Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
www.entomoljournal.com
JEZS 2021; 9(3): 119-124

Received: 13-03-2021
Accepted: 15-04-2021

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# Comparison of the insecticidal activity of calcium borate with mushroom-like structures and chitin synthesis inhibitor insecticide (Dimilin) against Egyptian cotton leaf worm

**Haytham A Ayoub, Mohamed Khairy, Farouk A Rashwan and Hanan F Abdel-Hafez**

**Abstract**
Egyptian cotton leaf-worm (*Spodoptera littoralis)* is one of the most destructive phytophagous insects not only in Egypt but also in the whole world. The extensive application of synthetic organic insecticides against *Spodoptera littoralis (S. littoralis)* cause raising resistance toward several insecticides. Thus, developing of an alternative novel class of insecticides is an urgent assignment. Herein, calcium borate nanoparticles (CB-NPs) with mushroom-like structures were synthesized *via* template assisted-hydrothermal route and applied against *S. littoralis* for the first time. The larvicidal activity of CB-NPs was investigated and compared with the insect growth regulator (dimilin), the calculated $LC_{50}$ values were 192.72 mg/L and 19.59 mg/L for CB-NPs and dimilin, respectively. The examination of the histological influences for cross-sections of 6$^{th}$ instar larvae showed a similar action mechanism on the cuticle layer for CB-NPs and dimilin. Thus, CB-NPs could be utilized in the agricultural programs instead of dimilin.

**Keywords:** nano-pesticides, calcium borate nanoparticles, insect growth regulators, dimilin, bioassay, *Spodoptera littoralis*

## 1. Introduction
Cotton leaf-worm, *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae), is considered one of the most phytophagous destructive insect pest in Egypt due to its high reproductive rate which causes a destructive loss in crops production. The larvae of *Spodoptera littoralis* are feeding on 40 plant families, containing 87 species of economic importance [1]. Although *Spodoptera littoralis* is native in Africa, it is distributed throughout the world. Therefore, European and Mediterranean Plant Protection Organization (EPPO) have listed it as an A2 quarantine insect pest [2]. The economic loss related to the damage of crops caused by *Spodoptera littoralis* makes its control a very important issue. In this context, chemical control with synthetic insecticides is a highly recommended strategy. However, the extensive usage of such traditional organic insecticides (i.e. organophosphate, carbamate, and pyrethroid) caused insect pesticide resistance. The rising resistance against many registered insecticides creates a compulsory limitation in the application of synthetic insecticides [3, 4].
Fortunately, the rapid development of nanotechnology endeavors to produce a novel class of plant protection products based on nano-materials "nano-pesticide". Nano-pesticide is any pesticide formulation intentionally including nano-sized entities (up to 100 nm) and claiming new properties arising from size. The utilization of nano-pesticides in crop protection helps to mitigate the drawbacks of traditional pesticides [5-7]. Recently, silica nanoparticles with varietal sizes and morphologies were fabricated and their toxic effect on *Spodoptera littoralis* was estimated. It was observed that the insecticidal activity is significantly affected by particle size and surface functionality of nano-silica [8]. Titanium dioxide nanoparticles ($TiO_2$-NPs) successfully applied against *Spodoptera littoralis*. The $LC_{50}$ values for 2$^{nd}$ and 4$^{th}$ instar larvae were found to be 62.5 mg/L, and 125 mg/L respectively. Further, the treated insects also suffered from malformations in larvae, pupae, and adult stages [9]. The acid and alkaline modification of natural kaolin exhibited potential entomotoxic effect against *Spodoptera littoralis* with $LC_{50}$ values 98.11 mg/L and 403.13 mg/L respectively [10]. On the other hand, the insertion of Ag and Zn ions in calcium borate enhances their bactericidal activities on

*Staphylococcus aureus* and *Escherichia coli* strains with a minimum inhibition concentration (MIC) of 75.0 mg/L and minimum bactericidal concentration (MBC) 50.0 mg/L [11]. Borate-based pesticides are multi-site inhibitors and used widely against several insects, mites, and diseases with low mammalian toxicity [12]. It might kill insects by disturbing the normal functions of several oxidized coenzymes. Once insects ingested borate-contaminated food, it interacts to form complexes with $NAD^+$, $NMN^+$, and $NADP^+$. It also can act as water balance disruptors. Besides, borate ions might absorb in cuticle wax causing destructive damage in the protective waxy layer, and then insects begin to lose the water content and die through desiccation [12, 13].

The insect growth regulators (IGRs) are chemical compounds could disturb the physiological processes of insects. Benzoylurea is one of the insect growth regulators, belongs to chitin synthesis inhibitors (CSI). CSI distresses the larval stage mainly by ingestion through inhibiting chitin formation causing abnormal endocuticular deposition and abortive molting [14-16]. Diflubenzuron is the first chitin synthesis inhibitors analog commercialized in 1975 under the trade name dimilin (Fig. 1) [17]. Dimilin affects the endocuticle layer by inhibiting the biosynthesis of chitin precludes the larvae to complete the molting process and finally results in death [18]. It is reported that, borate-based pesticide deteriorates the cuticle layer, while dimilin inhibits the synthesis of chitin leading to the uncompleted formation of the cuticle [13, 18]. Thus, in the present work we synthesized mushroom-like structures of calcium borate nanoparticles by template-assisted hydrothermal route to explore their insecticidal activity compared with dimilin insecticide against *S. littoralis*. The borate sample is characterized by wide-angle X-ray diffraction, Fourier-transform infrared spectroscopy, $N_2$ adsorption/desorption isotherms and scanning electron microscopy. It is reported recently that, calcium borate can act as nano-fertilizer [19]. Thus, the application of CB-NPs as bi-functional material in crop protection programs provides a sustainable inorganic insecticide and may help in plant growth.

## 2. Materials and Methods

### 2.1. Chemicals

All chemicals were of the highest analytical grades used as received from Sigma-Aldrich Ltd., Germany without any further purification. Boric acid ($H_3BO_3$), calcium chloride ($CaCl_2$), sodium hydroxide (NaOH), sodium acetate ($CH_3COONa$) polyvinylpyrrolidone (PVP), Diflubenzuron (N-[(4-chlorophenyl)carbamoyl]-2,6-difluorobenzamide),
Acetone ($(CH_3)_2CO$), Triton™ X-100. The solutions were prepared using bi-distilled water with resistivity >18.2 MΩ/cm at 25 °C.

### 2.2. Synthesis of calcium borate

Calcium borate NPs with mushroom-like structures were synthesized *via* template assisted-hydrothermal route by using PVP and sodium acetate as shown in (Scheme 1). In a typical procedure, 30 ml of boric acid (20 mmol) is prepared and marked as solution-A. A 30 mL of $CaCl_2$ solution (10.0 mmol) mixed with 0.5 g of polyvinylpyrrolidone (PVP) and 20 mmol of sodium acetate marked as solution-B. Solution-A was added drop wise to solution-B under continuous stirring and the final pH of the solution was adjusted to pH 8.0 by using 0.05 M NaOH at which white slurry was formed. The resultant slurry was transferred into a Teflon-lined stainless steel autoclave with a capacity of 60 mL. The autoclave was sealed, heated up to 160 ºC, and kept in an isothermal state for 6 h. Finally, the autoclave was cooled down to room temperature. The product was filtered, washed with water/ethanol three times, and then dried at 80 ºC for 12 h. The calcium borate with a mushroom-like structure is produced after annealing at 500 ºC for 5 h.

### 2.3. Characterization of calcium borate

Wide-angle X-ray diffraction (XRD) patterns of calcium borate powder was measured by using an X-ray diffractometer (Model D8 Advance, Bruker) with monochromatic CuKα radiation (λ = 1.54 Å), employing a scan rate of 0.06 $min^{-1}$. The diffraction data were analyzed using PDF-2 Release 2009. Fourier transform-infrared (FTIR) spectroscopy was recorded using the Bruker Alpha FTIR instrument.

The textural surface properties of calcium borate were determined by $N_2$ adsorption/desorption isotherms at 73 K with BELSORP apparatus, Japan. The specific surface area (SBET) was calculated using the *Brunauer-Emmett-Teller* (BET) method with multipoint adsorption data from the linear segment of the $N_2$ adsorption isotherm. The pore size distribution was determined from the analysis of the desorption branch of isotherm using the *Barrett-Joyner-Halenda* (BJH) method.

The morphology of calcium borate sample was investigated using scanning electron microscopy (SEM, JEOL model 5400 LV). Calcium borate powder was grinded and fixed onto a specimen stub using double-sided carbon tape. To obtain high-resolution micrographs, a 10 nm Au film was coated on the calcium borate using anion sputtering (Hitachi E-1030) at room temperature. The SEM was operated at 15 kV to obtain high-resolution SEM images.

### 2.4. Insect rearing

Susceptible laboratory strain of cotton leaf-worm (*S. littorals*) was reared under constant laboratory conditions in an incubator at a temperature of 25 ± 2 °C, relative humidity of 65 ± 5% and photoperiod of 8 h light: 16 h darkness in Plant Protection Research Institute, Giza, Egypt. Larvae were cultured on leaves of the castor bean plant (*Ricinus communis* L.) in a glass jar till pupation and emergence of adults. Emerged adults were supplied by a small piece of cotton saturated with a 10% sugar solution for feeding and as well by leaves of Tafla (*Nerium oleander*) for egg-laying. The collected eggs were incubated in distinct jars at 25 ºC till hatching [20].

### 2.5. Bioassay

The Larvicidal activities of CB-NPs and dimilin were evaluated *via* leaf-dip bioassay (ingestion) method under recommended experimental conditions [21]. Briefly, different concentrations of CB-NPs (150, 300, 600, 900, and 1200 mg/L) were dispersed in water at a measurable pH 8.6 by using an ultrasonic cleaner for 10 min. While, a stock solution of dimilin dissolved in acetone was diluted to prepare different concentrations (10, 20, 40, 60, and 80 mg/L). To increase the adhesion of the dispersed solutions, 0.1% Triton X-100 was added [22]. Then, leaf discs of a castor-bean plant with the same sizes were cleaned and dipped into the solutions. The treated and untreated leaves were introduced into drying container containing equal numbers (20 larva /replica) of new molting 2nd instar larvae of *S. littoralis*, each treatment has three replicas. The containers were maintained

and covered with a muslin cloth to allow aeration. Mortality percentages were calculated by using Equation (1), and natural mortality was corrected by using *Abbott's* formula Equation (2), where T and C are the numbers of live larvae after treatment experiments with CB-NPs and control solutions [23].

$$\text{Mortality percentage} = \frac{\text{Number of dead larvae}}{\text{Number of larvae introduced}} \times 100 \quad (1)$$

$$\text{Corrected mortality percentage} = \left[1 - \frac{T}{C}\right] \times 100 \quad (2)$$

### 2.6. Histological influences

The effects of sub-lethal concentrations ($LC_{50}$) of CB-NPs and dimilin on the histological influences were estimated. Leaf discs of a castor-bean plant with the same sizes were cleaned and dipped into solutions of their $LC_{50}$ values. The treated and untreated leaves were introduced to new molting 2nd instar larvae of *S. littorals via* leaf-dip bioassay method for three days [22]. The survived larvae of *S. littoralis* were collected and allowed to grow on untreated leaves till reaches to 6th instar larvae. Larvae washed with ethanol (70%) and then dehydrated according to series (70% - 100%) of ethanol. paraffin wax was used for infiltration embedding of the samples and then sections of 5.0 µm were stained with Haematoxylin-Eosin (H-E). The morphological alterations on the cuticle layer were examined by light microscopic and compared to the control group.

### 2.7. Statistical analysis

The median lethal concentration ($LC_{50}$) values were calculated using the probit analysis program [24]. Prior analysis, all-natural mortalities were corrected by using *Abbott's* formula as shown in Equation (2). The data were presented as the mean ± standard error (SE).

## 3. Results and Discussion

### 3.1 Characterization of calcium borate

The structural, functionality, textural properties and morphology of CB sample are carried out by wide-angle XRD, FTIR, $N_2$ adsorption/desorption isotherms and scanning electron microscopy, respectively. Figure 2a showed broad diffraction peaks are observed at 2θ of 30° and 45°. These diffraction peaks revealed amorphous phase of calcium borate even after thermal treatment at 500 °C. The FTIR showed several peaks indicating formation of calcium borate (Fig. 2b). The absorption peaks at 3442 $cm^{-1}$ related to stretching modes of $H_2O$ moisture in the sample [25]. The absorption signal at 1407 $cm^{-1}$ attributed to B-O stretching trigonal $BO_3$ units, while the signal at 981 $cm^{-1}$ related to B-O stretching of tetrahedral $BO_4^-$ [25, 26]. The absorption peaks at 704 $cm^{-1}$ related to B-O-B bending vibration in the borate ring [26].

According to IUPAC classifications of $N_2$ adsorption/adsorption isotherms CB sample revealed type IV isotherm with a pronounced H3 hysteresis loop which could characterize slit-like mesopore entrances (Fig. 2c). The specific surface area of CB sample was $S_{BET}$ = 8.24 $m^2/g$. CB sample exhibited mesoporous network architecture with pore diameters of 14.43 nm (Fig. 2d). This mesoporous structure might be suitable for the adsorption of biomolecules (i.e. cuticle wax) of the insect exoskeleton, which may help in determining the toxic effects related to the surface area.

Figure 2 (e, and f) also shows scanning electron microscopy (SEM) images of CB sample, the as-synthesized calcium borate are composed of aggregated nano-sheets with an average thickness of 65 nm interweaved in a beautiful mushroom-like macrostructure. The presence of PVP and sodium acetate plays a remarkable role in the formation and arrangement of nano-sheets. The surface energy of individual nano-sheets is quite high and thus tends to self-assembly to lower such surface energy by interweaved in a beautiful mushroom-like macrostructure [27].

### 3.2 Toxic effect

The larvicidal activity of CB-NPs and dimilin was investigated by utilizing feeding method. Accumulative mortalities for 2nd instar larvae of *S. littorals* were calculated after 11 days post-treatment (at the end of molting stage). The mortality rates of the treated larvae showed a positive correlation with CB-NPs and dimilin concentrations (Table 1 and Fig. 3). The calculated $LC_{50}$ values were 192.72 mg/L and 19.59 mg/L for CB-NPs and dimilin respectively. Despite the higher insecticidal activity of dimilin, CB-NPs with mushroom-like macrostructures provide an alternative inorganic insecticide and may contribute to plant fertility [19].

The histological influences of 6th instar larvae treated with CB-NPs and dimilin are investigated. Cross-sections of cuticle layers are examined by a light microscope as shown in Fig. 4. Insects in control group exhibited a normal histological structure of the body wall with intact layers. Meanwhile, the treated larvae suffered from detachment of endocuticle and exocuticle from the epidermis. CB-NPs exhibited a mesoporous network architecture with pore diameters of 14.43 nm (Fig. 2d) which enhances the absorption of cuticle waxes results in damaging for the protective exoskeleton (Fig. 3 (e and f)) [17]. In this context, chitin synthesis inhibitors (CSI) compounds affect the peritrophic membrane through inhibiting the biosynthesis of chitin hindering its role in protecting the secreting cells from damage (Fig. 3e and f) [28, 29]. Based on the histological study, the insecticidal action mechanism on the cuticle layer for CB-NPs is analogous to that for dimilin. Therefore, CB-NPs provide an effective alternative inorganic insecticide for cotton leaf-worm control.

**Table 1:** Toxicity data for 2nd in star larvae of *Spodoptera littorals* exposed to different concentrations of CB-NPs and dimilin *via* feeding bioassay method after 11 days post-treatment.

| Compound | LC50 (mg/L) | Confidence limit (95%) | | Slope ± SE | R | X2 | P |
|---|---|---|---|---|---|---|---|
| | | Lower | Upper | | | | |
| CB-NPs | 192.72 | 52.81 | 314.08 | 1.9903 ± 0.5796 | 0.8653 | 2.5217 | 0.4714 |
| Dimilin | 19.59 | 16.86 | 22.27 | 2.4061 ± 0.2077 | 0.9933 | 1.9697 | 0.5787 |

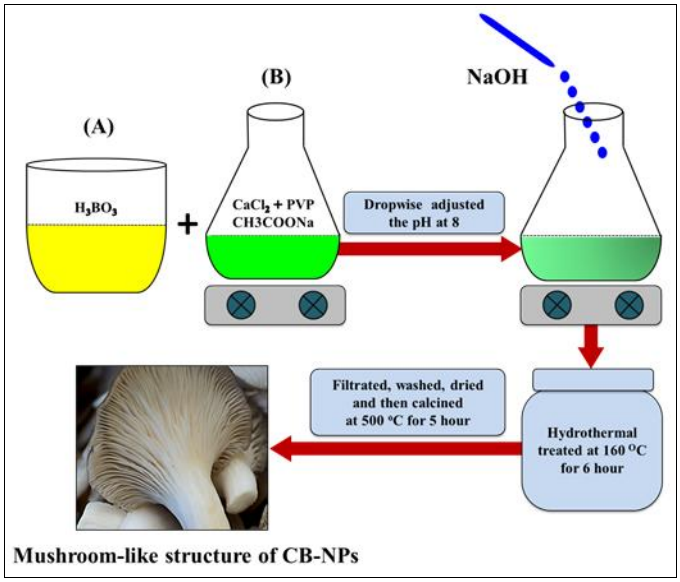


**Fig 1:** Scheme 1; Hydrothermal synthesis of mushroom-like structure of CB-NPs.

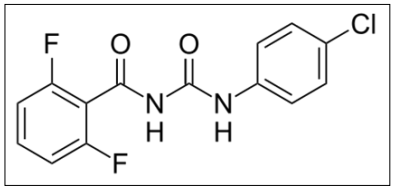


**Fig 2:** Chemical structure of dimilin.

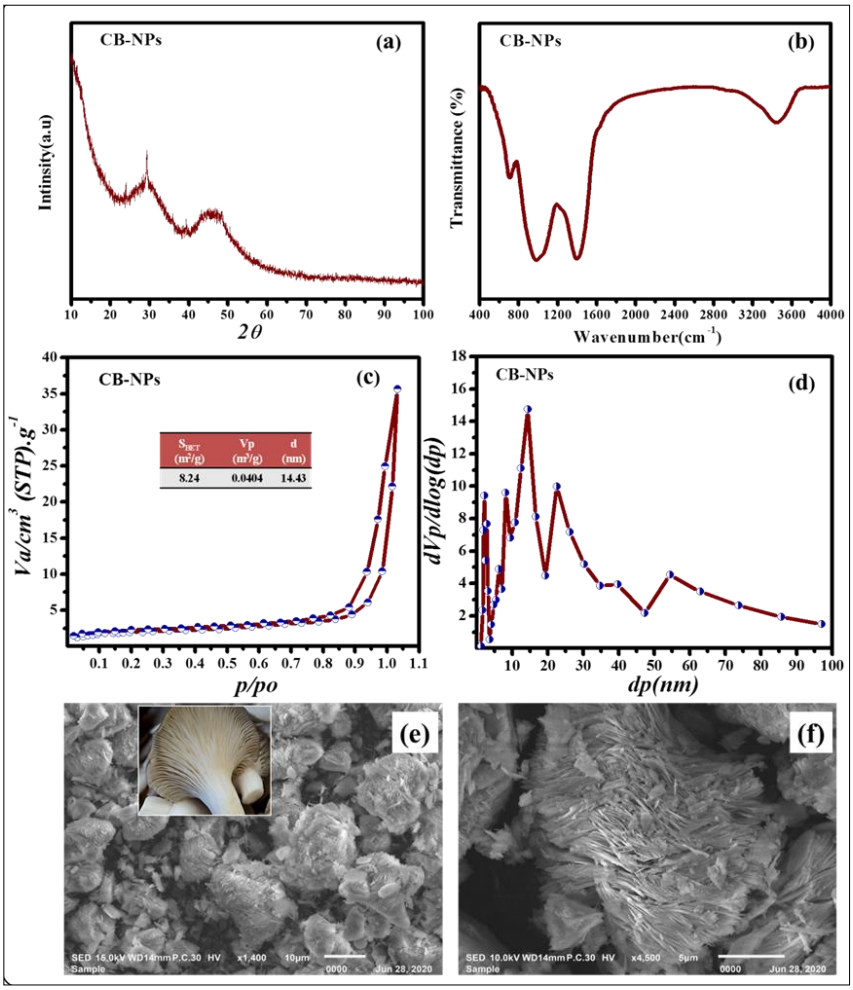


**Fig 3:** (a) Wide-angle XRD, (b) FTIR, (c) $N_2$ Adsorption/desorption isotherms, (d) Corresponding pore size distribution curves, and (f and e) SEM images of CB-NPs.

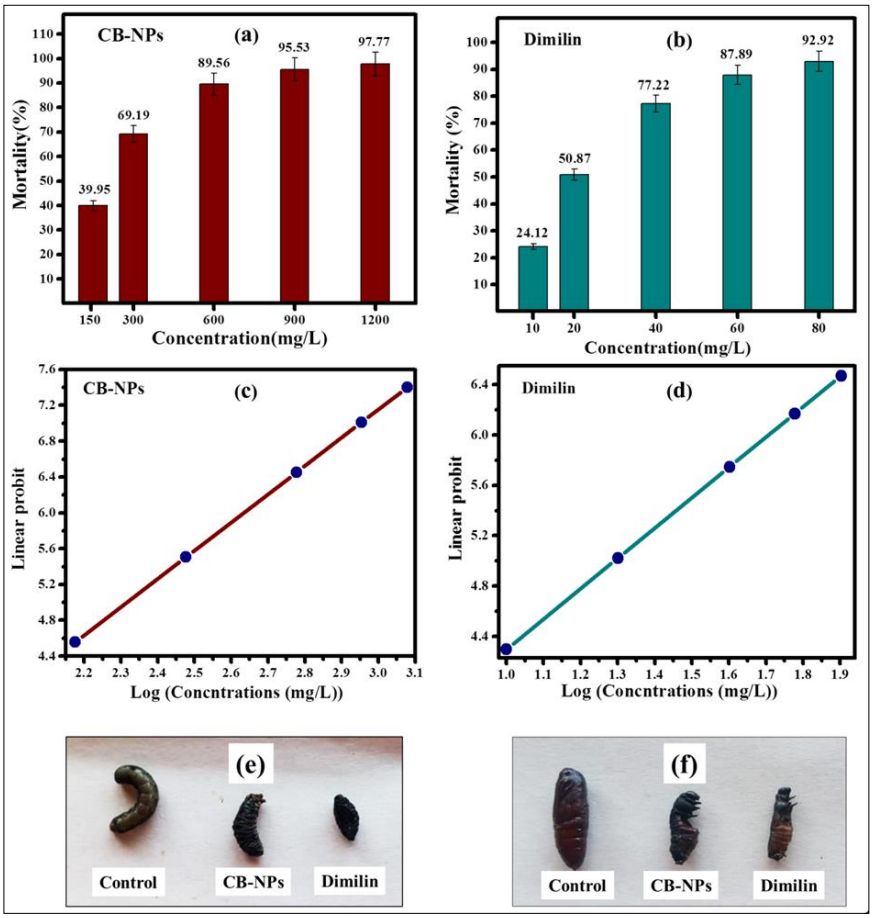


**Fig 4:** (a) Accumulative mortality for 2nd instar larvae of *S. littorals* exposed to different concentrations of CB-NPs, (b) dimilin, (c and d ) Toxicity lines for CB-NPs and dimilin, and (e and f) Photographic images for 4th instar larvae of *S. littorals* and pupa exposed to $LC_{50}$ of CB-NPs and dimilin. Data values are the mean of three independent replicates and vertical bars represent the standard error.

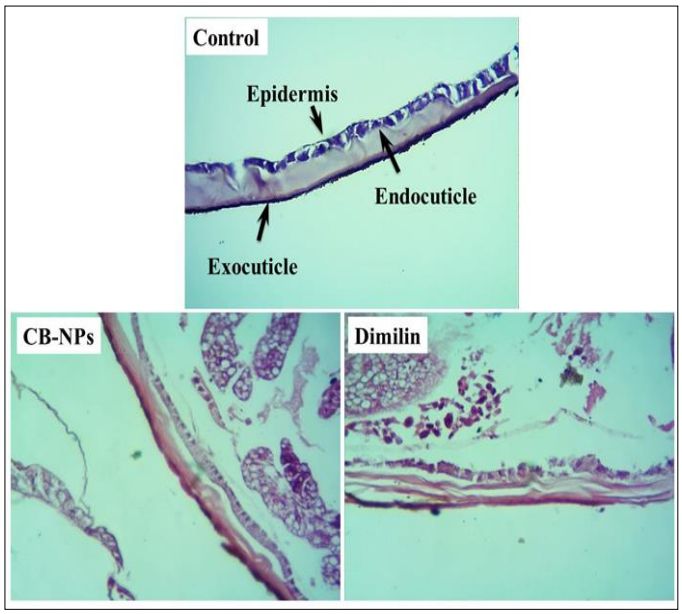


**Fig 5:** Cross-sections of cuticle layers of control and treated 6th instar larvae of *Spodoptera littoralis* (×40 H-E).

## 4. Conclusion

The calcium borate with mushroom-like macrostructures was synthesized *via* template assisted-hydrothermal route in the presence of PVP and sodium acetate as soft templates. Remarkable arrangement of nano-sheets with an average thickness of 65 nm is observed. The larvicidal activity of CB-NPs against *S. littorals* was investigated and compared with dimilin. The calculated $LC_{50}$ values were 192.72 mg/L and 19.59 mg/L for CB-NPs and dimilin, respectively. Based on the histological study, the insecticidal action mechanism on the cuticle layer for CB-NPs is analogous to that for dimilin. Thus, CB-NPs provide potent alternative inorganic insecticide for *S. littorals* control.

## 5. References

1. El-Sheikh ESAM, El-Saleh MA, Aioub AA, Desuky

WM. Toxic effects of neonicotinoid insecticides on a field strain of cotton leaf-worm, Spodoptera littoralis. Asian Journal of Biological Sciences 2018;11:179-185.
2. OEPP/EPPO. EPPO Standards PM 7/124 (1) Diagnostic Protocol for *Spodoptera littoralis*, *Spodoptera litura*, *Spodoptera frugiperda*, *Spodoptera eridania*. OEPP/EPPO Bull 2015;34:257-270.
3. Elghar GEA, Elbermawy ZA, Yousef AG, Abd Elhady HK. Monitoring and Characterization of Insecticide Resistance in the Cotton Leaf-worm, *Spodoptera littoral* is (Boisd.) (Lepidoptera: Noctuidae). Journal of Asia-Pacific Entomology 2005;8(4):397-410.
4. Vattikonda SR, Sangam SR. Effect of forskolin on the growth and differentiation of the ovary of *Papilio demoleus* L. (Lepidoptera: Papilionidae). International Research Journal of Environmental Sciences 2017;6:13-17.
5. Kah M, Hofmann T. Nanopesticide research: current trends and future priorities. Environment International 2014;63:224-235.
6. Anandhi S, Saminathan VR, Yasotha P, Saravanan PT, Venugopal Rajanbabu. Nano-pesticides in pest management. Journal of Entomology and Zoology Studies 2020;8(4):685-690.
7. Tripathi DK, Ahmad P, Sharma S, Chauhan DK, Dubey NK. Nanotechnology in Crop Protection. In Nanomaterials in Plants, Algae, and Microorganisms. Eds, Academic Press 2018;Chapter 16:345-391.
8. Ayoub HA, Khairy M, Rashwan FA, Abdel-Hafez HF. Synthesis and characterization of silica nanostructures for cotton leaf worm control. Journal of Nanostructure in Chemistry 2017;7:91-100.
9. Shaker AM, Zaki AH, Abdel-Rahim EF, Khedr MH. TiO2 nanoparticles as an effective nanopesticide for cotton leaf worm. Agricultural Engineering International: CIGR Journal 2017, 61-68.
10. Khairy M, Ayoub HA, Rashwan FA, Abdel-Hafez H. Chemical modification of commercial kaolin for mitigation of organic pollutants in environment via adsorption and generation of inorganic pesticides. Applied Clay Science 2018;1(153):124-133.
11. Arunachalam AL, Induja S, Sakthidasan J, Raghavan PS. Calcium borates incorporated with silver and zinc as promising antibacterial agents. Materials Letters 2020;9:128130.
12. Stirling R, Temiz A. Fungicides and insecticides used in wood preservation. In Deterioration and Protection of Sustainable Biomaterials. American Chemical Society 2014, 185-201.
13. Saldalgo V. BASF Insecticide mode of action technical training manual 2013.
14. Mulder R, Gijswijt MJ. The laboratory evaluation of two promising new insecticides which interfere with cuticle deposition. Pesticide Science 1973;4(5):737-745.
15. Ishaaya I, Casida JE. Dietary TH 6040 alters composition and enzyme activity of housefly larval cuticle. Pesticide Biochemistry and Physiology 1974;4(4):484-490.
16. Post LC, De Jong BJ, Vincent WR. 1-(2, 6-disubstituted benzoyl)-3-phenylurea insecticides: inhibitors of chitin synthesis. Pesticide Biochemistry and Physiology 1974;4(4):473-483.
17. Post LC, Vincent WR. A new insecticide inhibits chitin synthesis. Die Naturwissenschaften 1973;60(9):431-432.
18. Rao PA, Mehrotra KN. Effect of diflubenzuron treatment on chitin deposition in the wings of *Schistocerca gregaria* Forskal. Indian Journal of Entomology 1987;49:76-80.
19. Meier S, Moore F, Morales A, González ME, Seguel A, Meriño-Gergichevich C *et al*. Synthesis of calcium borate nanoparticles and its use as a potential foliar fertilizer in lettuce (*Lactuca sativa*) and zucchini (*Cucurbita pepo*). Plant Physiology and Biochemistry 2020;151:673-680.
20. Eldefrawi ME, Toppozada A, Mansour N, Zeid M. Toxicological studies on the Egyptian cotton leaf-worm, *Prodenia litura*. I. Susceptibility of different larval instars of Prodenia to insecticides. Journal of Economic Entomology 1964;57(4):591-593.
21. Zhu KY. Insecticide bioassay, Encyclopaedia of entomology: Springer, Berlin 2008, 1974-1976.
22. Ayoub HA, Khairy M, Elsaid S, Rashwan FA, Abdel-Hafez HF. Pesticidal activity of nanostructured metal oxides for generation of alternative pesticide formulations. Journal of agricultural and food chemistry 2018;66(22):5491-5498.
23. Abbott WS. A method of computing the effectiveness of an insecticide. Journal of Economic Entomology 1925;18(2):265-267.
24. Finney DJ. Probit analysis, Cambridge University Press. Cambridge, UK 1971.
25. Zhu W, Chen X, Sun P, Li J, Zhai P, Zheng Y *et al*. Facile hydrothermal synthesis of hierarchical porous priceite (Ca4B10O19· 7H2O) microspheres as high-efficiency adsorbents for heavy metal ions removal. CrystEngComm 2019;21(46):7141-7154.
26. Manam J, Sharma SK. Evaluation of trapping parameters of thermally stimulated luminescence glow curves in Cu-doped Li2B4O7 phosphor. Radiation Physics and Chemistry 2005;72(4):423-427.
27. Liu ZH, Xue L. Preparation of nanoplates assembled 4CaO·5B2O3·7H2O oval-like microspheres via a hydrothermal method. Materials Letters 2008;62(17-18):2692-2695.
28. Clarke L, Temple GH, Vincent JF. The effects of a chitin inhibitor—Dimilin—on the production of peritrophic membrane in the locust, *Locusta migratoria*. Journal of Insect Physiology 1977;23(2):241-246.
29. Shaurub ES, Zohdy NZ, Abdel-Aal AE, Emara SA. Effect of chlorfluazuron and flufenoxuron on development and reproductive performance of the black cutworm, *Agrotis ipsilon* (Hufnagel) (Lepidoptera: Noctuidae). Invertebrate reproductIon & development. 2018;62(1):27-34.