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Entomotoxic effects of calcium-based materials derived from bio-waste eggshells as alternative inorganic insecticides against cotton leafworm, *Spodoptera littoralis* (Lepidoptera: Noctuidae)

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Abstract
Recycling agricultural waste and changing it into value-added products is considered one of the most important issues. In this context, waste eggshells were utilized as an alternative bio-calcium source for preparing calcium-based materials. The prepared materials were characterized by using Fourier transform-infrared (FT-IR) spectroscopy and scanning electron microscopy (SEM). Their entomotoxic effects were estimated against *Spodoptera littorals*. Calcium-based materials provide an alternative inorganic insecticide for *Spodoptera littorals* besides their role in reducing the pollutant effects on the environment.

Keywords: Agricultural waste, Chicken eggshells, Inorganic insecticides, *Spodoptera littoralis.*

1. Introduction
Utilization of chicken eggshells as an alternative bio-calcium source is favored as it is cheap, readily available, and it is also environmental [1] .Eggshell is a composite material consisting of calcium carbonate (96%), magnesium carbonate (1%) calcium phosphate (1%), and also of organic substances and water [2]. The 'United Nations' Food and Agriculture Organization estimated that the world produced 70.4 million tons of eggs in 2015, which is projected to increase to 89 million in 2030. Taking into account that the shell occupies about 11% of the weight of each egg, the world will produce 8.1 million tons of eggshells in 2030 [1, 2]. The production of biocompatible material from agro-wastes has added a different dimension to the conversion of agricultural wastes into value-added products [3]. Thermal treatment of eggshells to prepare calcium oxide opens a door for countless applications as filler in feed, fertilizer, paper, printing ink, pharmaceutical and cosmetic products. As well as starting materials of dielectrics such as $CaSiO_3$, $CaTiO_3$, $CaAl_2O_4$, gypsum ($CaSO_4$), and bio-catalysts [4].

Calcium silicates (CS) are one of the inorganic compounds that are composed of Ca, Si, and O elements, and sometimes H element. Different forms of Calcium silicates with various chemical compositions were fabricated including $CaSiO_3$, Ca_2SiO_4, Ca_3SiO_5, and Ca_3Si_2O. The advantages of Calcium silicate materials such as bioactivity, biodegradability, high biocompatibility, high drug loading capacity, and pH-responsive drug release behavior increased their biological and biomedical applications [5, 6]. Calcium silicate ($CaSiO_3$) with a molar ratio of CaO: SiO_2 of 1:1 was synthesized *via* sol-gel method. The chicken eggshells were used as a starting material for the synthesis of calcium [7].

Cotton leafworm, *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae), is one of the most phytophagic damaging insects in Egypt. The high generative rate causes a damaging loss for many economic crops such as cotton, maize, potatoes, cereals, vegetables, and ornamental plants [8]. The larvae of *Spodoptera littoralis* are feeding on 40 plant families and more than 87 different plant species, as a result, the European and Mediterranean Plant Protection Organization (EPPO) listed it as an A2 quarantine insect pest [9]. Therefore, in this study chicken eggshells were used as an alternative precursor for preparing calcium-based materials (calcium carbonate (ECC), calcium oxide (ECO), and calcium silicate (ECS)). The prepared materials were applied in crop protection programs as inorganic insecticides for *Spodoptera littorals* control.

2. Materials and Methods

2.1 Chemicals

All chemicals were of the highest analytical grades used as received without any further purification from Sigma-Aldrich Co. Tetraethyl orthosilicate (TEOS) ≥99.0% (GC), Hydrochloric acid (HCL) 36%, Ethanol (EtOH) 96%, Triton™ X-100.

2.2 Materials preparation

The collected waste eggshells were washed with detergent and distilled water, dried at 80.0 °C overnight, and finally ground into a fine powder by using porcelain mortar. Eggshells powder was thermally treated at low temperature (300 ºC) to prepare calcium carbonate (ECC), while calcium oxide (ECO) was obtained at (800 ºC). A mixed solution of (10 ml TEOS, 40 ml EtOH, and 20 ml H_2O) was sonicated for 5 Min to complete the hydrolysis. After that, A 30 ml of 0.1 M $CaCL_2$ was prepared by dissolving an appropriate amount of ECO in concentrated HCL and added to silicate solution. All the solutions were prepared by using bi-distilled water and still under magnetic stirring for 4 hours at 50 ºC. The prepared calcium silicate was filtered, washed with water/ethanol three times, and then dried at 80 ºC for 12 hours, and finally calcined at 600 ºC for 6 hours to obtain ECS Scheme (1).

2.3 Materials characterization

Fourier transform-infrared (FT-IR) spectroscopy of calcium-based materials (ECC, ECO, and ECS) was recorded using the Bruker Alpha FTIR instrument. Scanning electron microscopy (SEM) model JEOL model 5400 LV was used to explore the morphology. Calcium-based material powders were ground 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 samples using anion sputtering (Hitachi E-1030) at room temperature. High-resolution SEM images were obtained by adjusting the acceleration voltage at 15 kV.

2.4 Insect rearing

Laboratory strain of cotton leaf worm (*Spodoptera littorals*) was reared under constant laboratory conditions in an incubator at 25 ± 2°C and 65 ± 5% relative humidity with 8 hour light: 16 hour darkness photoperiod in Plant Protection Research Institute, A.R.C., Dokki–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 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 25ºC till hatching [10].

2.5 Bioassay

Different concentrations of calcium-based materials (150, 300, 600, 900, and 1200 mg/L) were dispersed in water by using an ultrasonic cleaner for 10 minutes. Then, A 0.1% Triton X-100 was added to increase the leaves adhesion and stabilize the prepared formulations. The leaf discs of a castor-bean plant with equivalent sizes were washed and dipped into the prepared solutions. The treated and untreated leaves were introduced into a drying container containing equal numbers (20 larvae/replica) of new molting 2nd instar larvae of *Spodoptera littoralis* at a temperature of 25 ± 2°C, relative humidity of 65 ± 5%, and photoperiod of 8 h light/ 16 hours. The containers were maintained and covered with a muslin cloth to allow aeration, each treatment has three replicate.

2.6 Statistical analysis

The lethal concentration values of LC_{25} and LC_{50} were calculated using the probit analysis program [11]. The significant differences between the entomotoxic effects were calculated by using one-way ANOVA with post-hoc Tukey test by using SPSS 10.1 software at $p < 0.05$.

3. Results and discussion

3.1 Characterization of calcium-based materials

3.1.1 FT-IR analysis

FT-IR analysis was performed for ECC, ECO, and ECS samples (Fig.2a). The major absorption bands of ECC sample appeared at 1391 cm^{-1}, 865 cm^{-1} and 721 cm^{-1} which can be attributed to the presence of asymmetric stretch out-of-plane bend and in-plane bend vibration modes for $(CO_3)^{-2}$ [12]. While the absorption bans for ECO appeared at 1411 cm^{-1}, 870 cm^{-1} and 712 cm^{-1}. Thermal treatment for chicken eggshells at 800 ºC broken down the carbonate into CaO and the absorption bands of $(CO_3)^{-2}$ molecules migrated to higher energy corresponds to Ca–O bonds as represented at 665 cm^{-1}, 548 cm^{-1} and 477 cm^{-1}. Moreover the peak at 3644 cm^{-1} corresponding to OAH stretching vibration and bending hydroxyl groups present in $Ca(OH)^2$ [13]. Calcium silicate (ECS) showed characteristic absorption bands for the vibrational modes of the SiO_3 group that appear at 454 cm^{-1} for Si–O–Si and O–Si–O bonds, stretching modes of O–Si–O bonds at 795 cm^{-1} Si–O–Ca bonds. The symmetric stretching vibrations of Si–O–Si bonds and Si–O–Ca bonds were observed at 1065 cm^{-1} .The band at 3437 cm^{-1} is due to the absorption of moisture on the surface [14].

3.1.2 Scanning electron microscopy (SEM)

Surface morphology of prepared calcium-based materials (ECC, ECO, and ECS) was studied by scanning electron microscopy (SEM) as shown in (Fig.2b). Calcium carbonate (ECC) showed aggregated monolithic macroparticles with different sizes of 10.0 µm. Thermal treatment at high temperature for chicken eggshells at 800 ºC produces dense aggregated particles of calcium oxide (ECO) with particle size around 1.0 µm. SEM image of calcium silicate (ECS) showed highly dense aggregated sheets with different sizes less than 5.0 µm [14].

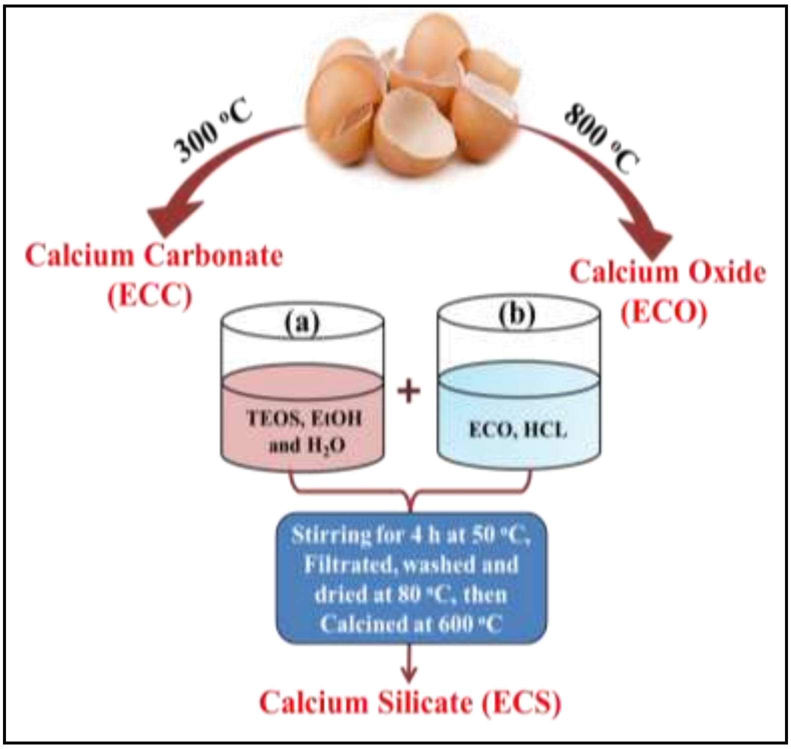
3.1.3 Entomotoxic effects of calcium-based materials

The entomotoxic effects of calcium-based material samples were estimated by using feeding bioassay method. The accumulative mortalities for 2nd instar larvae of *Spodoptera littorals* were calculated after 11 days of treatment. The mortality rates of the treated larvae showed a positive correlation with sample concentrations (Fig.2a). Lethal and sub-lethal concentrations (LC_{25}, LC_{50}) were calculated as recommended for pesticide formulations by using probit analysis program (Fig.2b) (Finney, 1971). Calcium carbonate (ECC) showed the lowest effect with LC_{25}= 282.88 mg/L and LC_{50}= 936.41 mg/L, while calcium silicate exhibited the highest effect with LC_{25}= 163.82 mg/L and LC_{50}= 517.00 mg/L. Calcium oxide (ECO) has moderate entomotoxic effect with LC_{25}= 227.91mg/L and LC_{50}= 685.01 mg/L as showed in (Tab.1). The mortality is attributed to the impairment of the digestive tract and surface enlargement of the integument as a consequence of dehydration or blockage of spiracles and tracheas. Such intensive damage sorption and abrasion might be due to the generation of reactive oxygen radicals in aqueous suspensions of calcium-based materials; the radicals stabilized as a surface-bound reactive oxygen species and then decay subsequently [15].

Table 1: Toxicity data of 2nd instar larvae of *Spodoptera littorals* treated with different concentrations of ECC, ECO, and ECS *via* feeding bioassay method after 11 days post-treatment.

Material	LC_{25}(mg/L) (95%CL)	LC_{50}(mg/L) (95%CL)	Slope ±SE	R	X^2	P
ECC	282.88 (198.19- 358.88)	936.41 (747.89-1292.39)	1.29 ± 0.19	0.991	0.93	0.82
ECO	227.91 (158.11-290.77)	685.01 (567.51-858.20)	1.41± 0.18	0.996	0.41	0.94
ECS	163.82 (103.07- 219.79)	517.00 (423.43- 633.12)	1.35± 0.18	0.996	0.42	0.95

CL Confidence Limits



Scheme 1: Schematic representation for the preparation of calcium-based materials derived from bio-west eggshells.

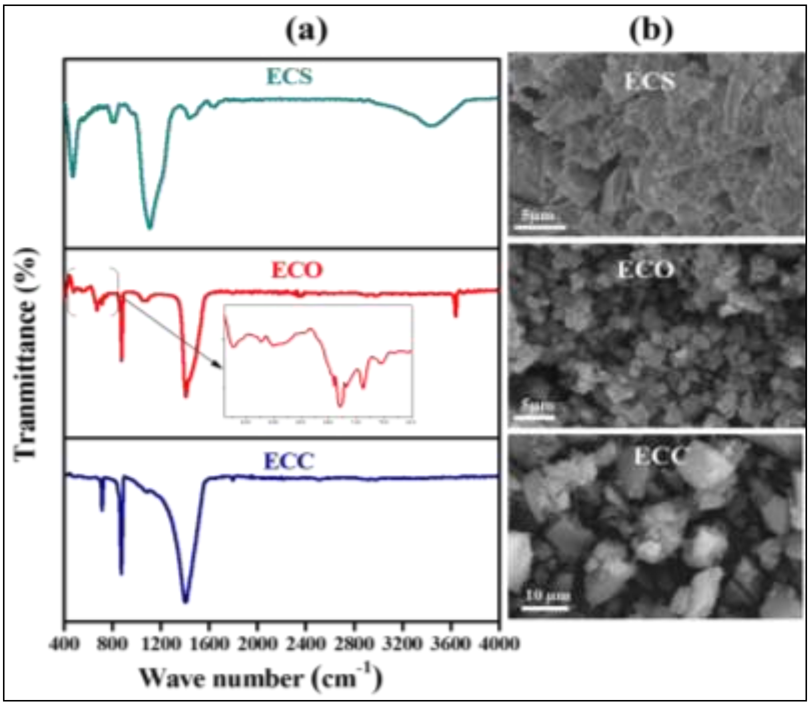


Fig 1: Fourier transform-infrared (FT-IR) spectroscopy (a), and (b) scanning electron microscopy (SEM) images of calcium-based materials (ECC, ECO, and ECS).

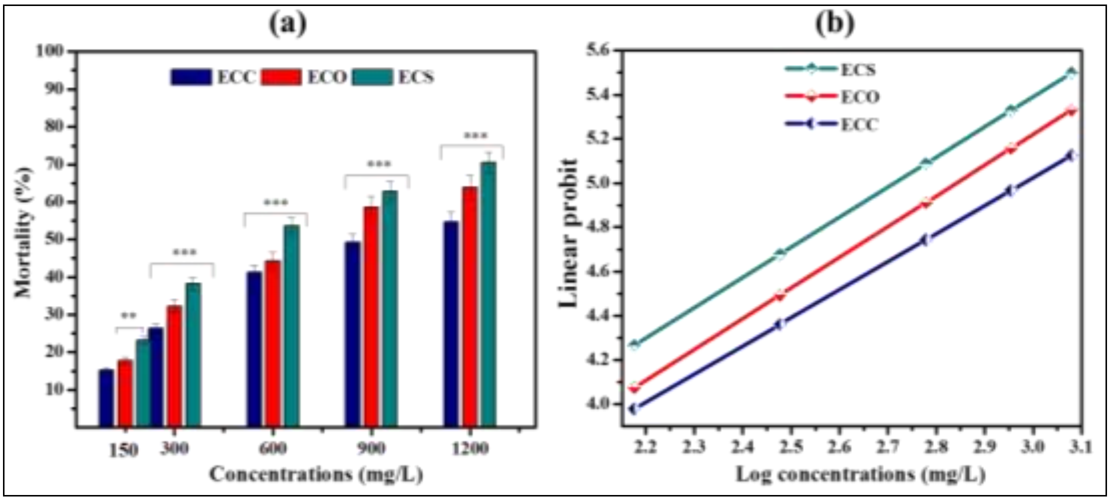


Fig 2: Accumulative mortalities (a),and toxicity lines (b) for 2nd instar larvae of *Spodoptera littorals* treated with different concentrations of calcium-based materials (ECC, ECO, and ECS). Data values are the mean of three independent replicates and vertical bars represent the standard error. [post-hoc Tukey test, ** only two materials are significant $P< 0.05$, *** the three materials are significant at $P< 0.05$].

4. Conclusions

Calcium-based materials (ECC, ECO, and ECS) were prepared by using chicken eggshells as an alternative bio-calcium source. The prepared materials were characterized by using fourier transform-infrared (FT-IR) spectroscopy and scanning electron microscopy (SEM). Their entomotoxic effects were estimated against 2nd instar larvae of *Spodoptera littorals* by using the feeding bioassay method. ECC showed the lowest effect with LC_{50}= 936.41, ECO has moderate entomotoxic effect LC_{50}= 685.01 mg/L, and ECS exhibited the highest effect LC_{50}= 517.00 mg/L. The application of calcium-based materials derived from waste eggshells provides an alternative insecticide for *Spodoptera littorals*, besides their role in reducing the pollutant effects on the environment.

5. References

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