nanoparticles against insect pests and pathogens. Likewise, El-bendary and El-Helaly (2013) evaluated the toxic effects of hydrophobic nano-silica application on the resistance of tomato plants of the neonates of *Spodoptera littoralis* were exposed daily to tomato leaves, under semi field conditions. The experiments were achieved with two treatments, consisting of nano-silica application, and a negative control (distilled water), with five replications. It was found that nano-silica LD_{50} be 212.045 ppm with slope 4.553, it was applied in six doses 100, 150, 200, 250, 300, and 350 ppm of 50 mL/plant, the mortality was detected after 15 days post application. It was found that the hydrophobic nano-silica treatment indicated high toxic action against the larvae at all used concentrations was parallel with concentrations. Also, Debnath et al. (2012) recorded that two types of SNP (Silica nanoparticles) (spherical, monosidperse SNP were synthesized from silicon alkoxide that functionalized in situ with 3- Mercaptopropyltr iethoxysilane and Hexamethyldisilazane) were effective in killing of the second instar of the *Spodoptera litura*. While, Vani and Brindhaa (2013) reported that the entomotoxic effect of silica nanoparticles were evaluated against the stored grain pest *Corcyra cephalonica*. They found that amorphous silica nanoparticles to be highly effective against this insect pest causing 100% mortality, indicating the effectiveness of silica nanoparticles to control insect pests. Hence, Araj et al. (2015) showed that silver nanoparticles (Ag NPs) were synthesized through reducing, stabilizing, and capping plant leaf extracts method at different concentrations (10, 50, 100, 200 ppm) which were tested on *Drosophila melanogaster* were highly effective on larvae mortality. Also, Goussain et al. (2002) recorded mortality increase and cannibalism in groups of fall armyworm, *Spodoptera frugiperda* of the second and sixth instars with silicon application. While, Mekewi et al. (2012) demonstrated insecticidal activity of nano zinc oxide samples (at four tested concentrations) that applied to artificial diet that the larvae of *Galleria mellonella* were reared on it. Whereas, Amal et al. (2015) studied the larvicidal activity of titanium dioxide nanoparticles (TiO_2 NPs) synthesized from the aqueous extract of *Moringa oleifera* against the *Rhynchophorus ferrugineus*. It was found that the mortality increased in the larvae stage by using this prepared solution of natural extract with titanium dioxde nanoparticles at concentration 75 mg L^{-1} and this was the best concentration used. Moreover, Stadler et al. (2010) showed that nano alumina could be successfully used to control stored grain pests. Also, Abd-EI-Salam et al. (2015) recorded that the concentration of 2 g kg^{-1} wheat grain had the highest effect based on the LC_{50} values ZnO was had the most effect compared to Al_2O_3 nanoparticles. They reported that Al_2O_3 and ZnO nanoparticles may used in integrated pest management programs as alternative to chemical insecticides where they are considered safe for humans if compared with synthetic insecticide.

Table 1 Insecticidal activity of TiO_2 nanoparticles against the second and fourth instar larvae of *Spodoptera littoralis*

Treatment	Con.	4th instar					2nd instar				
	ppm	Mortality %	LC50 values	Slope function	95% confidence limit Upper	95% confidence limit Lower	Mortality %	LC50 values	Slope function	95% confidence limit Upper	95% confidence limit Lower
TiO_2	100	1000	62.5	1.58	93.8	41.7	100	2.1	125	237.5	5.8
	100	500					100	2.1			
	100	250					80	125			
	90	125					50				
	50	62.5					40				
	40	31.5					30				

Control: All tested larvae were pupated.

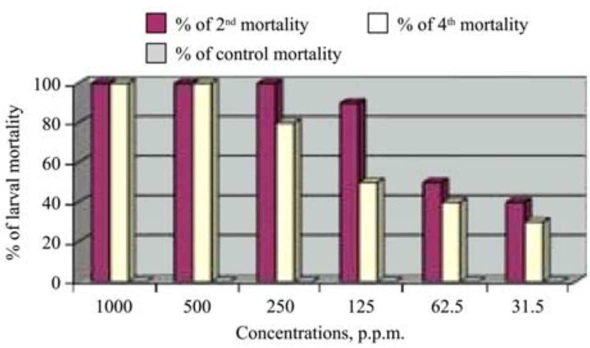

Figure 4 Insecticidal activity of TiO_2 nanoparticles against the second and fourth instar larvae of *Spodoptera littoralis*

3.3 Latent effect

3.3.1 Larval and pupal periods

Data in Tables 2 and Table 3 indicated that the treatment of second instar larvae of *S. littoralis* with the TiO_2 nanoparticles at its LC_{50} values had a higher significant (p<0.01) effect in larval period increasing to average 13.5 days, as compared to 10.2 days that of control. Conversely, the fourth instar treated with this material at the same concentration induced significant (p<0.01) decrease in the larval duration to average 9.4 days, as respect of that of control (10.6 days).

Also, Tables 2 and Table 3 showed that the larval treatment of the second instar larvae of *S. littoralis* with the TiO_2 nanoparticles at its LC_{50} values highly significantly (p<0.01) decreased the pupal duration to average 27.2 days, as compared to 29.6 days that of the control. While, the larval treatment of the 4^{th} instar with nano TiO_2 gave none significant (p>0.05) decrease of the pupal duration to average 26 days, as compared to 26.8 days of control.

Table 2 Latent effect of TiO_2 at its LC_{50} values against the 2nd instar larvae of *S. littoralis*

Treatment	Larval duration Mean±S.D. (days)	Larval Malfo.	Pupation % Normal	Pupation % Malfo.	Pupal duration Mean±S.D. (days)	Emergence % Mean±S.D. Normal	Emergence % Mean±S.D. Malfo.
TiO_2	13.5±0.9**	12.6±2.9**	60±5.5**	10±2.6**	27.2±0.7**	50±10**	22.6±2.1**
Control	10.2±0.7	0	100	0	29.6±0.9	99.8±0.1	0
F value	125.4	44.144	109.24	27.548	81.516	72.3	243.4
P value	0.01	0.0219	0.009	0.03444	0.00004	0.01355	0.0041
L.S.D at 0.05	0.6	5.6	10.5	5.3	0.5	16.1	4
L.S.D at 0.01	0.8	10.2	19.3	9.6	0.8	29.6	7.4

Note: ** = Highly significant (p<0.01); * = Significant (p<0.05); S.D. = Standard déviation; Malfo. = Malformation (%); L.S.D. = Least significant deviation.

Table 3 Latent effect of TiO_2 at its LC_{50} values against the 4th instar larvae of *S. littoralis*

Treatment	Larval duration Mean±S.D. (days)	Larval Malfo.	Pupation % (Mean±S.D.) Normal	Pupation % (Mean±S.D.) Malfo.	Pupal duration Mean±S.D. (days)	Emergence % Mean±S.D. Normal	Emergence % Mean±S.D. Malfo.
TiO_2	9.4±1.6**	7.2±0.7**	63.0±3.4**	7.2±0.7**	26±4 n.s.	52.2±7.8**	20.9±3.7**
Control	10.6±1.7	0	100	0	26.8±0.5	100	0
F value	14.189	338.77	584.211	338.77	0.6589	108.9	92.1
P value	0.0008	0.00293	0.00171	0.00293	0.4404	0.0091	0.01
L.S.D at 0.05	0.7	1.08	4.2	1.08	2.9	12.6	6
L.S.D at 0.01	0.9	1.98	7.7	1.98	4.2	23.1	11

Note: ** = Highly Significant (p<0.01); * = Significant (p<0.05); S.D. = Standard deviation; Malfo. = Malformation (%); L.S.D. = Least significant; n.s = None significant (p>0.05).

These results are similar to that obtained by El-bendary and El-Helaly (2013) who reported that the pupal duration of *S. littoralis larvae treated with silica nanoparticles* were not affected, as respect to that of control. Whereas, Meyer and Keeping (2005) showed that silica content increase reduced the growth rates and feeding efficiency of *Spodoptera exempta*. Also, Araj et al. (2015) indicated that none of the tested nanoparticles, silver nanoparticles (Ag NPs) and sulfur nanoparticles (SNPs) applied against the fruit fly *Drosophila melanogaster* has a significant effect on pupae longevity.

3.3.2 Pupation and adult emergence

Tables 2 and Table 3 demonstrated that the treatment of the 2nd and 4th instar larvae of *S. littoralis* with nano TiO_2 at its LC_{50} values significantly (p<0.01) reduced the pupation percentages which were 60% and 63% for pupae treated as 2nd and 4th larvae instar, respectively, as compared to 100% of the control.

Data in Tables 2 and Table 3 showed that the treatment of the second and fourth instar larvae of *S. littoralis* with nano TiO_2 at its LC_{50} values induced highly significant (p<0.01) reduction in the moths emergence rate. The treated second larvae instar had the highest effect on the moths emergence rate, where it averaged 50%, while it reached to 52% for adults treated as 4th larvae instar with this nano material.

These results are in agreement with those obtained by Mekewi et al. (2012) who found that among all four applied concentrations of nano oxide samples that applied to artificial diet that the larvae of *Galleria mellonella* were reared on it, the highest viable pupal production (6 pupae) was detected at the concentrations at which the lowest larval death values were achieved (1×10^{-6} and 4×10^{-6}), as compared to that of control (56 viable pupae). They reported that emergence of adult moths categorized into deformed and healthy adults. It was found that healthy moths were of high representation at the concentrations, at which the lowest larval death values were achieved. Also, Kubo-Irie et al. (2015) reported that pupation and emergence were not affected by the injection suspensions of 10 μL (100 μg mL^{-1}) titanium dioxide nanoparticles (TiO_2-NPs), 10 μL (100 μg mL^{-1}) zinc oxide nanoparticles (ZnO-NPs) or saline (control) against the 5th instar larvae of the sweet potato hornworm

(*Agrius convolvuli*).

3.3.3 Morphogenetic abnormalities

Data presented in Tables 2 and Table 3 demonstrated that the larval feeding of 2nd and 4th instars of *S. littoralis* on TiO_2 nanoparticles at its LC_{50} values induced increase in larval malformations percentage in relative to control. The treatment of 2nd larvae instar gave the highest percent, it reached 12.6%, as compared to 0% of control. While the 4th instar treated with TiO_2 nanoparticles induced 7.2%, as compared to that of control (0%). Also, the 2nd and 4th larvae instar treated with TiO_2 nanoparticles induced pupal malformations percent reached 10% and 7.2%, respectively, as compared to 0% of control. On the other hand, the second and fourth instar larvae treated with TiO_2 nanoparticles increased the malformed adults to reach 22.6% and 20.9%, respectively, as compared with 0% of the control.

Malformations of *S. littoralis* pupae resulting from the larval treatment of the 2nd and 4th instars of *S. littoralis* on TiO_2 nanoparticles at its LC_{50} values mostly appeared as a larvae keep with the moulting skin. While, pupal malformations percent appear as pupae failed to cast the old cuticle or deformed morphological pupa or pupa showing body shrinkage. Adult malformations percent occurred represent in moths bear deformed twisted wings and abnormal body or malformed adults with weekly wings and abnormal bodies or malformed adults had pupal bodies and adult wings, as compared to normal pupa and adult of control.

These results are in agreement with to those obtained by Mekewi et al. (2012) who reported that whenever the insecticidal efficacy of nano oxide samples (at four tested concentrations) against *Galleria mellonella* decreases, the chance of malformed individual's detection increases, and they found that larval pupal intermediates appeared at concentration 1×10^{-6}. Also, they recorded that the emergence of adult moths categorized into deformed and healthy adults.

3.3.4 Adult fecundity and eggs hatchability

Data presented in Table 4 demonstrated that the larval feeding of 4th instar larvae of *S. littoralis* on nano TiO_2 at its LC_{50} values induced a highly significant (p<0.01) reduction of the adult fecundity in respect of control to average 131.7 eggs/female, as compared to 505 eggs/female of control.

Table 4 Latent effect of TiO_2 at its LC_{50} values against the 4th instar larvae of *S. littoralis*

Treatment	Fecundity Mean±S.D. eggs/female	% Eggs hatching	Longevity Mean±S.D. (days)	Sex ratios %	
				Males	Females
TiO_2	131.7±32**	57	100	60	40
Control	505±200	100	10.7±1.2	40	60
F value	69.018		49		
P value	0.01		0. 000113		
L.S.D at 0.05	123.8		0.7		
L.S.D at 0.01	227.3		1.0		

Note: ** = Highly Significant (p<0.01); * = Significant (p<0.05); S.D. = Standard deviation; Malfo. = Malformation (%); L.S.D. = Least significant.

Likewise, the larval feeding of *S. littoralis* reduced the total number of viable eggs laid by adult females fed as 4th instar larvae on nano TiO_2 at its LC_{50} values to reach 57%, as compared to 100% of control.

These results are agreement with those obtained by El-bendary and El-Helaly (2013) who reported that the total number of eggs laid per female of *S. littoralis* was affected in all treatments (350, 300, 250, 200, 150, and100 ppm), as compared to that of control. Also, they recorded that the percentage of eggs hatchability was reduced with nano silica treatments, as respect of that of control.

3.3.5 Adult longevity

Data obtained in Table 4 showed that the treatment of the fourth instar larvae of *S. littoralis* with TiO_2 nanoparticles at its LC_{50} values highly significantly (p<0.01) reduced the adult longevity to average 8.3 days, as compared to 10.7 days that of control.

These results are in contract with that obtained by El-bendary and El-Helaly (2013) who mentioned that the longevity of adult treated as neonate larvae with nanosilica particles at different concentrations was not affected at all treatments (ranged 14 to 13 days), as compared to that of control (13days).

3.3.6 Adult sex ratio

Data obtained in Table 4 demonstrated that the larval treatment of the fourth instar of *S. littoralis* with TiO_2 nanoparticles at its LC_{50} values had the highest effect in the sex ratio shifting of adult males and females, it induced females decrease and males increase, as respect to that of control, it reached 40:60 for both females and males, respectively, as compared to 60:40 of adult females and males respectively that of control.

4 Conclusion

The results of the present work demonstrated that the nano titanium particles were effective against the survival of the 2nd and 4th instar larvae of *S. littoralis*. Also, some biological aspects (larval duration, pupation and adult emergence, fecundity and eggs hatching, adult longevity and sex ratios) of the insect were affected with the titanium dioxide treatment. Further, it caused malformations in larvae, pupae and adult stages. Thus, we conclude that the application of nano TiO_2 may minimized the problems caused by *S. littoralis* of the host crops. Recent findings showed the potential harmful effects of nanomaterial's on the digestive systems of a beneficial soil organism-earthworm (Ruitenberg, 2013). In addition to application of TiO_2 on food crops has been reported to promote plant growth, increase the photosynthetic rate, reduce disease severity and enhance yield by 30%. They also reported that application of TiO_2 significantly reduced the incidence of rice blast and tomato spray mold with a correspondent 20% increase in grain weight due to the growth promoting effect of TiO_2 nanoparticles (Mahmoodzadeh et al., 2000). A combination of titanium dioxide, aluminum and silica was reported to be effective in controlling downy and powdery mildew of grapes by Bowen et al. (1992), possibly through direct action on the hyphae, interference with recognition of plant surface and stimulation of plant physiological defences.

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