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Entomotoxicity Assessment of Locally Synthesized Nano-pesticide Formulations for Cotton Leafworm Control

A Thesis

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List of abbreviations	
FAO	Food and Agriculture Organization
NPs	Nanoparticles
EPA	Environmental Protection Agency
EO	Essential oils
RSM	Response surface methodology
AIs	Active ingredients
EMB	Emamectin-benzoate
PVA	Polyvinyl alcohol
DEPA	Diethyl Phenylacetamide
PEG	Polyethylene glycol
PCA	poly citric acid
AMK	Alkaline modified kaolin
PMK	Phosphate modified kaolin
SLNs	Solid lipid nanoparticles
NLC	Nanostructured lipid carriers
SC	Suspension concentrate
SDS	Sodium 1-dodecanesulfonate
PVP	Polyvinylpyrrolidone
MOs	Metal oxide nanoparticles
MSNPs	Mesoporous silica nanoparticles
bPEI	branched polyethyleneimine
DQ	Diquat dibromide
SPACEs	Screen-printed carbon electrodes
ROS	Reactive oxygen species
MIC	Minimum inhibiting concentration
MBC	Minimum bactericidal concentration
BBSV	Broad bean strain virus
CB	Calcium borates
CBM-A	Calcium borate monolithic microblocks(Amorphous phase)
CBM-C	Calcium borate monolithic microblocks (Crystalline phase)
CBSU-A	Calcium borate sea urchin-like microsphere (Amorphous phase)
CBSU-C	Calcium borate sea urchin-like microsphere (Crystalline phase)
CBMU-A	Calcium borate mushroom-like structure (Amorphous phase)
CBMU-C	Calcium borate mushroom-like structure (Crystalline phase)
CS	Calcium silicates
NCS-Ag	nano-structured calcium silicate-silver
ECS	calcium silicate from eggshells
TEOS	tetraethyl orthosilicate
XRD	X-ray diffraction
FTIR	Fourier transform infrared spectrometry

List of abbreviations

SEM	*Scanning electron microscopy*
MDA	*Malondialdehyde*
TBA	*Thiobarbituric acid*
H-E	*Haematoxylin-Eosin*
SE	*Standard error*
ANOVA	*one-way analysis of variance*
IGRs	*Insect growth regulators*
CSI	*Chitin synthesis inhibitors*
OPs	*Organic phosphorus*

Abstract

Agricultural practices (i.e. fertilizers or pesticides) are anthropogenic sources of organic pollutants through the contamination of the local-point or diffused sources. Although, the necessity of these organic species in the food production, the inconsiderate use of these organic species caused harmful impacts for environment and human. Thus, developing of alternative agrochemicals has attracted much attention to overcome these agricultural and environmental challenges. Many researchers and agrochemical companies devote their interest to provide efficient and sustainable strategies for controlling insect pests. Application of nano-sized particles in the agriculture sector might provide an environmentally sustainable solution for the shortage of annually food production. Numerous nano-agrochemical substances were developed. However, the efficacy, safety and applicability of these nano-agrochemicals onsite is still challenge.

In this context, we synthesized SiO_2, CuO and CaO nanostructures for cotton leafworm *(Spodoptera littoralis)* control. Interestingly, the CuO showed a faster entomotoxic effect than CaO. However, CaO showed lower median lethal concentration (LC_{50}). Thus, calcium-based nanopesticides will be developed in the current thesis. Thank to calcium as an essential nutrient elements for plants, thus it could enhance the soil fertility. Firstly, calcium borate particles with different shapes including monolithic micro-blocks (M), sea urchin microspheres (SU) and mushroom (MU) -like were synthesized by facile hydrothermal routes. Amorphous phases of calcium borates of CBM-A, CBSU-A, and CBMU-A were obtained by thermal treatment at 500 ^oC, while the crystalline phases of CBM-C, CBSU-C, and CBMU-C were produced after the calcination at 800 ^oC. The crystalline phase of CBM-C, CBSU-C, and CBMU-C exhibited typical diffraction peaks of orthorhombic calcium meta-borate (CaB_2O_4) with a crystal size of 42.54 nm, 35.66 nm, and 37.28 nm, respectively.

The insecticidal activity of calcium borate samples was explored against *Spodoptera littoralis.* Feeding bioassay method was utilized for evaluating larvicidal efficacy after eleven days post-treatment, biological aspects, oxidative stress, and histopathological effects. The larvicidal efficacy based on LC_{50} values for CBM-A, CBSU-A, and CBMU-A were found to be 406.92, 207.05 and 188.63 mg/L, respectively. While, CBM-C, CBSU-C, and CBMU-C showed LC_{50s} of 532.31, 303.61 and 276.38 mg/L, respectively, which is higher than that of the amorphous samples. These results indicated that the particle size and crystallinity of calcium borate samples are the significant key factors for *Spodoptera littoralis* control which mainly regarding to the availability of borate ions. The investigation of the biological aspects of calcium borate materials in terms of weight, pupation and adult emergency revealed that their lethal concentrations have significant effects on pupa and adult stages similar to insect growth regulator insecticides (i.e. dimilin). Thus, the influence of calcium borate samples on histopathological changes of mid-gut and cuticle cross-sections will be carefully studied. Interestingly, the calcium borate samples did not significantly effect on mid-gut section but cause partial destroy of cuticle layer leading to losing the protective exoskeleton and insects quickly begin to lose water and eventually die *via* desiccation.

Next attention was turned to utilizing calcium-rich waste (i.e. chicken eggshells) for preparing new insecticides at low cost with great environmental impacts. Thus, large scale of calcium silicate material was synthesized *via* template-free sol-gel method. Highly dense aggregated sheets with varied sizes less than 5.0 μm of amorphous calcium silicate (ECS) were obtained. The larvicidal efficacy of ECS was investigated by feeding bioassay method against

Spodoptera littoralis. The ECS exhibited lower larvicidal activity (LC_{50} = 517 mg/L) compared with calcium borates. Although, the lower insecticidal activity of ECS, it contains the most important soil nutrients (Ca and Si elements) as well as derived from environmental waste.

On the other hand, mitigation the usage of organic pesticides *via* combination with the nanostructured materials will be interesting trend. The synergistic combinations of CBSU-A, and ECS with cholinesterase-inhibiting insecticides such as methomyl and chlorpyrifos were explored. The combination of CBSU-A with methomyl and chlorpyrifos increased the toxicity to 2.4 and 2.6-folds. While, the combination of ECS raised the toxicity for methomyl and chlorpyrifos by 2.7 and 2.8-fold, respectively. The CBSU-A, and ECS could rupture the cuticle layer and then allows the organic insecticides to penetrate inside the insect body. Such miscellaneous mode of action increases the insecticidal efficacy against *Spodoptera littoralis,* which sequentially reduces the intensive usage of highly toxic nervous system insecticides. In conclusion, the synthesized calcium borates and calcium silicate nanostructures can be employed as a multifunctional nanoagrochemical to boost various agricultural programs in terms of soil fertility and plant growth.