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Titanate-Bacillus Nanocomposite as a Novel Nanopesticide for Cotton Leafworm

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ABSTRACT

The cotton crop is one of the most important strategic crops in Egypt, but unfortunately there are several types of insect pests which are very harmful to this crop. One of these pests is cotton leafworm Spodoptera littoralis (Boisd.) (Lepidoptera: Noctuidae) which causes damage to leaves and hence decreases the crop yield. One of the commonly used biopesticides is Bacillus thuringiensis subspecies Kurstaki (BTK), but the main problem of using this biopesticide is that; it gives low activity towards cotton leafworm. Nanotechnology makes good use of some ecofriendly nanomaterials with their unique physical, chemical and electrical properties to enhance the activity of B. thuringiensis. The current study focused on the use of Titanate nanotubes (TNT), and Nanosheets (TNS) and their composites with Bacillus Thuringiensis (Bt), as a new nanopesticides and to study insecticidal activity and their impacts on different biological of cotton leafworm such as; adult longevity, adult sex ratio, pupation, fecundity and percent of eggs hatching. All samples were characterized using Field emission scanning electron microscope (FESEM), X-ray diffraction (XRD), FTIR-spectroscopy and Zetasizer for zeta potential measurements.

Keywords: Titanate, Nanoparticles, Nanopesticide, Leafworm, Cotton, Agriculture

INTRODUCTION

Cotton leafworm, *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae) is one of the most dangerous pests which injures cultivated crops like corn, cotton, beet, tomato and many other crops [1]. It causes damage to the leaves and hence influences the crop yield. Several types of traditional synthetic chemical pesticides such as: Parathion, organophosphorous and synthetic pyrethroid have been used in this field to resist this pest. Farmers use great amounts of these toxic chemical compounds which cause soil, air and aquatic pollution [2,3]. Several studies were carried out using alternative safe biopesticides to replace these toxic chemical compounds [4]. These biopesticides are naturally occurring micro-organisms like: Bacteria, fungi, protozoa and viruses. They are considered to have minimal or no harmful impacts on the environment, so they are called green biocides or eco-friendly biocides [5]. One of the widely used micro-organisms as a biopesticides is *Bacillus thuringiensis* subspecies Kurstaki (Btk) which is a Gram-positive bacteria exist in soil, food, aquatic environment and in the gut of caterpillars of various kinds of moths and butterflies [6].

B. thuringiensis is used as a biopesticide against cotton leafworm (*S. littoralis*). It provides long time pest control and longer cycling in the host pest. Under optimal conditions, *B. thuringiensis* sporulates to form parasporal bodies containing toxic insecticidal proteins called delta-endotoxins [7]. The first one who described *B. thuringiensis* was Berliner [8], and the first isolation was in 1901 when a Japanese biologist Ishiwata Shigetane discovered and described a harmful bacterium to silkworms [6]. Edward Steinhaus performed several studies on *B. thuringiensis* and paid attention to its potential impact on pests [6]. The insecticidal effect of *B. thuringiensis* was found to be related to the crystalline protein formed during sporulation stage [9]. Inspite of all of these ecological and insecticidal advantages of *B. thuringiensis*, it has some disadvantages such as; a lack of broad spectrum activity, slow rate of killing pests and low mortality percent [7]. The mortality of *B. thuringiensis* after 48 h laboratory experiment was found to be about 10% only [10].

One of the revolutionary and unique technology being used in the agriculture field is nanotechnology which offers the synthesis of materials having a particle size in the nanoscale (10^{-9} m), with enhanced or new chemical, physical, optical and magnetic properties. In addition to these properties, they have also antimicrobial and antipesticidal activities towards a variety of microorganisms and pests. Nanosized particles have wide applications in biological, physical, chemical, environmental, agricultural, industrial and pharmaceutical science [11]. Nanopesticides, nanofertilizers and agricultural nanosensors are good examples for the use of nanotechnology in agricultural field [5,10].

Nanomaterials exist in different formulations such as: Suspensions, emulsions and capsules. The nanoparticles can be prepared in different morphologies: like tubes, sheets, rods, fibers and wires. This variation gives these materials a variety of surface to volume ratios which has a great effect on surface activity. Several nanomaterials like: SiO_2, ZnO, CuO, MnO and Ag nanoparticles have been used as nanopesticides [5,10,12-14]. Nanopesticide is defined as any formulation that includes particles in the nanosize range and exhibit novel properties associated with this size. Nanopesticides may consist of organic ingredients (e.g. polymers) and/or inorganic ingredients (e.g. metal oxides) in various forms [4]. One of the highly reactive nanomaterials with good optical and insecticidal properties is the nontoxic nanosized TiO_2 which is has different applications in plant protection and improving plant growth. The main goal of the present research is to increase the activity and of *B. thuringiensis* against 2nd and 4th instars of *S. littoralis* larvae through the formation of different titanate-*Bacillus* nanocomposites.

MATERIALS AND METHODS

Insect rearing

The cotton leafworm, *S. littoralis* was reared in the laboratory for several generations at room temperature ranged between 25-28°C and 60-65% R.H. Larvae were fed on castor bean leaves, *Ricinus communis* (L.) in 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 with 10% sugar solution as a feeding source for the emerged moths and branches of Tafla (*Nerium oleander* L.) or castor bean leaves as an ovipositor site [15]. Egg masses were kept in plastic jars until hatching. The obtained second and fourth instars larvae were used for bioassay tests. The bioassay evaluations were performed under the same laboratory condition for 12 h photo phase.

Materials

TiO_2 powder was purchased from Luba Chemie-India, sodium hydroxide and hydrochloric acid were purchased from EL Nasr Company- Egypt and *Bacillus* was obtained from Biopesticides unit-Agricultural research center (ARC) -Egyptian ministry of agriculture.

Synthesis of H-Titanate Nanotubes (TNT) and H-Titanate Nanosheets (TNS)

All the reactants used were of analytical grade and were used without further purification. As in our previously published work [16], but with some modifications, 5 g of pure bulk anatase TiO_2 powder was mixed with 500 ml 10 M aqueous NaOH solution under magnetic stirring for about 45 min till a milky white solution was obtained. Then, this solution was transferred to 1000 ml capacity teflon-lined stainless steel autoclave and after that temperature treatment was carried out at 160°C for 6 and 23 h to prepare nanosheets and nanotubes, respectively. The autoclave chamber was allowed to cool down. The formed white precipitates were collected and washed several times with distilled water, and finally washed with 0.1 M HCl. The formed nanosheets and nanotubes were left to dry overnight at 80°C.

Synthesis of titanate-*Bacillus* nanocomposites

One g of each morphology was added separately to 0.5 g *Bacillus* dispersed in 100 ml distilled water for each to prepare *B. thuringiensis*-Titanate Nanotubes (Bt-TNT) and *B. thuringiensis*-Titanate Nanosheets (Bt-TNS). The two mixtures were sonicated for 10 min to increase particles dispersion, then were mixed for 1 h using magnetic stirrer. The obtained composites were dried at 40°C for 24 h [21].

Characterization

All samples were characterized by X-Ray Diffraction (XRD), Field Emission Scanning Electron Microscope (FESEM), Zetasizer for zeta potential measurements and Fourier Transform Infrared (FTIR) spectroscopy.

Application of the prepared nanocomposites on *S. littoralis* larvae

A concentration of 1 g/l of TNT, TNS, Bt-TNT, Bt-TNS nanocomposites and *B. thuringiensis* were prepared. Castor bean leaves *(R. communis)* were immersed in each sample for 20 sec and then allowed to dry in air. Six replicates of 2nd and 4th instars of *S. littoralis* were prepared to contain 20 larvae for each replicate, i.e., 10 larvae for each treatment. The replicates were placed over sawdust inside a transparent plastic can ($10 \times 10 \times 4$ cm^3). The larvae in the first five replicates were fed on the recinus leaves immersed in suspensions of prepared materials, whereas those in the sixth one were fed on untreated leaves (control sample).

Statistical analysis

The total percent of the larval mortality of the 2nd and 4th instars larvae until pupation were recorded and corrected according to Abbott's formula [17]. The different biological parameters such as larval and pupal duration, pupation and adult's emergence %, adult fecundity %, hatchability (% of eggs hatching), adult longevity and adult sex ratios were evaluated at the tested concentration. The obtained data were statistically treated to determine the F-value, P-value, and Least Significant Difference (LSD) at 0.05 or 0.01 freedom degrees.

RESULTS

Physical characterization of the prepared materials

Figure 1 shows the X-ray Diffraction (XRD) patterns of all samples, it is clear from patterns that the mean peaks of the as prepared samples H-TNT), H-TNS; at 9.8°, 24.2°, 28.2°, 38.9°, 48.2°; 61.7° are the same as that of hydrogen titanium oxide hydrate [17,18]. The crystal structure of nanosheets which are observed as an intermediate product during nanotube or nanofibre synthesis is attributed to either the hydrated form of delaminated anatase [19] or lepidocrocite-type titanates consisting of shared TiO_6 octahedrons [20] have similar diffraction peak positions. The figure also shows the XRD pattern of *Bacillus* bacteria where many peaks are observed at 18°, 22°, 31.9°, 45.3° which may be due to the presence of crystalline proteins in this bacteria. Whereas in case of Bt-TNT and Bt-TNS only the peaks of TNT and TNS can be observed with some change in their intensities and this may be attributed to the coverage of the Bacteria surfaces with TNT and TNS.

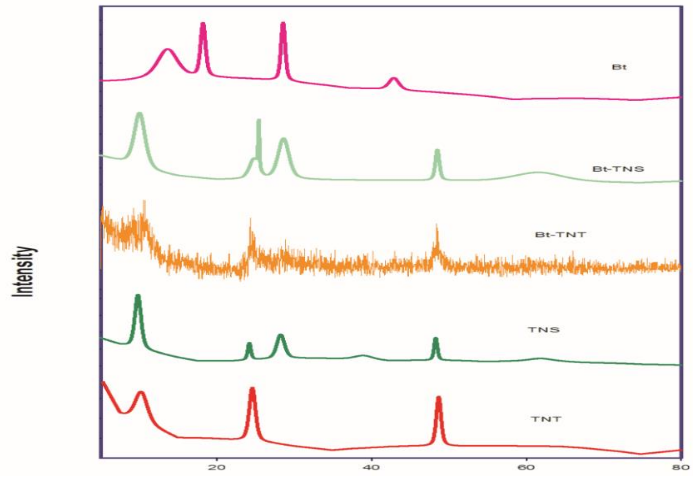

Figure 1: XRD patterns of TNT, TNS, Bt-TNT, Bt-TNS and Bt

Figure 2 shows FTIR spectrum of H-TNT, H-TNS, Bt -TNT and Bt-TNS. All spectra of titanate nanostructures and *Bacillus* titanate composites show peaks characteristic of the OH group at ~3400 and 1620 cm^{-1} [19] which indicates the presence of large amount of water and hydroxyl groups in all samples. The broader peaks centered at 3400 cm^{-1} are O–H stretching vibrations while the peaks at 1620 cm^{-1} are due to physically adsorbed water molecules H–O–H [19]. While in case of *Bacillus*, the following bacterial characteristic peaks were observed at 1460 cm^{-1} due to CH_2 bending of lipids, 1651 cm^{-1} due to protein C=O stretching, α helices at 2857 cm^{-1}, CH_2 symmetric stretch of lipids at 2922 cm^{-1} CH_2 symmetric stretch of lipids and at ~ 3400 due to the presence of amount of water and hydroxyl groups.

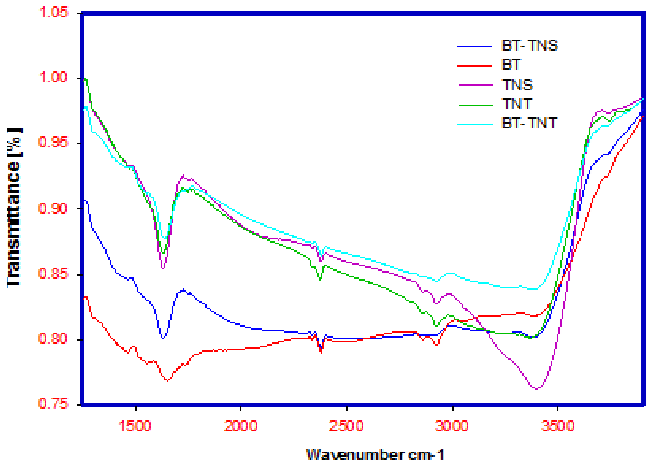

Figure 2: FT-IR spectra of TNT, TNS, Bt-TNT, Bt-TNS and Bt

The FESEM images of BT, TNT, TNS, Bt-TNT) and Bt-TNS are shown in Figures 3a-3e, respectively. In Figure 3a the Bacteria size appeared to be in the micron scale. In Figure 3b it is clear that the nanotubes are randomly oriented, appear to have a uniform dimensions and cross-linked with each other forming network-like shape. Figure 3c shows that the nanosheets seem to be agglomerated in appearance due to the absence of surfactant during the preparation process. In Figures 3d and 3e it is clear that the nanotubes and nanosheets are well distributed and cover the *Bacillus* surface and this confirms the results obtained by XRD analysis where no peaks were detected for the bacteria in both composites due to this well distribution.

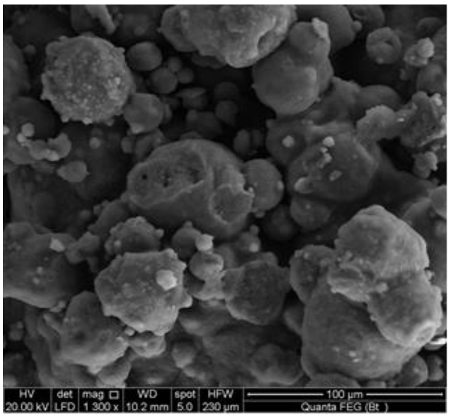

Figure 3a: FESEM image of *Bacillus*

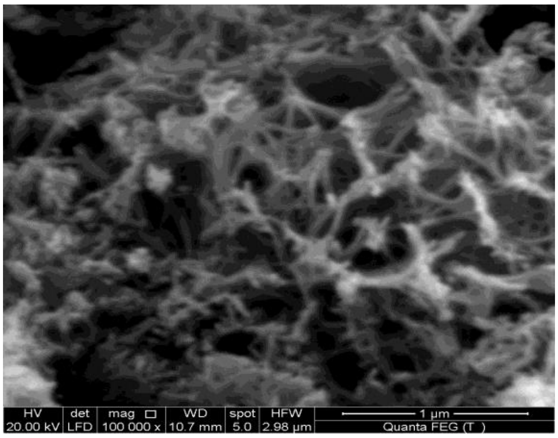

Figure 3b: FESEM image of H-Titanate Nanotubes (TNT)

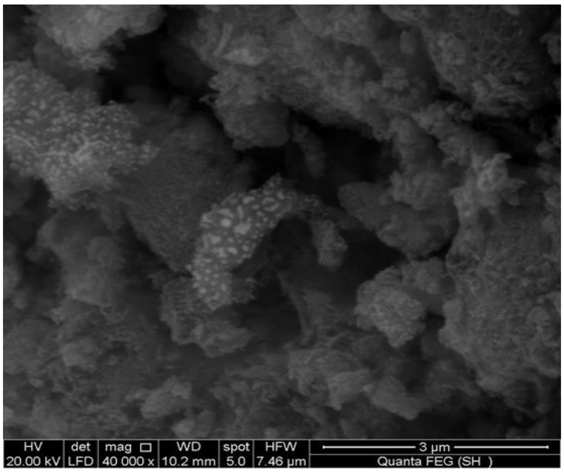

Figure 3c: FESEM image of multilayered H-Titanate Nanosheets (TNS)

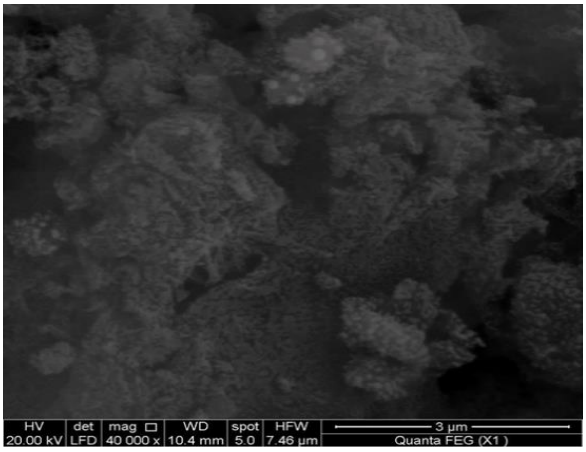

Figure 3d: FESEM image of *Bacillus* -H-Titanate Nanotubes Composite (BT-TNT)

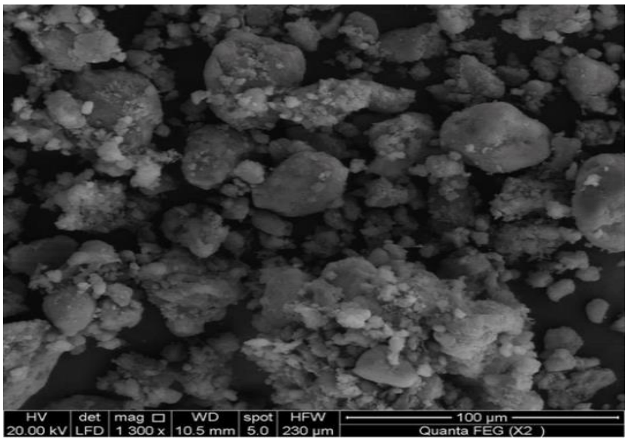

Figure 3e: FESEM image of *Bacillus*-H-Titanate Nanosheets Composite (BT-TNS)

Figure 4 shows the average zeta-potentials of TNT, TNS, *B. thuringiensis* and their composites. From this figure we can notice the increase of *B. thuringiensis* negative potential from (-17.55 mv) to (-25.25 mv) and (-24.80 mv) in case of Bt-TNT) and Bt-TNS), respectively, which reveals the adsorption of the prepared nanomaterials on the surface of bacteria as confirmed previously through XRD and FESEM analyses and this may be the reason for increasing the bacterial activity toward the targeted cotton leafworm.

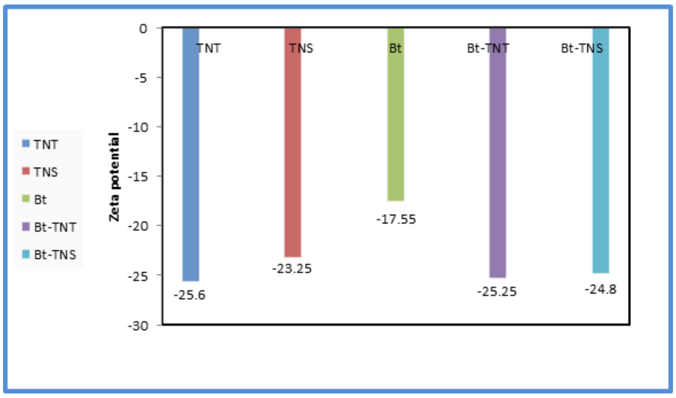

Figure 4: The average zeta-potentials of TNT, TNS, Bt and their composites

Latent effect

Total mortality and pupation

The total mortality % is the sum of larval and pupal mortality % of the 2nd and 4th instars of cotton leafworm. It is clear from Figure 5 that TNT and Bt-TNT caused 30% total mortality for both 2nd and 4th instars, compared to 10% mortality for both instars treated with Bt. Figure 5 also shows that Bt-TNT and TNS were able to cause 20% and 10% mortality for the 4th instar respectively. It is clear from these data that Bt-TNT) caused the highest mortality % for both instars, while TNT is more effective towards the 2nd instar only. Figure 6 illustrates the pupation % of the surviving larvae. It is clear from data that there is no direct effect of all materials on the pupation %.

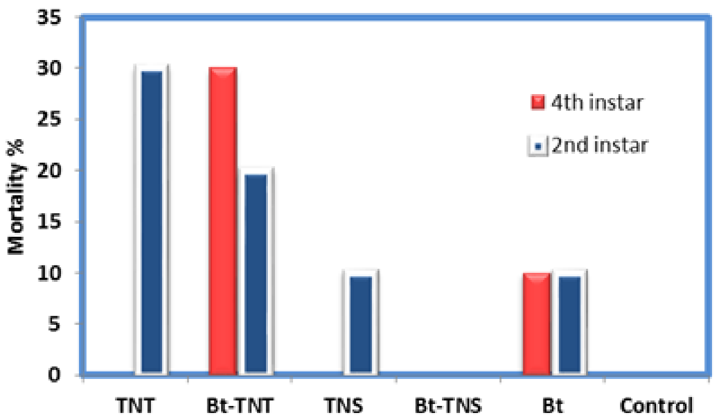

Figure 5: Effect of the TNT, TNS, Bt-TNT, Bt-TNS and Bt on total mortality % of 2nd and 4th instars

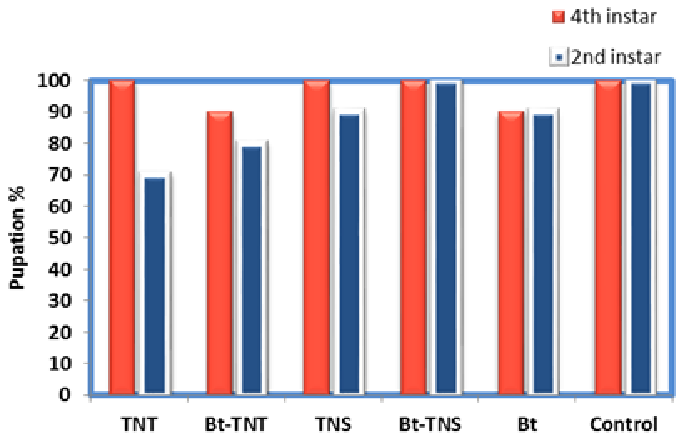

Figure 6: Effect of TNT, TNS, Bt-TNT, Bt-TNS and Bt on pupation %

Adult emergence

Figure 7 illustrates the emergence deviation % which is the deviation of the percent of adult moths emerged from the pupae compared to the control. It is obvious that there is no effect on the emergence % after using all materials except (Bt-TNT) which resulted in 70% emergence compared to 100% in case of control.

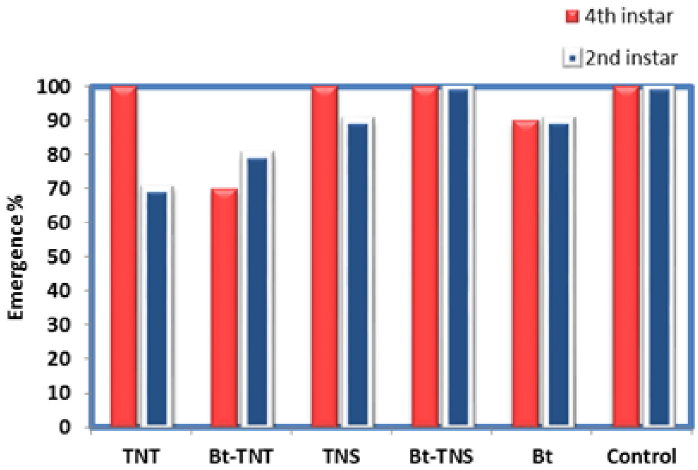

Figure 7: Effect of TNT, TNS, Bt-TNT, Bt-TNS and Bt on emergence %

Larval duration

As shown in Figure 8 the larval duration for the 2nd instar decreased below normal average by 42, 15 and 2% after using TNT, Bt-TNT and Bt-TNS, respectively. On the other hand, TNT, Bt-TNT, TNS, Bt-TNS and *B* Bt caused 12.5, 37, 71, 57 and 40% larval duration increase for the 4th instar compared to that of control.

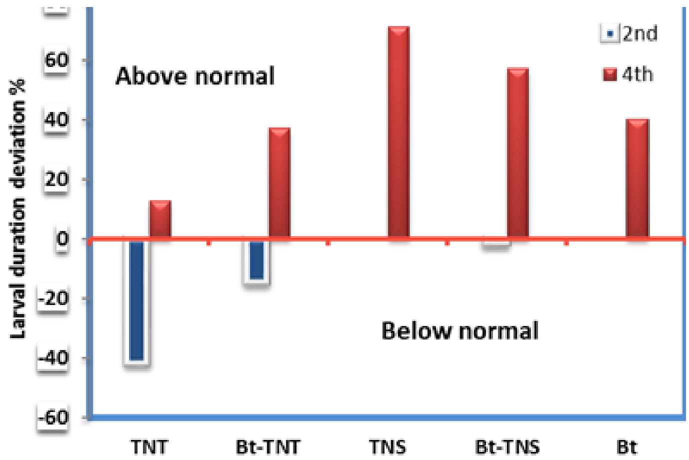

Figure 8: Effect of TNT, TNS, Bt-TNT, Bt-TNS and Bt on larval duration %

Pupal duration

In case of the 2nd instar as shown in Figure 9, TNT and Bt-TNS increased the pupal duration above normal average by 27 and 10%, respectively. While TNT, Bt-TNT, TNS, Bt-TNS and Bt decreased the pupal duration for the 4th instar by 5, 2, 19, 66 and 9%, respectively. It is clear from Figure 9 also that Bt-TNT and Bt decreased the pupal duration of the 2nd instar by 9 and 10%, respectively, compared to that of control.

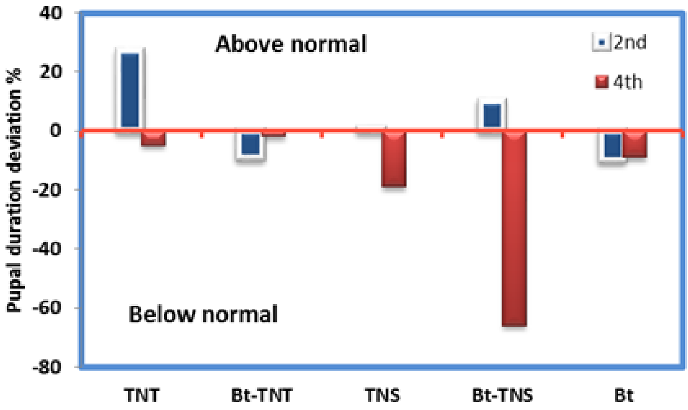

Figure 9: Effect of TNT, TNS, Bt-TNT, Bt-TNS and Bt on the pupal duration %

Pupal weight

The pupal weight of the 2nd instar Figure 10 decreased by 9 and 2% as a result of using TNT and Bt-TNS, respectively. While they increased the weight of the 4th instar by 8 and 14%, respectively, compared to that of control. On the other hand Bt-TNT, TNS and Bt increased the weight of the 2nd instar by 6, 0.96 and 13%, respectively, they also increased the weight of the 4th instars by 3, 7 and 4%, respectively, compared to that of control.

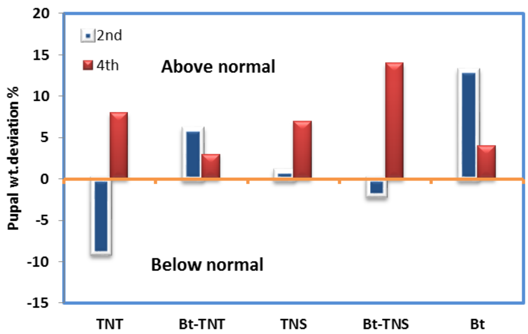

Figure 10: Effect of TNT, TNS, Bt-TNT, Bt-TNS and Bt on the pupal weight

Adult fecundity (fertility) and eggs hatchability

It is clear from data in Table 1 that the treatment of 4th instar of *S. littoralis* with Bt-TNT and Bt-TNS strongly affected the fecundity of the 4th instar, where the total number of eggs per female reached to 0 compared to 111.3 eggs/female of control. In case of TNT, Bt and TNS the adult fecundity decreased to 33.3, 43 and 50 eggs/female, respectively. The treatment of 4th instar of *S. littoralis* with Bt-TNT and Bt-TNS had the highest effect on reduction the total number of eggs per female, and eggs hatching reached 0% compared to that of control (100%).

Table 1: Effect of Bt, TNT, TNS, Bt-TNT and Bt-TNS on the adult fecundity and eggs hatching

Sample	Fecundity (eggs/female) Mean+S.D	Eggs hatchability%
TNT	33.3+9	100
Bt-TNT	0+0	0
TNS	50 + 3.5	100
Bt-TNS	0+0	0
Bt	43+2.6	100
Control	111.3+3.5	100
F value	1107.9	
P value	0.0019	
L.S.D at 0.05	8.225	
0.01	15.2	

Adult longevity

The data presented in Figure 11 demonstrate the effect of the prepared materials on the life period of adult moths. The presented data show 21, 7, 12.5 and 31.5% adult longevity decrease for the 2nd instar after using TNT, Bt-TNT, TNS and Bt-TNS, respectively. On the other hand, there is only 3.4 and 2.6% adult longevity decrease for the 4th instar adults after using TNS and Bt-TNT compared to that of control. There was also 3.4 and 12% adult longevity decrease for the 4th instar after using TNT and Bt-TNT, respectively, while Bt resulted in 11.5 and 0.86% adult longevity decrease for the 2nd and 4th instars, respectively, compared to that of control.

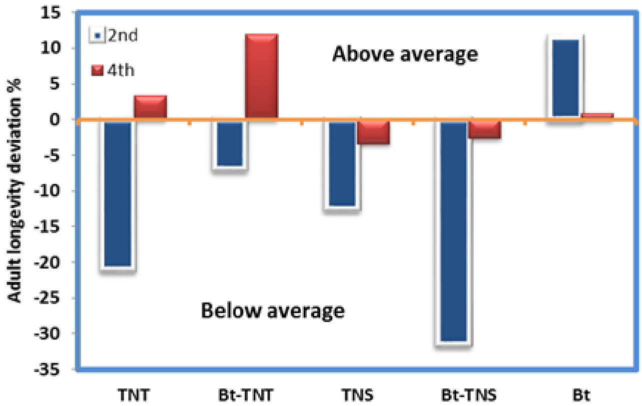

Figure 11: Effect of TNT, TNS, Bt-TNT, Bt-TNS and Bt on adult longevity

Sex ratio

Figure 12 illustrates the effect of the different materials on the percent of females (F) to males (M) of adult moths compared to that of control. It is obvious from these data that Bt-TNS resulted in a significant change in sex ratio to be (50 F: 50 M) for the 2nd instar compared to (60 F: 40 M) of control, while it became (60 F: 40 M) for the 4th instar compared to (40 F: 60 M) of control. Also, Bt-TNT and TNS changed the sex ratio for the 4th instar to be (37.5 F: 62.5 M) and (55.6 F: 44.4 M), respectively.

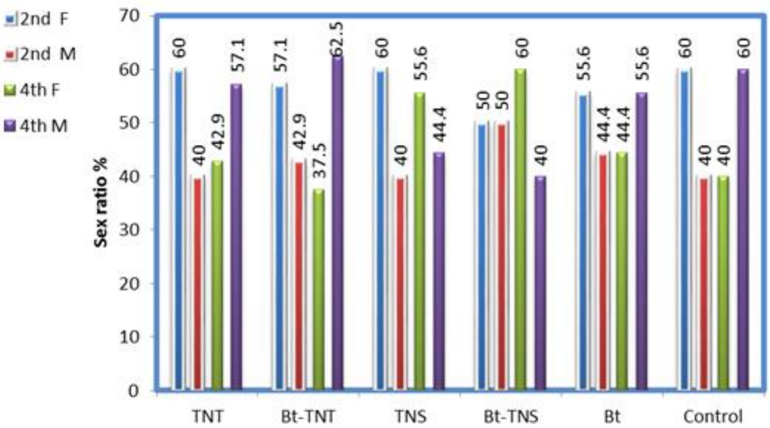

Figure 12: Effect of TNT, TNS, Bt-TNT, Bt-TNS and Bt on adult sex ratio

CONCLUSION

Titanate nanosheets, nanotubes and Bt-TNS were successfully synthesized. pesticidal acvtivity of TNT, TNS and their composites with *B. thuringiensis* towards cotton leafworm *S. littoralis* was tested. It was found that these materials affected on different biological features of cotton leafworm like: Adult longevity, adult sex ratio, pupation %, fecundity and percent of eggs hatching. These results revealed that TNT, TNS and their composites with bacillus can be used as new nanopesticides against cotton leafworm.

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