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EFFECT OF GREEN SYNTHESIZED ZNONPS USING *CALPURNIA AUREA*(AIT.) BENTH. (CEEKAA) LEAF EXTRACT AGAINST MAIZE WEEVIL, *SITOPHILUS ZEAMAI*S MOSTCH (COLEOPTERA: CURCULIONIDAE)

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ABSTRACT

It has been considered a green technique to synthesize nanoparticles using leaf extract. In this study, the potential for preparing Zinc Oxide Nanoparticles (ZnO NPs) using a leaf extract from Ceekaa, *Calpurnia aurea* (Ait.) Benth. The source of zinc was Zinc Nitrate Hexahydrate ($Zn(NO_3)_2 \cdot 6H_2O$). UV-Vis spectroscopy, X-ray diffraction, scanning electron microscopy, and photoluminescence (PL) were used in the characterization of investigation. The synthesis of ZnO NPs is shown by the surface plasmon resonance at 336 nm from the UV-Vis qualitative evaluation result. The morphology of nanoparticles is shown in the XRD result, corroborated by SEM results, and as a result, the ZnO NPs scale was anticipated. ZnONPS were developed to work against *Sitophilus zeamais* adults. The 14-day mortality count showed that all 3 dosages (0.2g, 0.4g, and 0.6g) of pesticide were effective against the test insect. Compared to the untreated check, the appearance of F1 progeny was significantly reduced. The use of the treatment led to the effective germination of maize seeds.

Key words: *Calpurnia aurea*, ZnOPs, Seed germination, Maize weevil, *Sotophilus zeamais*

Introduction

Maize, wheat, millets, rice, and other cereal crops are the most widely grown worldwide (Awika, 2011; Ye and Fan, 2021). Because they are high in proteins, carbohydrates, fats, vitamins, minerals, and oils and these crops are an important part of our diet (Sarwar et al., 2013; Oso and Ashafa, 2021). Because of the presence of certain nutritional components, the grains of these crops are susceptible to insect pest infestation during storage. In extreme cases, losses owing to pest infestation in storage might reach 50 to 60% (Kumar et al., 2017; Luo et al., 2020). Post harvest losses can sometimes outweigh crop losses in the field (Mesterházy et al., 2020). Direct losses include direct consumption of kernels, whereas indirect losses include the creation of webbing, exuviae, frass, and insect cadavers, all of which drastically reduce seed quality and render grains unfit for human consumption (Kumar and Kalita., 2017; Mesterházy et al., 2020).

Storage insect pest species inflicting quantitative and qualitative losses on grains primarily belong to two insect orders: Coleoptera (about 600 species), Lepidoptera (70 species), and mites (approximately 355 species) (Rajendran, 2002; Rajendran and Sriranjini, 2008). Insect pests of stored grains are classified into two types: primary and secondary pests (Banga et al., 2020). Primary pests cause harm to sound or whole grains, whereas secondary pests cause damage to broken or previously damaged grains. Primary pests are divided into internal and exterior

feeders based on where they attack (Deshwal et al., 2020). There are many ways to control insects, including physical, mechanical, chemical, and biological methods. However, fumigation is the most widely used approach because it can be used in a wide range of storage environments, including bags, silos, warehouses, and mills (Nguyen et al., 2015; Nayak et al., 2020). However, there are commercially available synthetic pesticides for the control of storage pests; however, these pesticides are expensive, ineffective, and hazardous to both human and environmental health (Jallow et al., 2017; Poudel et al., 2020).

Innovative nanotechnology has paved the door for the creation of nano-sized formulations with lower residual toxicity and greater environmental friendliness for pest management (Yadav et al., 2021). The physical properties of these chemically modifiable particles with large surface-to-volume ratio enable them to target pest organism (Madhuri et al., 2010; Mustafa and Hussein, 2020; Sahoo et al., 2021).

Nanomaterials may therefore aid in the development of novel pesticides and insecticides. It is anticipated that nanopesticides will allow for more efficient insect pest control with fewer doses and application times. The use and manufacturing of hazardous materials can be decreased with the help of green nanomaterial synthesis. According to Pulit-prociak et al. (2016), zinc oxide has a history as a food and feed ingredient. The toxicity of zinc oxide (ZnO) and aluminum

oxide (Al_2O_3) nanoparticles against adults of *Sitophilus oryzae* (Linnaeus), *Sitotroga cerealella* (Olivier), and *Tribolium castaneum* (Herbst) was assessed by Keratum et al. (2015), Salem et al. (2015), and Ibrahim et al. (2022). According to their findings, both nanomaterials had a moderate to strong harmful effect on the studied insects and dramatically reduced the number of offspring produced. The current study aims to evaluate the toxicity of green synthesized ZnONPs using leaf extracts of *Calpurnia aurea* against *Sitophilus zeamais* under laboratory conditions.

2. Materials and Methods

2.1. Materials

Calpurnia aurea (ceekaa) leaves were collected from around Dambi Dollo University campus, Zinc nitrate hexahydrate ($\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$, 99%, 297.49 M.W, AR grade) was purchased from Yeshadam Trading PLC, Addis Ababa, Ethiopia and distilled water.

2.2. Preparation of Plant Extract

Leaf extract of *Calpurnia aurea* was prepared. Briefly, 40-50g of leaves were collected and washed using distilled water and air dried and fine powdered 10g was added to 100 ml distilled water and macerated. After 72 hours, the extracts were filtered with filter paper Whatman No. 1.

2.3. Green Synthesis of ZnO NPs

Following the procedures of Ibrahim et al. (2022), ZnO NPs were produced by combining a previously prepared plant extract in a 1:1 ratio with 1M zinc nitrate hexahydrate ($\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$) and vigorously agitating the mixture for two

hours to produce a white precipitate. The precipitate was dried at 300°C overnight after being centrifuged at 3000rpm for 15 minutes to yield the powder. After that, the samples kept for additional characterization and toxicity investigations.

2.4. Characterization of zinc oxide nanoparticles

Structural, optical and morphological studies of these nanocomposites were carried out using Ultraviolet-visible spectroscopy (UV-vis), scanning electron microscope (SEM), X-ray diffraction (XRD) and Photoluminescence (PL) spectrum of the nanoparticles.

2.5. Insecticidal effect of synthesized ZnONPs

Twenty gram of Limu variety maize grains was added to 3 glass jar of 500 ml and 3 levels of ZnONPs (0.2, 0.4 and 0.6 g) were added to each jar and mixed. To each jar, 10 unsexed adult maize weevils were introduced. The experiment was replicated thrice with completely randomized design and kept under $23 \pm 3^\circ\text{C}$ and $64.8 \pm 9\%$ RH laboratory conditions for 14 days.

2.6. Effect of ZnONPs on F1 progeny emergence and seed germination

Mortality count of test insect was carried out at day 2, 7 and 14 days after treatment application. After 14 days, all dead and live maize weevils were removed and the jar containing the grains were kept for additional 14 days for F1 progeny emergence. After F1 progeny emergence count, seed germination test was carried out by taking 5 maize seeds randomly from

each jar and placed on moistened filter paper with 10 ml distilled water in petri dish of 9 cm diameter that replicated 3 times. Germinated seeds were counted after four days.

2.7. Data Analysis

Obtained data on maize weevil mortality, F1 progeny emergence and seed germinations were analyzed using one-way ANOVA and the significance level was set at $P < 0.05$. The data was analyzed using IBM SPSS (version 25). The data obtained from characterization of ZnONPs were analyzed using ORIGINPRO Graphing and Analysis 2022.

3. Results and Discussion

3.1. Characterization of green synthesized ZnO nanoparticle

3.1.1. X-Ray Diffraction Analysis

The Zinc Oxide nano-composites' phases as well as crystallinity are confirmed by bagging their X-ray diffraction patterns. Fig.1 showed the X-Ray Diffraction graph for the Zinc Oxide Nanoparticle that was gained. For the hexagonal wurtzites Zinc Oxide phases (JCPDS cards: 36-1451 (Mydeen et al., 2020). A certain line expansion of the X-ray diffraction peaks specifies that the synthesized material consist of particles in nanometer scale ranges. Using XRD graph analysis, it is determined peaks intensities, positions and width, full-width at half-maximum (FWHM)

datas. The diffracted peaks positioned at $2\theta = 27.50038^\circ$, 28.43905° , 43.74373° , 45.53062° , 53.71939° and 72.97486° have been intensely indexed as hexagonal wurtzites phases of Zinc Oxide and further it also Intensity (a.u.) as well as 2θ (degrees) (111), (101), (110), (100) and (112) approves the prepared nano powder was free of impurity as it didn't contains any physical characteristics X-ray diffraction peaks other than Zinc Oxide peak. The prepared Zinc Oxide nanoparticle size was designed using Debye Scherer equation (Aklilu and Aderaw, 2022).

$$D(nm) = \frac{K\lambda}{\beta \cos\theta}$$

(1)

where 0.91 is Scherrer's constant, λ (0.154nm) is the wave length of X-rays, θ is peak position, and β stands for the full width at half-maximum (FWHM) of the diffraction peaks matching to plane (111), (101), (110), (100) and (112). The average particle size of the sample was found to be 4.13 nm which is resulting from the full width at half-maximum (FWHM) of more powerful peak agreeing to (111), (101), (110), (100) and (112) planes located at 27.50038° , 28.43905° , 43.74373° , 45.53062° , 53.71939° and 72.97486° using Scherer's formula. Similar works reported with present study confirmed that the prepared sample is suitable for insecticidal activity (Umar et al., 2023; Azizi et al., 2017; Saka et al., 2022).

Table 1. Crystalline parameters calculated from XRD data along with scherer formula

2θ	FWHM	D(nm)	Average crystalline size(nm)
28.50038	0.96112	8.025648	4.134466
29.43905	6.00308	1.282323	
43.74373	21.14637	0.348503	
45.53062	1.07498	6.811808	
53.71939	1.61503	4.386583	
72.97486	1.61557	3.95193	

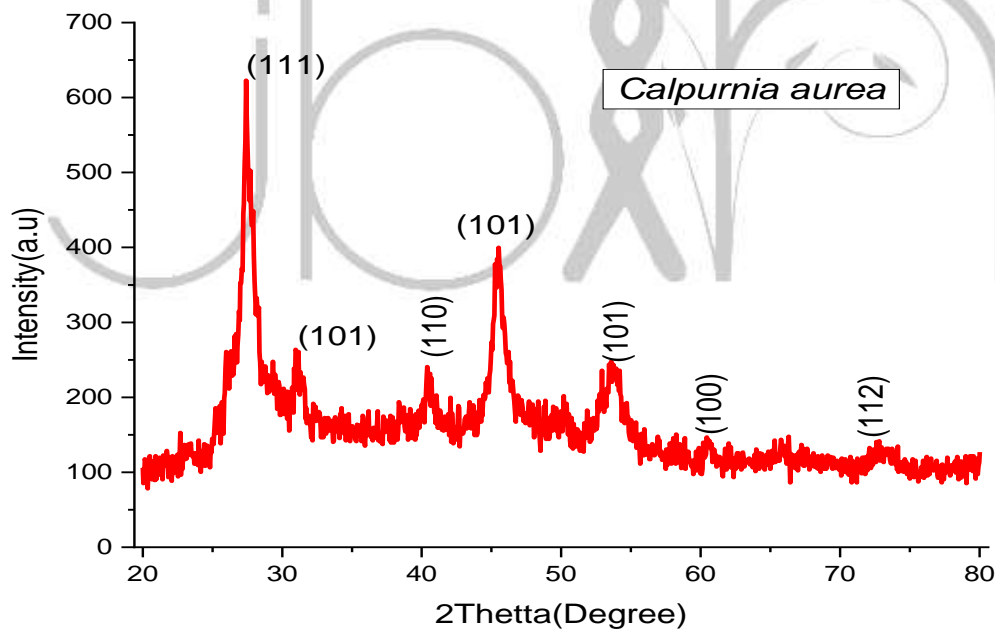


FIGURE 1. XRD graph of ZnO Nanoparticle synthesized by using *Calpurnia aurea* leaf extracts

3.1.2. Surface Morphological Analysis

Scanning Electron Microscope (SEM) was prepared to probe the samples and the data were utilized to study the superficial

landscape and organizations of particles in the biosynthesized ZnO Nanoparticles. The surface morphology of biosynthesized ZnO Nanoparticles confirmed cubic cluster like

structure with agglomerations. SEM studies under 20000 x magnifications (Figure 2 of the biosynthesized ZnO Nanoparticle reveal comparatively random sphere-shaped shapes of nanoparticle as well as average crystalline size of 4.13nm which is very effective in insecticidal activity. Correspondingly, they demonstrate random sphere like shaped particle, which is similar to those discovered in the beforehand reported literatures (Awan et al., 2023). The SEM micrograph displays micro meter and nano meter sized particles of ZnO Nanoparticles. The scanning electron micrograph image tells no snaps even in the glance at of greater

area. For the ZnO NPs, arbitrarily concerned with cubic cluster like structures are showed all over surface (El-Naggar et al., 2022). Due to the oxidations of Zn atoms, the extraordinary annealing temperatures support a rapid and grain free development of ZnO crystalline. In interesting way, the structures of ZnO don't get improved its consistencies with the XRD analyses. The gained values are initiated to be in virtuous agreement with the anticipated stoichiometry ratios. The SEM consequences cares that the equipped ZnO Nanoparticles through green method has an important role in the anti-insecticidal (Dinga et al., 2022).

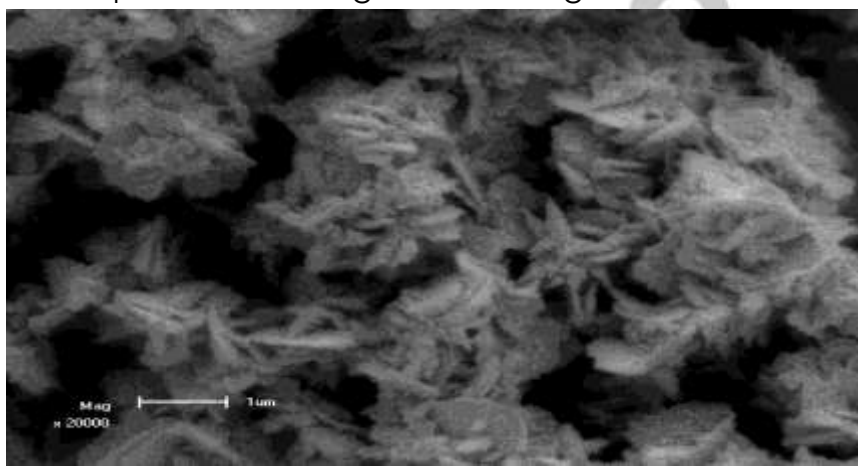


Figure 2. SEM images of green synthesized ZnO NPs by using *Calpurnia aurea* leaf extract under 20000X magnification

3.1.3. UV-VIS Spectroscopy Analysis

The UV-visible absorption spectrum of ZnO NPs between 200 and 800 nm was analyzed.

The Fig. 4 demonstrates the absorption spectrum of the synthesized ZnO Nanoparticles from the leaf extracts. In the UV-Vis spectrum ranges, the ZnO nano particle shows a good absorption around the value of found at 336nm (Fig. 3) in the

UV-Vis absorption spectrums. The peak specified an already recorded superficial Plasmon resonances for different metallic nano particles from 2- 100 nm in sizes (Nour et al., 2022). Therefore, the existence of peak at 336 nm shown productions of ZnO nanoparticles by UV analyzes. A sole surface Plasmon resonances band indicates the improvement of a sphere-shaped Nanoparticle, whereas the

occurrence of twofold or extra bands signifies the synthesis of different sized and shaped nanoparticles. A bluish shift in absorptions verge is evidently observable in the absorption spectrums of the biosynthesized ZnO Nanoparticles. These values are allocated to the absorptions of characteristic band-width deviation. This peak can be described via the transitions of valence band electrons to conduction bands (Lahbib et al., 2023).The optical energy band gap if the green synthesized ZnO nanoparticles can be calculated using the UV-Vis spectrums, from using Tauc relation. The equation (2) below is known as Tauc relation.

$$(aE_{\text{photon}})^n = K(E_{\text{photon}} - E_g)^n \quad \text{-----} \quad (2)$$

Here, a represents the co-efficient of absorption, which is dividing absorbance by thickness of the particle, $E_{\text{photon}} = h\nu$ which is the energy of photon and K is a constant. Replacing all these variables we

can get the energy gap E_g (Kaviti and Akkala, 2023).The Tauc plots were used to define the energy gap of biosynthesized ZnO nanoparticles. The Fig. 3(a) shows the plot of absorbance against wave length and Fig.3(b) shows $(ah\nu)^n$ against $E(h\nu)$. When $n = 2$ specifies directly allowable transition and agreeing intercept on Energy ($h\nu$) axis provide the energy band gaps values. From the extrapolations of the honest line portions of the curves on to the x-axis, the band gap value was found to be 2.8eV for the prepared ZnO nanoparticles. The resulted value of energy band gap is very much agrees well investigated reports by (Hosseinzadeh, 2022; Akash et al., 2022). The energy gap (E_g) value of Zinc Oxide nanoparticles were obtained to get reduced through green synthesize methods, which is very suitable for different applications like insecticidal and antimicrobial (Kaur et al., 2023; Tryfon et al., 2023).

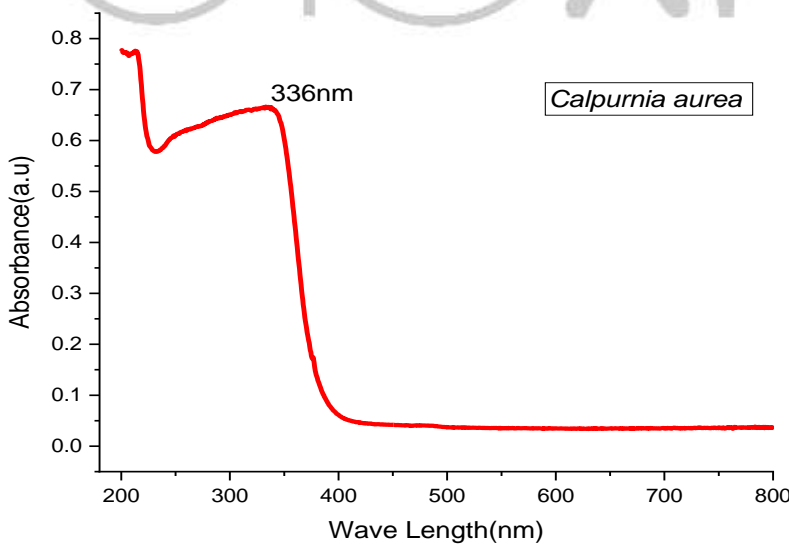


Figure 3a. UV-Vis spectrum of ZnO nanoparticles prepared by *Calpurnia aurea* leaf extract, inset: Tauc plot of the same UV-Vis spectrum

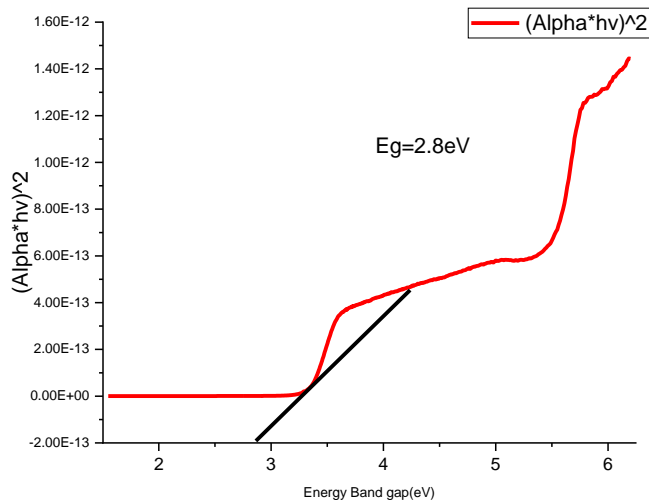


Figure 3b. Energy band gap of ZnO nanoparticles prepared by *Calpurnia aurea* leaf extract, inset: Tauc plot of the same UV–Vis spectrum

3.1.4. Photoluminescence (PL) Spectroscopy Analysis

PL analyses are one of the further most inescapable approaches to investigate the structural imperfections in the prepared samples such as vacancy as well as special effects because of reduced sizes. Figure 4 showed the photoluminescence spectra of the synthesized ZnO NPs from *Calpurnia aurea* leaf extracts. The UV emission peak was observed at 336 nm. This nearest band edge emissions in the nanoparticle synthesized is because of the recombination occurred straight from

exciton to exciton dusting. This could be attributed to the internal transitions of exciton's from the conduction bands to the valence bands. The peaks of emissions of the Zinc Oxide Nanoparticle perform at 414, 435nm (Ramesh *et al.*, 2023). This is because of the formation of oxygen vacancy in the prepared material. This oxidation vacancy remains accountable; growing the defect levels in the crystals, thereby attractive the visible-emission spectrums. For biosynthesized ZnO Nanoparticle, the peaks of emissions were observed. The obtained results agree with recently reported works (Saha *et al.*, 2022).

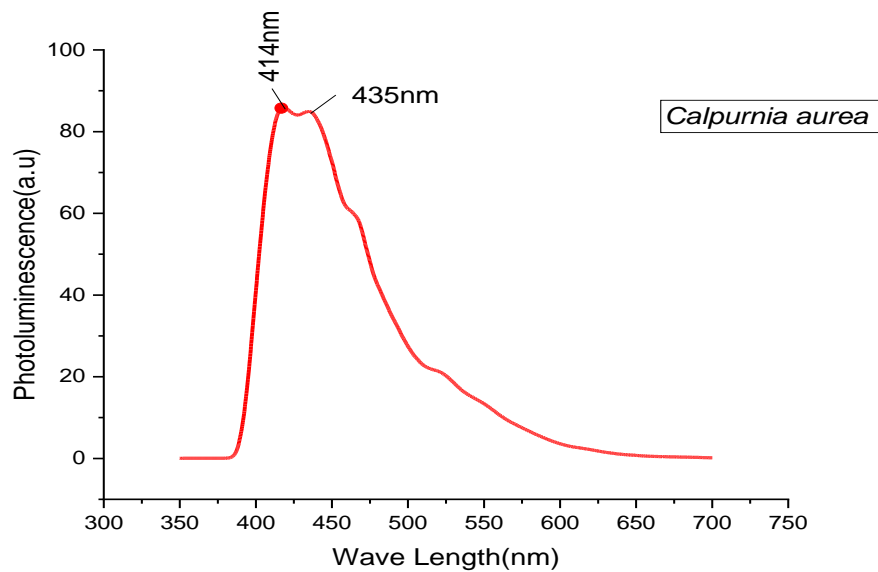


Figure 4. Photoluminescence spectrum of green synthesized ZnO nanoparticle using leaf extract of *Calpurnia aurea*.

3.2. Insecticidal effect of synthesized ZnONPs

Table 2's summary of the toxicity experiment's findings revealed that there was no statistically significant difference ($P > 0.05$) between the three concentrations and that mortality of *Sitophilus zeamais* adults treated with green produced ZnO NPs was generally higher than that of untreated controls at all concentrations. Adult maize weevils were successfully killed by green ZnO nanoparticles. In our study, it was found that the applied ZnO NPs dust stuck to the body of *Sitophilus zeamais* when it was viewed under a dissecting microscope at a magnification of 20X

(Figure 5). Compared to untreated maize weevil, the collected photos demonstrated a significant amount of nanoparticle body covering on the dorsal and ventral surfaces. According to Debnath *et al.* (2011) and Rumbos *et al.* (2016), the abrasion that the particles generate is also a part of the way that inert dusts work. As a result, the insects are exposed to lose water, which causes them to die from desiccation (Ebeling, 1971). Such inert dusts and nanocides are advantageous for usage as dust protectants against many storage insect pests due to the physical mechanism of action of these substances.

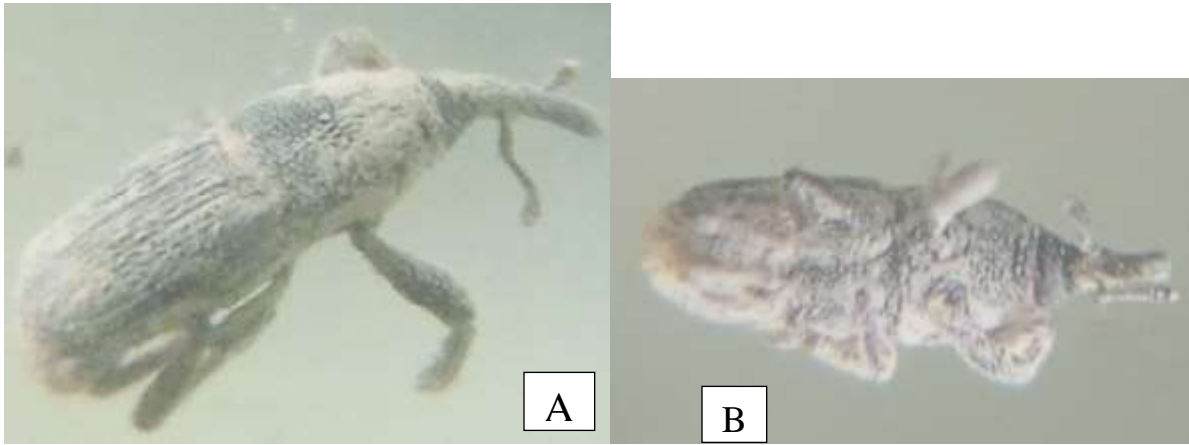


Figure 5. Adult *Sitophilus zeamais* under dissecting microscope A) dorsal view B) ventral view

Table 2. Mortality effect of synthesized ZnONPs using leaf extract of *Calpurnia aurea* after 14 days of exposure

	Cumulative mortality after treatment application (Mean±SD)			
Dosage	0.6g	0.4g	0.2g	Untreated control
Percent mortality	100±0.00 ^a	100±0.00 ^a	99.65±0.61 ^a	0.00±0.00 ^b

3.3. Effect of ZnONPs on F1 progeny emergence and seed germination

3.3.1. Effect of ZnONPs on F1 progeny emergence

In comparison to the untreated control, *Sitophilus zeamais* F1 progeny count was considerably (P <0.05) impacted after exposure to three dosages of ZnO NPs (Table 3). With application of the three dosages, the mean number of emerged

adults did not differ significantly (P>0.05). This outcome is consistent with the findings of Salem et al. (2015), who found that applying ZnO and Al₂O₃ nanoparticles to wheat grains greatly reduced the quantity of *Tribolium castaneum* progeny. Ibrahim et al. (2022) also showed that green generated ZnO NPs prevented the adult progeny of *S. oryzae* and *S. cerealella* from emerging.

Table 3. Number of F1 progeny emergence after green synthesized ZnO nanoparticles using *Calpurnia aurea* leaf extract

	Mean number of F1 progeny emergence			
Dosage	0.6g	0.4g	0.2g	Untreated control
No of F1 progeny (Mean±SD)	0.00±0.00 ^b	0.00±0.00 ^b	0.33±0.58 ^b	31.67 ^a

3.3.2. Effect of ZnONPs on seed germination

The results in Table 4 showed maize seed germination after insecticidal effect of ZnONPs. Seed germination after treatment application was significantly different ($P<0.05$) compared to untreated check but no significant difference ($p>0.05$) among the 3 dosages of ZnONPs

application. Due to insecticidal effect of green synthesized ZnONPs, treated maize seeds were not damaged by insects and also Itrotwar *et al.* (2020) reported in their result that ZnONPs enhanced maize seed germination.

Table 4. Effect of green synthesized ZnO nanoparticles using *Calpurnia aurea* leaf extract on seed germination

Dosage	Seed germination after treatment application (Mean±SD)			
	0.6g	0.4g	0.2g	Untreated control
Percent germination (Mean±SD)	100±0.00 ^a	100±0.00 ^a	93.33±11.55 ^a	6.67±11.55 ^b

4. Conclusion

This research was done to develop a greener method of synthesising ZnO nanoparticles. Leaf extract from *Calpurnia aurea* has been utilized successfully as a surfactant and reducing agent. The characterizations of the generated nanoparticles' morphology, optical properties, and structural characteristics were examined. ZnO nanoparticles were created with an average crystal size of 4.134466 nm. Investigated was the insecticidal potential of ZnO NPs as an efficient, environmentally benign control agent against *Sitophilus zeamais*, one of the most significant pests of stored goods. In this study, the insecticidal impact of ZnO nanoparticles against *Sitophilus zeamais*

was observed with high insect pest mortality rates when ZnO NP dosages were used. The emergence of new progeny was likewise decreased by synthesized ZnO NPs. Compared to the untreated check, treated maize seeds germinated more successfully. In the current work, ZnO nanoparticles were applied to maize grains in a very little quantity, however they were highly poisonous to *Sitophilus zeamais*. Future research is needed to evaluate the potential toxicity of nanoparticles on human health and the environment.

Data Availability

The data used to support the findings of this study are included within the article

Conflicts of Interest

The authors declare no conflict of interest.

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