

1 **Biological preparation, characterization of CaO nanoparticles**  
2 **from egg shell waste and insecticidal activity against**  
3 **seed weevil, *Sitophilusoryzae* L. in maize**  
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8 **ABSTRACT**  
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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, *Sitophilusoryzae* in 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  $2\theta = 32.320, 37.48^\circ, 48.40^\circ, 53.96^\circ, 64.18^\circ, 67.52^\circ$  and exhibited polycrystalline nature. In the FTIR spectrum, a broad band at  $1410\text{ cm}^{-1}$ , 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 came to the conclusion 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.

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11 *Keywords: CaO nanoparticles, storage pest, Sitophilusoryzae, Contact toxicity.*  
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13 **1. INTRODUCTION**

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

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26 The main cause of damage to the maize seeds is storage insect pests. Grain damage is  
27 caused by both the larva and the adult. Larvae eat inside the seed, hollowing it out, then  
28 exiting via a circular hole on the surface. Adults may cause damage on a variety of seeds by  
29 cutting a differently lined circular hole through which they feed on the kernel. Rice weevil,  
30 *Sitophilusoryzae* (L.), is a global insect-pest that causes 10-65% damage under moderate  
31 storage settings and 80% damage under prolonged storage conditions. In storage, more  
32 than 37 different species of insects cause harm (Tadesse, 1997). The seed weevil,  
33 *Sitophilusoryzae* L. is the one that has the most qualitative and quantitative impact on the  
34 economy. The use of chemicals insecticides to eradicate the pest caused negative impacts

35 on the ecosystem and the emergence of insect resistance. Botanicals are plant derived  
36 products possessing bio-active constituents involving in pest control. The extracts from  
37 plants are widely used as repellent, antifeedant and arrestant. They also have effect on  
38 insect growth either by affecting the oviposition or feeding behaviour. Common botanicals  
39 used in storage ecosystem are sweet flag(Paneru, Le Patourel, & Kennedy, 1997),  
40 neem(Islam & Talukder, 2005), pepper mint(Lashgari et al., 2014), sweet basil(Mishra,  
41 Tripathi, & Tripathi, 2012) and bael(Kumar et al., 2008); despite, botanicals lack immediate  
42 mode of action and mortality.  
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44 Recently, nanoparticles can be considered as an alternative for chemical insecticides,  
45 particularly under storage environments and also improve the germination of seed during  
46 storage.(Padmasri et al., 2017)observed that, at the end of nine months of storage, *Acorus*  
47 *calamus* rhizome powder @ 10 g kg<sup>-1</sup> seed had the highest germination percentage (85.67),  
48 seedling vigour index (2354), minimum infestation (0.18%), and weight loss (0.2%).  
49 (Padmasri et al., 2018)Nano silica used at a dose of 500 ppm kg<sup>-1</sup> exceeded all other  
50 treatments, resulting in 100% mortality one day after treatment. (Mallavadhani et al., 2016)  
51 observed that, after 14 days of treatment, aloin A at 0.0024 mg/cm<sup>2</sup> shown 58.0% mortality  
52 against *S. oryzae*, followed by sample AV 13b (leaf peel) at 0.6 mg/cm<sup>2</sup> (56.0%). (El-Naggar  
53 et al., 2020) found that, SiO<sub>2</sub>-NPs can be used as a growth stimulant as well as a potent  
54 unconventional insecticide for crops during storage at a very low and safe  
55 dose.Nanotechnology is an emerging field of science with wide range of applications. It  
56 offers new methods for designing novel active ingredients with nanoscale dimensions, as  
57 well as their formulation and delivery that are collectively referred to as 'Nanopesticides'.

58 An interesting fact adding to their insecticidal property is that nanoparticles possess higher  
59 surface area to volume ratio(Debnath et al., 2011). Nanoparticles bring about insect mortality  
60 through physical mode of action even at very low concentration. Major advantages include  
61 nanoparticles bring quick mortality and eco-friendly too since it can be synthesized from  
62 green route, with minimum residues in the produce and wide scale adaptation. In addition to  
63 serving as a structural component of cell walls and membranes and regulating plant growth  
64 and development, calcium is a crucial macronutrient for plants. The structural integrity of  
65 stems that support flowers and fruit, as well as the quality of the generated fruit, are highly  
66 influenced by calcium availability. Additionally, calcium improves a plant's resistance to  
67 bacterial and viral infections. For treating the calcium deficit in groundnuts, calcium oxide  
68 nanoparticles (CaO-Nanoparticles) can be employed. Hence, an attempt has been made to  
69 prepare calcium oxides nanoparticle from egg shell waste to assess the insecticide against  
70 *S. oryzae*.

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## 72 **2. MATERIAL AND METHODS**

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### 74 **2.1 Biological synthesis of CaO Nanoparticles**

75 Egg shell waste were collected from kitchen. Collected egg shell waste was washed using  
76 tap water for 3-5 times to clean the shell and remove white membrane which is present  
77 inside the egg shell. Egg shells are then dried using hot air oven at 70°C for 2 hours and  
78 grinded using pestle and mortar to get fine powders rich in calcium carbonate. The egg shell  
79 powder is finally calcinated at 900°C for 4 hrs using muffle furnace to get CaO  
80 nanoparticles(Sree et al., 2020).

### 81 **2.2 Characterisation of nanoparticles**

82 The size and surface morphology of the CaO nanoparticles was characterized using  
83 Scanning Electron Microscope (SEM). The presence of CaO nanoparticles were confirmed

84 by absorbed specific wavelength of the nanoparticles using UV-Vis spectroscopy. The  
85 crystalline nature of the biologically synthesized CaO nanoparticle were observed by X-ray  
86 Diffractometer (XRD). The functional groups present in CaO nanoparticles were analysed  
87 using the Fourier Transform Infrared Spectrometer (FTIR).

## 88 **2.3 Mass culturing of test insects**

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

## 94 **2.4 Toxicity assessment of test materials against *S. oryzae***

95 Bioassay was conducted by filling each tiny plastic container with 100g of maize seeds. A  
96 control (without any treatment) was maintained while maize seeds were exposed to  
97 CaONanoparticles at various concentrations (1 mg, 5 mg, 30 mg, 50 mg, 60 mg, 80 mg, 120  
98 mg, 140 mg, 160 mg, 200 mg and 240 mg) and insecticide treatment (Cypermethrin 0.25%)  
99 was used for comparison. To achieve equal dispersion, the containers were then manually  
100 shaken for about a minute (Subramanyam & Roesli, 2000) and then fifteen pairs of adults  
101 were placed in each container with four replications in a completely randomized design. The  
102 experimentation was done at 27°C and 70% RH and mortality was observed in every 24  
103 hours for seven days (lack of movement and/or responsiveness to repeated probing). Abbott  
104 formula was used to calculate the corrected mortality. The observations on the mortality  
105 percentage were put through a probit analysis, and the LD50 value was calculated.

106 Corrected (%) mortality =  $(X - Y) / (100 - X) \times 100$

107 X = Percentage mortality in test material treated treatments

108 Y = Percentage mortality in the untreated check

## 109 **2.5 Statistical analysis**

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

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## 115 **3. RESULTS AND DISCUSSION**

116 The size and surface morphology of the biologically synthesized CaO nanoparticles was  
117 observed by Scanning electron microscope (figure 1). The results are revealed that the  
118 particles are granular in shape with 189.0 nm in size. The results of the CaO molecules or  
119 atoms absorbing UV light, their electrons are stimulated to higher energy levels. In the  
120 present study, a sharp peak in UV-visible spectra at 320 nm suggested the presence of  
121 calcium oxide nanoparticle (Figure 2). The diffraction peaks (Figure 3) were observed at  $2\theta =$   
122 32.320, 37.48°, 48.40°, 53.96°, 64.18°, 67.52°. The CaONanoparticles' exhibited excellent  
123 polycrystalline nature which shown by the XRD pattern's sharp peaks and smaller spectrum  
124 width. Similarly, the FT-IR spectrum (Figure 4) of the biosynthesized CaO nanoparticles  
125 revealed a broad band at 1410 cm<sup>-1</sup>, a sign of a C-O bond that indicates an association  
126 between the oxygen atom of carbonate and the calcium atom. In addition, a sharp peak  
127 around 876 cm<sup>-1</sup> corresponds to C-O bond denoting the carbonation of biosynthesized CaO  
128 nanoparticles. The results are in agreement with the findings of (Pasupathy & Rajamanickam,  
129 2019) and they have also prepared, characterized CaO nanoparticles from chicken egg  
130 shells.

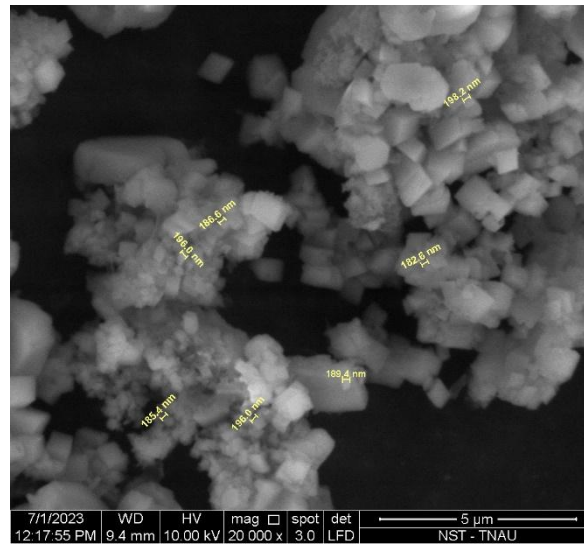


Figure 1: SEM analysis of CaO nanoparticles

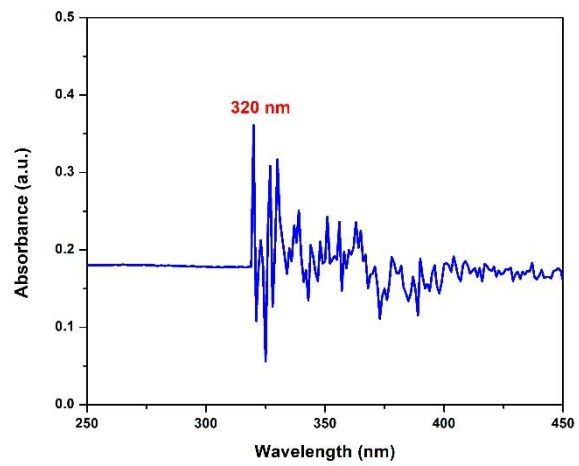


Figure 2: UV-Vis spectra of CaO nanoparticles

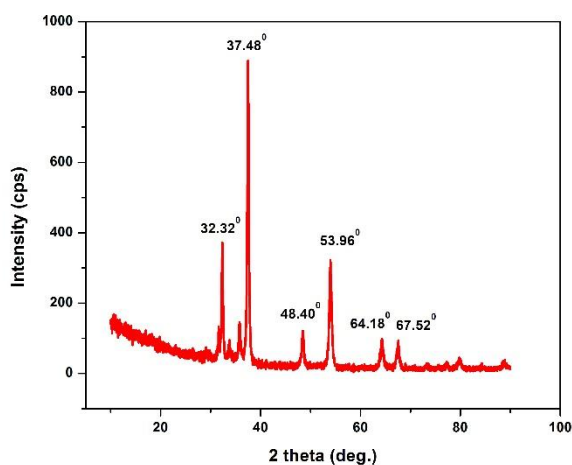


Figure 3: XRD spectrum of CaO nanoparticles

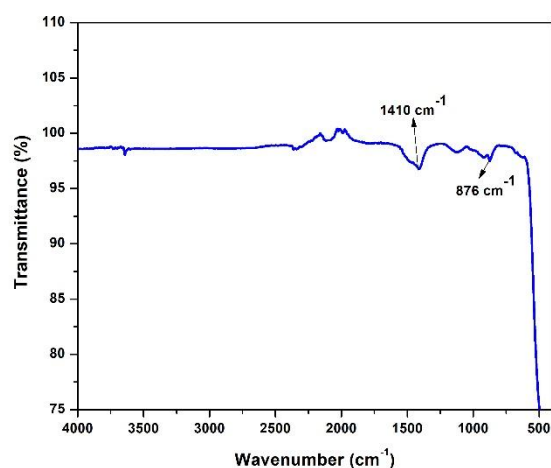


Figure 4: FT-IR spectrum of CaO nanoparticles

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### 3.1 Toxicity assessment against *S. oryzae*

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The bioassay results demonstrated the entomotoxic potential of CaONanoparticles against *S. oryzae*. The LD<sub>50</sub> and LD<sub>90</sub> of CaONanoparticles (72.62 and 511.10 mg/100 g, respectively) and Cypermethrin 0.25%, (12.63 mg/100 g and LD<sub>90</sub> 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 CaONanoparticles 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(Ayoub et al., 2018), where CaONanoparticles



1	1	0.00 <sup>d</sup> ± 0.00	0.00 <sup>d</sup> ± 0.00	0.00 <sup>f</sup> ± 0.00	0.00 <sup>e</sup> ± 0.00	6.67 <sup>g</sup> ± 0.06	6.67 <sup>i</sup> ± 0.10	13.33 <sup>k</sup> ±0.26	13.33
2	5	0.00 <sup>d</sup> ± 0.00	0.00 <sup>d</sup> ± 0.00	0.00 <sup>f</sup> ± 0.00	6.67 <sup>d</sup> ± 0.07	13.33 <sup>f</sup> ±0.25	13.33 <sup>h</sup> ±0.04	20.00 <sup>j</sup> ±0.26	20.00
3	30	0.00 <sup>d</sup> ± 0.00	0.00 <sup>d</sup> ± 0.00	0.00 <sup>f</sup> ± 0.00	6.67 <sup>d</sup> ± 0.12	13.33 <sup>f</sup> ±0.10	20.00 <sup>g</sup> ±0.16	26.67 <sup>i</sup> ±0.01	26.67
4	50	0.00 <sup>d</sup> ±0.00	0.00 <sup>d</sup> ± 0.00	6.67 <sup>e</sup> ± 0.15	13.33 <sup>c</sup> ±0.26	20.00 <sup>e</sup> ±0.42	26.67 <sup>f</sup> ±0.32	33.33 <sup>h</sup> ±0.45	33.33
5	60	0.00 <sup>d</sup> ±0.00	0.00 <sup>d</sup> ± 0.00	6.67 <sup>e</sup> ± 0.16	13.33 <sup>c</sup> ±0.06	20.00 <sup>e</sup> ±0.39	33.33 <sup>e</sup> ±0.43	40.00 <sup>g</sup> ±0.94	40.00
6	80	6.67 <sup>c</sup> ± 0.02	6.67 <sup>c</sup> ± 0.09	6.67 <sup>e</sup> ± 0.04	13.33 <sup>c</sup> ±0.05	26.67 <sup>d</sup> ±0.36	40.00 <sup>d</sup> ±0.33	53.33 <sup>f</sup> ±0.11	53.33
7	120	0.00 <sup>d</sup> ± 0.00	6.67 <sup>c</sup> ± 0.05	20.00 <sup>d</sup> ±0.07	40.00 <sup>b</sup> ±0.02	53.33 <sup>c</sup> ±1.28	60.00 <sup>c</sup> ±1.50	66.67 <sup>e</sup> ±1.67	66.67
8	140	0.00 <sup>d</sup> ± 0.00	6.67 <sup>c</sup> ± 0.17	26.67 <sup>c</sup> ±0.04	40.00 <sup>b</sup> ±0.92	53.33 <sup>c</sup> ±0.31	60.00 <sup>c</sup> ±0.53	73.33 <sup>d</sup> ±1.37	73.33
9	160	13.33 <sup>b</sup> ±0.26	20.00 <sup>b</sup> ±0.44	26.67 <sup>c</sup> ±0.04	40.00 <sup>b</sup> ±0.75	53.33 <sup>c</sup> ±0.17	60.00 <sup>c</sup> ±0.00	80.00 <sup>c</sup> ±0.21	80.00
10	200	13.33 <sup>b</sup> ±0.03	20.00 <sup>b</sup> ±0.26	33.33 <sup>b</sup> ±0.03	53.33 <sup>a</sup> ±1.08	60.67 <sup>b</sup> ±1.04	80.00 <sup>b</sup> ±0.62	93.33 <sup>b</sup> ±0.58	93.33
11	240	20.00 <sup>a</sup> ±0.32	33.33 <sup>a</sup> ±0.61	40.00 <sup>a</sup> ±0.27	53.33 <sup>a</sup> ±0.69	73.33 <sup>a</sup> ±1.34	86.67 <sup>a</sup> ±0.05	100 <sup>a</sup> ± 0.31	100
12	Control	0.00 <sup>d</sup> ± 0.00	0.00 <sup>d</sup> ± 0.00	0.00 <sup>f</sup> ± 0.00	0.00 <sup>e</sup> ± 0.00	0.00 <sup>h</sup> ± 0.00	0.00 <sup>j</sup> ± 0.00	0.00 <sup>l</sup> ± 0.00	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	-

180 Mean of 30 observations, Means followed by different letters within a column indicate  
181 significant differences ( $P < 0.05$ ; LSD (Least significant difference test); DAR= Day(s) after  
182 insect release

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#### 4. CONCLUSION

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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.

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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.

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Biologically synthesized CaO nanoparticles also possessed higher insecticidal activity with

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193 LD<sub>50</sub> dose of 72.62 mg/100 g of maize seeds. In the current study, we seek to utilize the  
194 benefits of nanostructured engineering surfaces that contribute to the effects of metal oxide  
195 nanostructures (CaO) for the creation of alternative and effective pesticides against rice  
196 weevil in maize seeds.  
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