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**Research Article**

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# The efficacy effect of using chitosan and nano-chitosan against *Tuta absoluta* (Lepidoptera: Gelechiidae)

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**ABSTRACT**

*The autumn tomato variety A-V262 were planted in two Egyptian governorate Behyra and Qalubyia to control the tomato pin worm Tuta absoluta. under laboratory conditions, the LC50 of T. absoluta, recorded , 49, 67, 87, 101, 122,133 and 135 ppm for newly hatched larvae, 1st larval instars, 2nd larval instars, 3rd larval instars, adult males and adult females, respectively The effect of nano chitosan under laboratory conditions showed that, the LC50 of the corresponding stages of T. absoluta, 19, 27, 29,43, 44,66 and 69, respectively When the tomato crop cultivated in green house, and treated with the testes chitosan , the infestations means number recorded significantly decreased to 16.0±8.1 individuals after nano chitosan treatme nts as compared to 99±9.6 individuals in the contro l.*

**Key wards:** chitosan, nano. *Tuta absoluta*, *tomato*

## INTRODUCTION

The Egyptian tomato crop, *Lycopersicon esculentum* (Mill) is a considered among very important vegetable crop of the world. The tomato leafminer, *Tuta absoluta* Meyrick, (Lepidoptera : Gelechiidae) is one of a harmful serious pest of both the greenhouse, and field tomatoes. The harmful pest, *T. absoluta* considered among serious insect pests of the tomato crop. Tomatoes fruits lost by the tomato leaf minor in the field [1-3]. *Tuta absoluta* infect the leaves and soluneouses fruits which leads to drying the plant [4-7]. This insect pests migrate from tomato crop to any other solanouse crops which leads to damaging and loss of it [8-11]. The new pest of the tomato leaf minor have a serious threat to such efforts and needs to be kept in check as early as possible. During the past three years and while expanding eastward from Spain along the North African coast, *Tuta absoluta* have caused havoc in agricultural production , devastating crops in all countries on the way, elevating prices beyond the capability of average consumer. [1] controlled the tomato leaf miners by the microbial fungi and *Trichogramma evanesens*. Under laboratory condition the percentage eggs parasitoid of *T. evanesens* were significantly decreased after treatments with *M. anisopliae to 93.2% as* compare to 98.2 in the control. Under green house conditions the means number of infestation were significantly decreased [1] . Damage to fruit allows e.g. fungal diseases to enter, which leading to rotting fruit before or after harvest , [1,2] . In Egypt. tomato grown in green house and open field. *T. absoluta* are severely attack the tomato fruits which causing a lose of their commercial value. 50–100% losses have been reported on tomato [3,4]. [5, 6, 7] , used the Biocontrol agent bacteria or fungi for controlling the Tomato Pinworm *T. absoluta* (Meyrick) (Lepidoptera: Gelechiidae) in Egypt. [7,8] control the tomato insect pests by using isolated *Bacillus thuringiensis* and the entomopathogenic fungi. Nano pesticides, nano fungicides and nanoherbicides are being used efficiently in agriculture [9,10, 11, 12, 13].

The aim of this work to evaluate of chitosan and nano-chitosan against *T. absoluta* under laboratory, greenhouse effect and field.

## EXPERIMENTAL SECTION

**2.1. Rearing insect pests:**
The tomato pinworm were reared on tomato leaves under laboratory conditions 22±2Co and RH 60-70% *T. absoluta* used in the trials were obtained from laboratory cultures. The experiments were repeated 4 times. The percentages of mortality were calculated and corrected according to [14], while LC50 was calculated through probit analysis, [15]. The experiments were carried out under laboratory conditions 22 ± 2o C and 60-70% R.H. Twenty individuals of the third larvae of *T. absoluta* were put on them, covered with muslin. Control (untreated) was made by feeding the larvae on untreated leaves (sprayed by water only).

**2.2.Green house trials:**
The autumn tomato variety A-V262 was planted in the green house in 40 plots in each artificial infestation was made by spraying the plant with the bioinsecticides chitosan at the concentrations of and 30ppm. Control samples were sprayed by water only. The plants were examined every two days, the percentage of infestation was calculated until the end of the experiment. Each treatment was replicated 4 times. The percent mortality was counted and corrected according to [14]; while Lc50s were calculated through probit analysis after [15].

2.5.**Bioassays**
The insecticidal efficacy of chitosan and nano- chitosan were tested at three dose rates 10, 25, 50 ppm against the 3rd instar larvae of *T. bsoluta*. For each case, four glass jars as replicates were used. Each replicate was treated individually with the respective nano- chitosan quantity and then shaken manually for one minute to achieve equal distribution of the chitosan and nano chitosan Subsequently, ten 3rd instar larvae of the two tested species were introduced into each glass jar and covered with muslin for sufficient ventilation. Twelve replicates glass jars containing untreated wheat served as control. Mortality was assessed after 7 d of exposure in the treated and untreated jars. Mortality was corrected according to [13]. All tests were conducted at 27 ± 2 °C and 65 ± 5% relative humidity (RH). All the experiments were repeated three times.

**2.4. Nanoencapsulation**
The Nanoencapsulation is a process through which a chemical is slowly but efficiently released to the particular host for insect pests control. Release mechanisms include dissolution, biodegradation, diffusion and osmotic pressure with specific pH [26]. Encapsulated of the tested bioinsecticides nano-chitosan, nano-emulsion is prepared by high-pressure homogenization of 2.5% surfactant and 100% glycerol, to create stable droplets which that that increase the retention of the oil and cause a slow release of the nano materials . The release rate depends upon the protection time; consequently a decrease in release rate can prolong insect pests protection time [27].

## RESULTS

Table 1 show that under laboratory conditions, the LC50 of *T. absoluta*, recorded , 49, 67, 87, 101, 122,133 and 135 ppm for newly hatched larvae, 1st larval instars, 2nd larval instars, 3rd larval instars, adult males and adult females, respectively (Table 1).

The effect of nano chitosan under laboratory conditions showed that, the LC50 of the corresponding stages of *T. absoluta*, 19, 27, 29,43, 44,66 and 69, respectively(Table2).

When the tomato crop cultivated in green house, and treated with the testes chitosan , the infestations means number recorded significantly decreased to 16.0±8.1 individuals after nano chitosan treatments as compared to 99±9.6 individuals in the control (Table 3).

After the tomato harvest time the weight of the tomato crop recorded , 2631± 36.80, 2939± 76.60.

In the tomato treated with chitosan and nano chitosan plots as compared to 2334± 46.81Kg/ feddan in the control in Qlubyia governorate. In Behyra governorate the weight of tomato recorded, for the corresponding treatments**,** 3091± 46.40, 3651± 76.70 as compared to 2231± 56.60 kg/ feddan in the control (Table 4).

**Table 1.Effect of Chitosan against the different stages of *T. absoluta* under laboratory conditions**

| Insects | $LC_{50}$ | slope | variance | 95%confidence limits |
|---|---|---|---|---|
| Newly hatched larvae | 49 | 0.1 | 1.01 | 39-66 |
| 1st larval instars | 67 | 0.2 | 1.01 | 55-98 |
| 2nd larval instars | 87 | 0.4 | 1.01 | 77-99 |
| 3rd larval instars | 101 | 0.3 | 1.01 | 97-122 |
| 4th larval instars | 122 | 0.2 | 1.01 | 101-133 |
| Adult males | 133 | 0.1 | 1.02 | 111-148 |
| Adult females | 135 | 0.1 | 1.02 | 118-155 |

**Table2 .Effect of Nano-Chitosan against the different stages of *T. absoluta* under laboratory conditions**

| Insects | $LC_{50}$ | slope | variance | 95%confidence limits |
|---|---|---|---|---|
| Newly hatched larvae | 19 | 0.1 | 1.01 | 39-66 |
| 1st larval instars | 27 | 0.2 | 1.01 | 55-98 |
| 2nd larval instars | 29 | 0.4 | 1.01 | 77-99 |
| 3rd larval instars | 43 | 0.3 | 1.01 | 97-122 |
| 4th larval instars | 55 | 0.2 | 1.01 | 101-133 |
| Adult males | 66 | 0.1 | 1.02 | 111-148 |
| Adult females | 69 | 0.1 | 1.02 | 118-155 |

**Table (3): Effect of chitosan against *T. absoluta* under greenhouse conditions**

| Treatments | Days after treatment | Means of infestations (Means ± S.E.) |
|---|---|---|
| Control | 20 | 29.2±4.7 |
| | 50 | 58±5.5 |
| | 90 | 78±9.9 |
| | 120 | 99±9.6 |
| Chitosan | 20 | 11.0±6.1 |
| | 50 | 22±5.1 |
| | 90 | 33±3.8 |
| | 120 | 57±7.9 |
| Nano-chitosan | 20 | 3.0±4.1 |
| | 50 | 8.0±3.1 |
| | 90 | 11.2±6.1 |
| | 120 | 16.0±8.1 |
| F –test | 14.4 | |
| LSD 5% | 13.8 | |

**Table (4): Weight of harvested tomato into two Egyptian regions after chitosan and nano-chitosan treatment against *T. absoluta* during seasons 2015**

| Treatments | Qalubyia Weight tomatoes (Kg/feddan) | Behayira Weight tomatoes (Kg/feddan |
|---|---|---|
| Control | 2334± 46.81 | 2231± 56.60 |
| Chitosan | 2939± 76.60 | 3091± 46.40 |
| Nano-chitosan | 3531± 39.89 | 3651± 76.70 |
| F –test | 34.4 | |
| LSD 5% | 18.8 | |

**Table 5. the effect of biocontrol chitosan on the tomato crop production in two different governorates**

| Governorate | the current situation | | Projected the situation | | | |
|---|---|---|---|---|---|---|
| | | | Treatments with Chitosan | | Treatments with Nano-chitosan | |
| | The amount of production in tons | The amount of water / m3 per tonne | The amount of production in tons | The amount of water / m3 per tonne | The amount of production in tons | The amount of water / m3 per tonne |
| Behyra | 515245 | 183 | 713614 | 132 | 842941 | 112 |
| Qalubia | 50504 | 183 | 63585 | 145 | 76413 | 120 |
| Total | 565749 | | 777199 | | 919354 | |

**Fig1. infestation percentages of *T. absolua* under green house conditions**

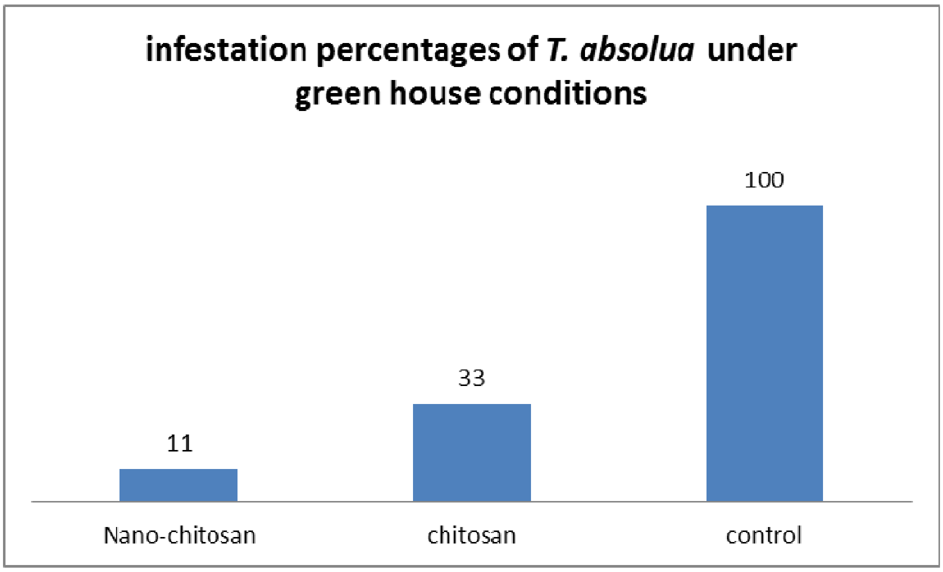
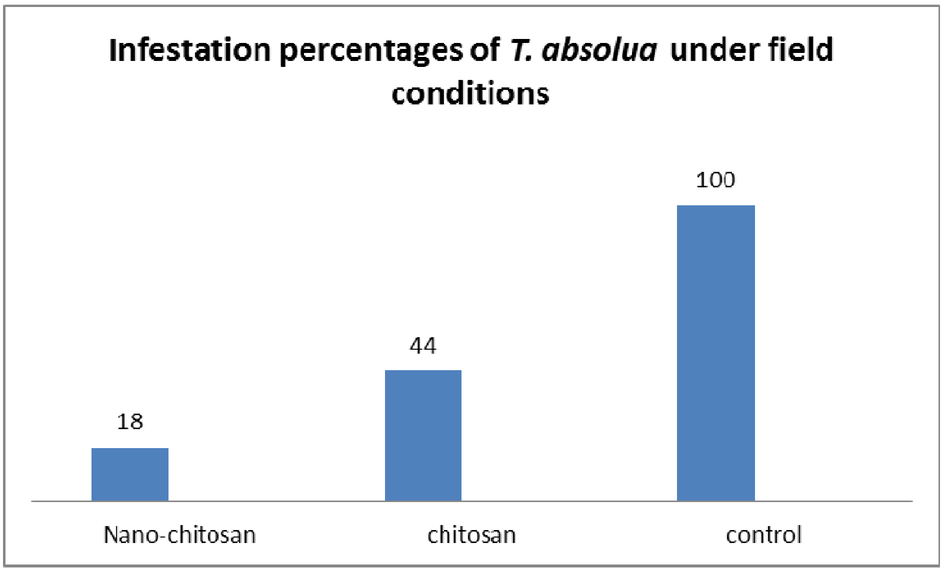



**Fig 2. Infestation percentages of *T. absolua* under field conditions**




The efficiency of Productivity means: - Get the biggest production of the crop equally user of various elements of production of it , or get the same production less than the factors of production as much as used in the production process. It is clear from Table (4) which show, the increase of tomato crop productivity in all of the regions of the study when the treatment of the crop treated with, Chitosan, and the Nano-chitosan by about 38.5%, 63.6% in Behyra governorate , and about 25.9%, 51.3% in Qaliubiya govrnorate each, respectively, compared the control (untreated) productivity. Table (5) also that the impact of bio-resistance to raise the productive efficiency of the crop of tomatoes and by raising the productive efficiency of the varieties used in production, as well as raise the rated efficiency of water per ton of product yield

Figures 1 and 2 showed that, the infestations of the target insect pests of T. absoluta not only significantly decreased when treated with chitosan solution but also highly significantly decreased when treated with nano-chitosan under green house and field conditions treatments ( figre, 1 & 2).

Figure 3 a show the scanning electron microscopy particles at 50 nano meter .Also, figure 3 b, show the chitosan nano particles at 100 nano meter**.**

**Figure 3 show the particles of the chitosan under scanning electron microscopy**

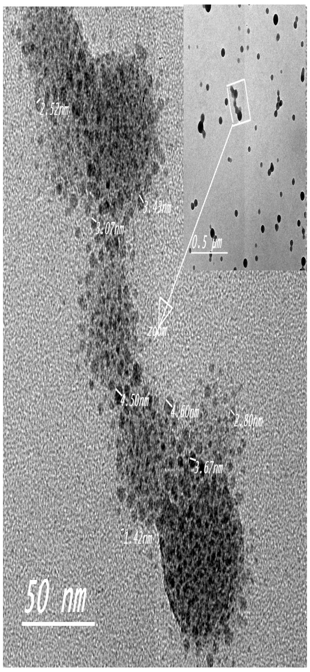
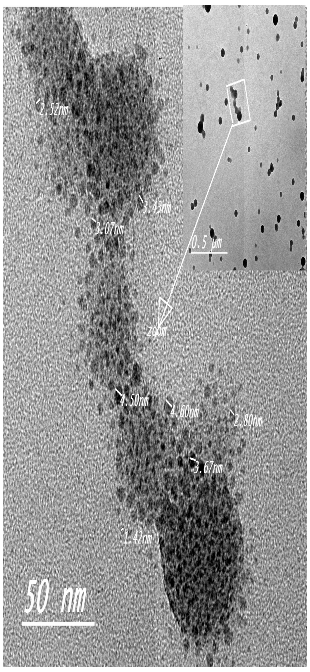
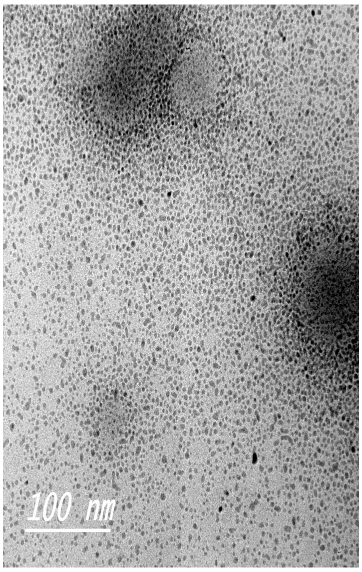



**Figure. ( 3 a andb), scanninigng electron microscopy**

**a. Scanning electron microscopy 50 nano meter**



**b. Scnning electron microscopy 100 nano meter**




## DISCUSSION

Author found that the bio insecticide control many vegetables pests [15 ,16, 17].The same results obtained [17, 18, 19,20, 21,22] who found that the nano microbial insecticide decrease the amount of insecticides used. . [23] found the insecticidal activity the nano-chitosan (CS-g-PAA) showed highest effect against the three insect of soybean. [24, 25, 26, 27] reported that the means number of eggs deposited /female were significantly decreased. Under laboratory and semifield condition, *Aphis gossypii* were significantly decreased to 20.9±9.1 and 28.9±9.2 eggs/female respectively as compared to 97.3±4.9 and 90.3±4.9 eggs/female in the control, respectively. The same trends were also observed against *Callosobruchus maculatus* .

[28,29,30,31] found that the nano insecticides of Imidacloprid and fungi strains cases a higher mortality for insect infestations. Our results agree with[32,33,34,35,36] who find that the nano pesticide decrease the infestation percentage of different pests.

[26-29] agree with our results and control a lot of pests with nano materials. [30-41] have the same results obtained and show that the nano pesticides is a perfect for controlling many pests and diseases . [40-45] found the insecticidal activity the nano-chitosan (CS-g-PAA) showed highest effect against the three insect of soybean. as the means number of eggs deposited /female were significantly decreased. Under laboratory and semifield condition, *Aphis gossypii* were significantly decreased to 20.9±9.1 and 28.9±9.2 eggs/female respectively as compared to 97.3±4.9 and 90.3±4.9 eggs/female in the control, respectively. The same trends were also observed against *Callosobruchus maculatus* . [28, 29, 30, 331, 32, 33] found that the nano insecticides of Imidacloprid and fungi strains cases a higher mortality for insect infestations. Our results agree with[34,35, 36, 41,42] who find that the nano pesticide decrease the infestation percentage of different pests.

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