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Biological Preparation, Characterization of CaO Nanoparticles from Egg Shell Waste and Insecticidal Activity against Seed Weevil, *Sitophilus oryzae* L. in Maize

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Calcium oxide (CaO) nanoparticles was biologically synthesized by calcinating egg shells from kitchen waste and characterized using SEM, UV-Vis, XRD and FT-IR. Biosynthesized CaO nanoparticles are found to be an effective insecticide against seed weevil, *Sitophilus oryzae* in

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maize. The indiscriminate use of already available commercial pesticides has led to develop resistance among the pests and also occurrence of pesticide residues in produce. The SEM study revealed that the particles are granular in shape with a size of 189 nm. The diffraction peaks of XRD were observed at 2θ = 32.320, 37.48°, 48.40°, 53.96°, 64.18°, 67.52°and exhibited polycrystalline nature. In the FTIR spectrum, a broad band at 1410 cm^{-1}, a sign of a C-O bond which indicated the association between the oxygen atom of carbonate and the calcium atom. The insecticidal activity of CaO nanoparticles was assessed against *S. oryzae* by contact toxicity bioassay method. The outcomes of the bioassay studies showed that the lethal dose (LD_{50}) for maize seeds was 72.62 mg/100 g. and within seven days of exposure, 100% death of *S. oryzae* was attained at a dosage of 240 mg/100 gm of maize seeds. The current study concluded that biosynthesized CaO nanoparticles may be used as an effective management technique to successfully manage the *S. oryzae* under storage conditions as an alternative to chemical pesticides.

Keywords: CaO nanoparticles; storage pest; Sitophilus oryzae; contact toxicity.

1. INTRODUCTION

Maize (*Zea mays* L.) is one of the most versatile developing crops, with a wide range of adaptation under a variety of agro-climatic situations. Maize is renowned as the "Queen of Cereals" because it has the largest genetic yield potential compared to any grain. Maize is a tall, determinate, annual C4 plant that grows from 1 to >4 metre tall and has big, thin, opposing leaves that grow alternately down the length of a sturdy stem. To satisfy the human population's need for food, cereals are cultivated to greater extents. Among these, maize is regarded as a crucial crop because it provides both human food and animal feed. It is cultivated all over the world and widely used in the trade of coarse grains. In the global trade of coarse grains, maize is a key player. The biggest issue with maize cultivation is post-harvest loss, which results in a significant yield loss in terms of production and productivity. Post-harvest losses are caused by both biotic and abiotic causes.

The main cause of damage to the maize seeds is storage insect pests. Grain damage is caused by both the larva and the adult. Larvae eat inside the seed, hollowing it out, then exiting via a circular hole on the surface. Adults may cause damage on a variety of seeds by cutting a differently lined circular hole through which they feed on the kernel. Rice weevil, *S. oryzae* (L.), is a global insect-pest that causes 10-65% damage under moderate storage settings and 80% damage under prolonged storage conditions. In storage, more than 37 different species of insects cause harm [1]. The seed weevil, *Sitophilus oryzae* L. is the one that has the most qualitative and quantitative impact on the economy. The use of chemicals insecticides to eradicate the pest caused negative impacts on the ecosystem and the emergence of insect resistance. Botanicals are plant derived products possessing bio-active constituents involving in pest control. The extracts from plants are widely used as repellent, antifeedant and arrestant. They also have effect on insect growth either by affecting the oviposition or feeding behaviour. Common botanicals used in storage ecosystem are sweet flag [2], neem [3], pepper mint [4], sweet basil [5] and bael [6]; despite, botanicals lack immediate mode of action and mortality.

Recently, nanoparticles can be considered as an alternative for chemical insecticides, particularly under storage environments and also improve the germination of seed during storage. Padmasri et al. [7] observed that, at the end of nine months of storage, *Acorus calamus* rhizome powder @ 10 g kg-1 seed had the highest germination percentage (85.67), seedling vigour index (2354), minimum infestation (0.18%), and weight loss (0.2%). Padmasri et al. [8] Nano silica used at a dose of 500 ppm kg-1 exceeded all other treatments, resulting in 100% mortality one day after treatment. Mallavadhani et al. [9] observed that, after 14 days of treatment, aloin A at 0.0024 mg/cm2 shown 58.0% mortality against *S. oryzae*, followed by sample AV 13b (leaf peel) at 0.6 mg/cm2 (56.0%). El-Naggar et al. [10] found that, SiO_2-NPs can be used as a growth stimulant as well as a potent unconventional insecticide for crops during storage at a very low and safe dose. Nanotechnology is an emerging field of science with wide range of applications. It offers new methods for designing novel active ingredients with nanoscale dimensions, as well as their formulation and delivery that are collectively referred to as 'Nanopesticides'.

An interesting fact adding to their insecticidal property is that nanoparticles possess higher surface area to volume ratio [11]. Nanoparticles bring about insect mortality through physical mode of action even at very low concentration. Major advantages include nanoparticles bring quick mortality and eco-friendly too since it can be synthesized from green route, with minimum residues in the produce and wide scale adaptation. In addition to serving as a structural component of cell walls and membranes and regulating plant growth and development, calcium is a crucial macronutrient for plants. The structural integrity of stems that support flowers and fruit, as well as the quality of the generated fruit, are highly influenced by calcium availability. Additionally, calcium improves resistance to bacterial and viral infections. For treating the calcium deficit in groundnuts, calcium oxide nanoparticles (CaO-Nanoparticles) can be employed. Hence, an attempt has been made to prepare calcium oxides nanoparticle from egg shell waste to assess the insecticide against *S. oryzae.*

2. MATERIALS AND METHODS

2.1 Biological synthesis of CaO Nanoparticles

Egg shell waste were collected from kitchen and washed using tap water for 3-5 times to clean the shell and remove white membrane which is present inside the egg shell. Egg shells are then dried using hot air oven at 70°C for 2 hours and grinded using pestle and mortar to get fine powders rich in calcium carbonate. The egg shell powder is finally calcinated at 900°C for 4 hrs using muffle furnace to get CaO nanoparticles [12].

2.2 Characterisation of Nanoparticles

The size and surface morphology of the CaO nanoparticles was characterized using Scanning Electron Microscope (SEM). The presence of CaO nanoparticles were confirmed by absorbed specific wavelength of the nanoparticles using UV-Vis spectroscopy. The crystalline nature of the biologically synthesized CaO nanoparticle were observed by X-ray Diffractometer (XRD). The functional groups present in CaO nanoparticles were analysed using the Fourier Transform Infrared Spectrometer (FTIR).

2.3 Mass Culturing of Test Insects

S. oryzae weevils were collected from the infested grain products in godowns at the Department of Millets, Tamil Nadu Agricultural University (TNAU), Coimbatore. The weevils were raised on maize grains in a 27±3°C room environment. To ensure a constant supply of insects, subculturing was carried out at intervals of 15 to 20 days. For bioassay studies, uniform aged adults that were one week old were employed.

2.4 Toxicity Assessment of Test Materials against *S. oryzae*

Bioassay was conducted by filling each tiny plastic container with 100g of maize seeds. A control (without any treatment) was maintained while maize seeds were exposed to CaO Nanoparticles at various concentrations (1 mg, 5 mg, 30 mg, 50 mg, 60 mg, 80 mg, 120 mg, 140 mg, 160 mg, 200 mg and 240 mg) and insecticide treatment (Cypermethrin 0.25%) was used for comparison. To achieve equal dispersion, the containers were then manually shaken for about a minute [13] and then fifteen pairs of adults were placed in each container with four replications in a completely randomized design. The experimentation was done at 27°C and 70% RH and mortality was observed in every 24 hours for seven days (lack of movement and/or responsiveness to repeated probing). Abbott formula was used to calculate the corrected mortality. The observations on the mortality percentage were put through a probit analysis, and the LD50 value was calculated.

Corrected (%) mortality = (X - Y) / (100-X) x 100

X = Percentage mortality in test material treated treatments

Y = Percentage mortality in the untreated check

2.5 Statistical Analysis

Using AGRES statistical software, the data were analysed using a completely randomized design (CRD). Finney's approach (1971) was used to perform the probit regression analysis. Analysis of variance (ANOVA) was carried out to see if there are any treatments that differ significantly from one another.

3. RESULTS AND DISCUSSION

The size and surface morphology of the biologically synthesized CaO nanoparticles was observed by Scanning electron microscope (Fig. 1). The results are revealed that the particles are granular in shape with 189.0 nm in size. The results of the CaO molecules or atoms absorbing UV light, their electrons are stimulated to higher energy levels. In the present study, a sharp peak in UV-visible spectra at 320 nm suggested the presence of calcium oxide nanoparticle (Fig. 2). The diffraction peaks (Fig. 3) were observed at 2θ = 32.320, 37.48°, 48.40°, 53.96°, 64.18°, 67.52°. The CaO Nanoparticles' exhibited excellent polycrystalline nature which shown by the XRD pattern's sharp peaks and smaller spectrum width. Similarly, the FT-IR spectrum (Fig. 4) of the biosynthesized CaO nanoparticles revealed a broad band at 1410 cm^{-1}, a sign of a C-O bond that indicates an association between the oxygen atom of carbonate and the calcium atom. In addition, a sharp peak around 876 cm^{-1} corresponds to C-O bond denoting the carbonation of biosynthesized CaO nanoparticles. The results are in agreement with the findings of [14] and they have also prepared, characterized CaO nanoparticles from chicken egg shells.

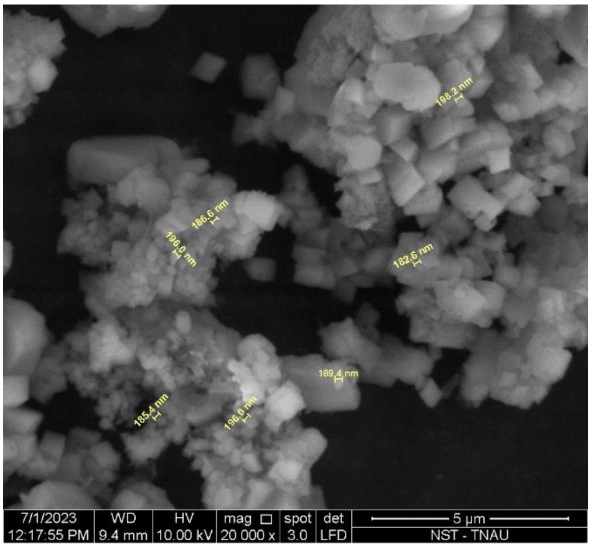

Fig. 1. SEM analysis of CaO nanoparticles

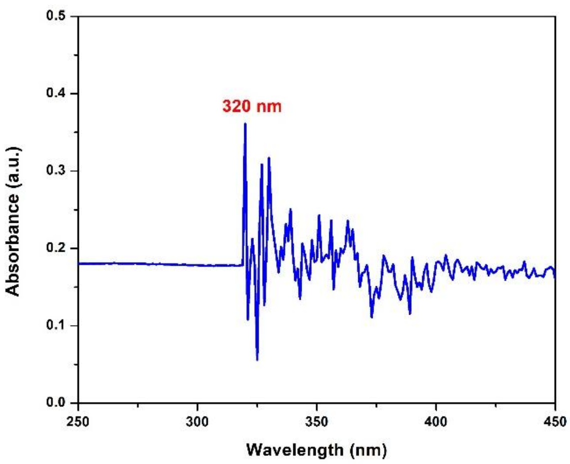

Fig. 2. UV-Vis spectra of CaO nanoparticles

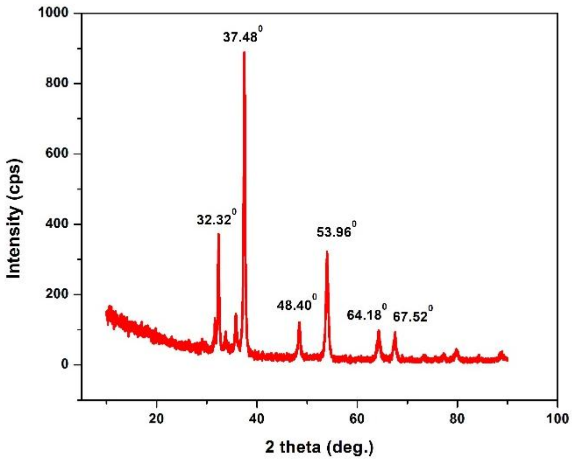

Fig. 3. XRD spectrum of CaO nanoparticles

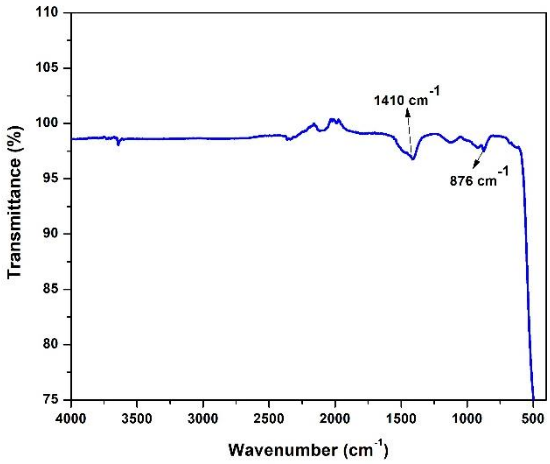

Fig. 4. FT-IR spectrum of CaO nanoparticles

3.1Toxicity Assessment against *S. oryzae*

The bioassay results demonstrated the entomotoxic potential of CaO Nanoparticles against *S. oryzae*. The LD_{50} and LD_{90} of CaO Nanoparticles (72.62 and 511.10 mg/100 g, respectively) and Cypermethrin 0.25%, (12.63 mg/100 g and LD_{90} was 543.56 mg/100 g) was determined by probit regression analysis (Table 1). The calculated chi-square value was lower than the tabular value, showing that the data fit the probit model exactly. In 100 g of seeds, 72.62 mg of CaO NP killed 50% of the insect population over the course of 7 days. When rice weevil comes into contact with CaO Nanoparticles treated seeds, Nanoparticles sticks on the surface. *viz.,* head, thorax, abdomen, and its appendages and causes mortality. The results are in consistent with the results of [15], where CaO Nanoparticles showed a slow entomotoxic effect against *Spodoptera littoralis* with LC_{50} of 129.03 mg/L after 11 days of post-treatment.

3.2 Toxicity Pattern of Test Materials against *S. oryzae*

The cumulative pattern of CaO Nanoparticles mortality against *S. oryzae* in maize seeds at various doses were revealed in Table 2. Generally, mortality increased with dose and time of exposure. Total mortality (%) observed on seven days after release in various doses *viz.,* 1.0 mg, 5.0 mg, 30 mg, 50 mg, 60 mg, 80 mg, 120 mg, 140 mg, 160 mg, 200 mg and 240 mg / 100 g of maize seeds (13.33, 20.00, 26.67, 33.33, 40.00, 53.33, 66.67, 73.33, 80.00, 93.33, 100.00 and 0.00, respectively). Bioassay results

Table 1. Probit regression analysis of mortality data against *S. oryzae*

S.No.	Particulars	LD_{50} mg/100 g of seeds (95 % fiducial limits)	LD_{90} mg/100 g of seeds (95 % fiducial limits)	Slope	χ2*	Degrees of freedom
1.	CaO Nanoparticles	72.62 (57.88-91.13)	511.10 (205.10-1273.62)	1.944	0.8221	5
2.	Cypermethrin 0.25%	12.63 (7.72-20.65)	543.56 (83.73-3528.49)	1.140	1.3942	5

***In each case χ^2 value from the goodness of fit test was less than the tabular value, ($p = 0.05$), indicating that the data fit the probit model*

Table 2. Insecticidal activity and toxicity pattern of biologically synthesized CaO nanoparticles against *S. oryzae*

S. No.	Dose (mg/100gof maize seeds)	Cumulative mortality (%)							Total mortality (%)
		1 DAR	2 DAR	3 DAR	4 DAR	5 DAR	6 DAR	7 DAR	
1	1	$0.00^{d}\pm0.00$	$0.00^{d}\pm0.00$	$0.00^{f}\pm0.00$	$0.00^{e}\pm0.00$	$6.67^{g}\pm0.06$	$6.67^{i}\pm0.10$	$13.33^{k}\pm0.26$	13.33
2	5	$0.00^{d}\pm0.00$	$0.00^{d}\pm0.00$	$0.00^{f}\pm0.00$	$6.67^{d}\pm0.07$	$13.33^{f}\pm0.25$	$13.33^{h}\pm0.04$	$20.00^{j}\pm0.26$	20.00
3	30	$0.00^{d}\pm0.00$	$0.00^{d}\pm0.00$	$0.00^{f}\pm0.00$	$6.67^{d}\pm0.12$	$13.33^{f}\pm0.10$	$20.00^{g}\pm0.16$	$26.67^{i}\pm0.01$	26.67
4	50	$0.00^{d}\pm0.00$	$0.00^{d}\pm0.00$	$6.67^{e}\pm0.15$	$13.33^{c}\pm0.26$	$20.00^{e}\pm0.42$	$26.67^{f}\pm0.32$	$33.33^{h}\pm0.45$	33.33
5	60	$0.00^{d}\pm0.00$	$0.00^{d}\pm0.00$	$6.67^{e}\pm0.16$	$13.33^{c}\pm0.06$	$20.00^{e}\pm0.39$	$33.33^{e}\pm0.43$	$40.00^{g}\pm0.94$	40.00
6	80	$6.67^{c}\pm0.02$	$6.67^{c}\pm0.09$	$6.67^{e}\pm0.04$	$13.33^{c}\pm0.05$	$26.67^{d}\pm0.36$	$40.00^{d}\pm0.33$	$53.33^{f}\pm0.11$	53.33
7	120	$0.00^{d}\pm0.00$	$6.67^{c}\pm0.05$	$20.00^{d}\pm0.07$	$40.00^{b}\pm0.02$	$53.33^{c}\pm1.28$	$60.00^{c}\pm1.50$	$66.67^{e}\pm1.67$	66.67
8	140	$0.00^{d}\pm0.00$	$6.67^{c}\pm0.17$	$26.67^{c}\pm0.04$	$40.00^{b}\pm0.92$	$53.33^{c}\pm0.31$	$60.00^{c}\pm0.53$	$73.33^{d}\pm1.37$	73.33
9	160	$13.33^{b}\pm0.26$	$20.00^{b}\pm0.44$	$26.67^{c}\pm0.04$	$40.00^{b}\pm0.75$	$53.33^{c}\pm0.17$	$60.00^{c}\pm0.00$	$80.00^{c}\pm0.21$	80.00
10	200	$13.33^{b}\pm0.03$	$20.00^{b}\pm0.26$	$33.33^{b}\pm0.03$	$53.33^{a}\pm1.08$	$60.67^{b}\pm1.04$	$80.00^{b}\pm0.62$	$93.33^{b}\pm0.58$	93.33
11	240	$20.00^{a}\pm0.32$	$33.33^{a}\pm0.61$	$40.00^{a}\pm0.27$	$53.33^{a}\pm0.69$	$73.33^{a}\pm1.34$	$86.67^{a}\pm0.05$	$100^{a}\pm0.31$	100
12	Control	$0.00^{d}\pm0.00$	$0.00^{d}\pm0.00$	$0.00^{f}\pm0.00$	$0.00^{e}\pm0.00$	$0.00^{h}\pm0.00$	$0.00^{j}\pm0.00$	$0.00^{l}\pm0.00$	0.00
	SE(d)	0.14	0.19	0.31	0.30	0.48	0.85	1.17	-
	CD (0.05)	0.40	0.4	0.65	0.63	1.00	1.77	2.43	-

Mean of 30 observations, Means followed by different letters within a column indicate significant differences ($P < 0.05$; LSD (Least significant difference test); DAR= Day(s) after insect release revealed the significant differences among the treatments (doses) (1.0 mg, 5.0 mg, 30 mg, 50 mg, 60 mg, 80 mg, 120 mg, 140 mg, 160 mg, 200 mg and 240 mg / 100 g of maize seeds). On first DAR (Day After Release), the highest mortality (20.00%) was obtained at the dose of 240 mg/100g followed by 13.33 (%) in 160 and 200 mg/100g and the per cent mortality increased in the consecutive days in the order of 73.33, 86.67, 100.00 at the highest dose of 240 mg/100g of maize seeds on 7DAR and lowest mortality was seen at the dose of 1 mg/100g and control.

The results are agreement with those of [16], who found that adults of *S. oryzae* were 90% more likely to die after exposure to ZnO nanoparticles at a concentration of 2 g/kg. Haroun et al. [17] reported similar outcomes as well. When insects (*S.oryzae*, *Callosobruchus maculatus*, and *Tribolium castaneum*) exposed to ZnO Nanoparticles, they observed increased toxicity, decreased enzyme activity (midgut -amylase, cysteine protease, -glucosidase, -glucosidase, and lipase), and a delay in the developmental period. The present study's findings are consistent with those of [18], who reported that 250 mg/kg of Ag Nanoparticles from aqueous extracts of *E. prostrata* leaves might result in more than 90% mortality. Additionally, the outcomes are consistent with those of [19], who found that mortality increased after the fourth day of exposure to Ag Nanoparticles against *S. oryzae*. Ag Nanoparticles made from curry leaves, according to [20], were also efficient against the pulse beetle (*Callosobruchus chinensis*) on soybean seeds and caused 100% mortality at a concentration of 70 ppm on 14 days after treatment.

4. CONCLUSION

NPs have a bright future and the potential to create safer and more effective pesticide formulations, which might lead to revolutionary advances in agriculture. In the future, nano particles will play a significant role in pest control at a cheap cost and with great efficiency. An essential inorganic substance known as calcium oxide (CaO) is employed in many different industries as a catalyst, toxic-waste remediation agent, adsorbent, etc. When applied topically to leaves, calcium oxide nanoparticles greatly accelerated germination and growth because they penetrate the phloem more deeply than their bulk counterparts. Biologically synthesized CaO nanoparticles also possessed higher insecticidal activity with LD_{50} dose of 72.62 mg/100 g of maize seeds. In the current study, we seek to utilize the benefits of nanostructured engineering surfaces that contribute to the effects of metal oxide nanostructures (CaO) for the creation of alternative and effective pesticides against rice weevil in maize seeds.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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