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Entomotoxicity of ZnO NPs synthesized using *Clausena anisata* Hook.f. ex Benth(ulmaayii) leaf extract against maize weevil, *Sitophilus zeamais* Mostch (Coleoptera: Curculionidae)

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ABSTRACT

The application of leaf extract to synthesize nanoparticles has been taken as a green method. In this study, the potential for synthesizing zinc oxide nanoparticles (ZnO NPs) from *Clausena anisata* Hook.f. ex Benth. leaf extract was investigated. The source of zinc was zinc nitrate hexahydrate ($Zn(NO_3)_2.6H_2O$). The characterization study was done by Ultraviolet–visible (UV-Vis) spectroscopy, X-ray diffraction (XRD), scanning electron microscope (SEM), Transmission electron microscope (TEM) and atomic force microscopy (AFM). The crystalline shape of nanoparticles is disclosed inside the XRD result, morphology is confirmed through SEM effects, and consequently, the ZnO NPs scale was predicted. ZnO NPs were synthesized to work against *Sitophilus zeamais* adults. A mortality count was carried out in 14 days and all the 3 dosages (0.2 g, 0.4 g and 0.6 g) were effective in killing *S. zeamais*. F1 progeny emergence was highly reduced in comparison to untreated control. Maize seeds were successfully germinated after treatment application with ZnO NPs.

KEYWORDS: Characterization, *Clausena anisata*, Entomotoxicity, Maize weevil, Nanoparticles

INTRODUCTION

Maize, wheat, millet, rice, and other cereal crops are the most widely grown worldwide (Awika, 2011; Ye & Fan, 2021). Because they are high in fats, vitamins, proteins, minerals, carbohydrates, and oils, these crops are an important part of our diet (Sarwar *et al.*, 2013; Oso & Ashafa, 2021). They contain certain nutritional components due to this the grains of these crops are susceptible to insect pest attacks 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). Postharvest losses can outweigh crop damages in the field (Mesterházy *et al.*, 2020). Direct losses include direct feeding of seeds, whereas indirect losses include the creation of exuviae, webbing, insect cadavers, and frass, all drastically reduce the quality of seed and make grains unhealthy for human consumption (Kumar & Kalita, 2017; Mesterházy *et al.*, 2020).

Storage insect pest species imposing quantitative and qualitative losses on grains primarily are Coleopteran species and Lepidopteran species (Rajendran, 2002; Rajendran & Sriranjini, 2008). Primary and secondary pests are two types of stored grain insect pests (Banga *et al.*, 2020). Primary insect pests cause harm to whole grains, whereas secondary pests cause damage to broken or previously damaged grains. Based on where they attack, primary insect pests are divided into internal and exterior feeders (Deshwal *et al.*, 2020). Insect pest control tactics include physical, mechanical, chemical, and biological methods. However, fumigation is an extensively used insecticide because it can be used in a wide range of storage environments (Nguyen *et al.*, 2015; Nayak *et al.*, 2020). However, there are commercially available synthetic pesticides for the control of storage pests; which are expensive 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 ratios enable them to target pest organisms (Madhuri *et al.*, 2010; Mustafa & Hussein, 2020; Sahoo *et al.*, 2021). Nanomaterials may, therefore, aid in the development of novel pesticides and insecticides.

In the beginning of nanotechnologies, numerous nanoscale policies have been industrialized using several approaches, like physically, chemically, and biologically (green) approaches. Green synthesis aims to promote innovative chemical technologies to reduce or eliminate the use and production of hazardous substances in the design, manufacture, and use of chemical products. This involves minimizing or, if possible, eliminating the pollution produced in the synthesis processes, avoiding the consumption and wastage of nonrenewable raw materials, using hazardous or polluting materials in product manufacturing, and reducing the synthesis time (Singh *et al.*, 2018). Until now, biosynthesis of nanoparticle is a means of choices that can be simply organized and prepared (Jabbar *et al.*, 2022). There are numerous limitations of conservative methods for the preparation of nanoparticle, with long terms of processing, too expensive, difficult procedures, as well as in specific the usage of poison chemicals. Furthermost of the applicable investigation has been focused to ecologically friendly and debauched synthesis procedures for the productions of nanoparticle because to these drawbacks (Saravanan *et al.*, 2022). In this admiration, biosynthesis of NPs, specifically through extracts from different plant's parts, is a rising tendency that is measured simple, cost effective, and non-toxic in green chemistry (Sen & Mukherjee, 2023). Nano-technology has also improved the human standards of alive by give a talk many normal lives matter, such as the contributions to energy adequacy; climate changes; attractiveness, textiles, and health manufacturing as well as the cures of poisonous diseases such as cancers (Barabadi *et al.*, 2022). Because of their numerous uses in various technical fields, inclusive investigations into metallic oxide nanoparticle have been focused in the past decades. Amongst these, with multi-layered assistances, ZnO NP is exciting inorganic material (Islam *et al.*, 2022). ZnO NP might be used in numerous segments, like energy conservations, textile, opto-electronics, health care, catalysis, make-ups, semi-conductors, and chemical sensors. The nanoparticles are non-toxic and biologically well-matched and displays outstanding bio medicinal application, such as anti-cancer, anti-inflammatory, and anti-microbial characteristics, in besieged drug distribution, twisted curative, and bio-imaging (Ali *et al.*, 2022). Nano-products can be formed from many approaches (chemical, physical, as well as bio-synthesis) with numerous characteristics and enormous application (Popa *et al.*, 2022). Plant based synthesis of ZnO NP has beforehand been reported however, insufficient literature is present on their various biological characteristics such as anti-microbial, anti-larvicidal, protein kinases, and anti-cancer applications (Abdelghany *et al.*, 2023). The restorative uses of *Myristica fragrans* (Jaiphal) are well known, and it is mainly used as an anti-inflammatory, antidiarrheal, analgesic, and sex stimulating agents. Here, we report plant grounded synthesis of ZnO NP using *Clausena anisata* leaf extracts. Green method of ZnO-NP has ecologically friendly aspects as well as various bio-medical application. The bioactive copmounds or molecules found in the extract of *C. anisata* act as an oxidizing, reducing and capping agent for the synthesis of ZnO NP.

It is anticipated that nano pesticides 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.

Review report of Benelli (2018) indicated precise information on the mechanisms of action of nanoparticles against insects and mites are limited. However, Ebeling and Wagner (1959) proposed that insecticidal property of dusts gets increased if the particles are finer. This was also evident from experiments conducted by Das *et al.* (2019) where it was observed that nanoparticles of aluminium, titanium and zinc oxide materials were much more effective than their micron sized counterparts. They also reported, because of their enormously increased exposed surfaces these NPs can more effectively interact with the protective cuticular wax layer of the insects. The insects get killed as their wax layer gets damaged due to both abrasion and adsorption of lipids and they start losing water from their body and ultimately die because of desiccation (Debnath *et al.*, 2011). As the insecticidal effect of NPs are mostly physical in nature, there is probability that the insects are unlikely to become resistant to this mode of action very soon. In the current study, the *C. anisata* leaf extract acted as reducing, capping and stabilizing agent in ZnO NPs formation. Therefore, this study aims to evaluate the toxicity of green synthesized ZnO NPs using leaf extracts of *C. anisata* against *Sitophilus zeamais* in the laboratory on maize seeds.

MATERIALS AND METHODS

Materials

C. anisata (ulmaayii) leaves were collected from around Dambi Dollo University campus, Zinc-nitrate hexa hydrate ($Zn(NO_3)_2.6H_2O$, 99%, 297.49 M.W, AR grade) was purchased fromYeshadam Trading PLC, Addis Ababa, Ethiopia and distilled water.

Rearing of Test Insect

The study was conducted at Dambi Dollo University, Ethiopia, in the zoological science laboratory. The adult maize weevils, *S. zeamais*, were collected from the farmers' storage facilities and brought laboratory and cultured at 23±3 °C and 64.8±9% RH laboratory conditions. Limmu variety maize seeds were bought from Dambi Dollo university research center and disinfected in an oven at 40 °C for 4 h from any prior infestation before using them as a substrate for insect rearing following Bekele (2002) procedures. The grains were used as food substrate for the test insect, *S. zeamais*. Fifty test insects were placed in 4, 500 mL capacity glass jars containing 200 g seeds. The jars were covered with nylon mesh to allow ventilation and put in place with rubber bands to prevent the escape of weevils and to protect the interference of other insects into the jars. The parent maize weevils were removed by sieving after two weeks of oviposition period and the maize grains were kept under laboratory conditions until the emergence of the F1 progeny.

Preparation of Plant Extract

Leaf extract of *Clausena anisata* was prepared. Briefly, 40-50 g of leaves were collected and washed using distilled water and air-dried and fine powdered 10 g was added to 100 mL of distilled water and macerated following Ileke (2014) with slight modification. After 72 hours of frequent agitation, the leaf extracts were filtered with filter paper Whatman No. 1. Then, the extract was kept for further synthesis of nanoparticles.

Synthesis of Zinc Oxide Nanoparticles

Following the procedures of Ibrahim *et al.* (2022), ZnO NPs were synthesized by combining a previously prepared plant extract in a 1:1 ratio(v/v) with 1 M zinc nitrate hexahydrate ($Zn(NO_3)_2.6H_2O$) and vigorously agitating the mixture for two hours to produce a white precipitate and centrifuged. The precipitate was dried at 300 °C overnight after being centrifuged at 3000 rpm for 15 minutes to yield the powder. After that, the samples were kept for additional characterization and toxicity investigations. Figure 1 briefly expresses the flow chart of ZnO NPs synthesis.

Characterization of Zinc Oxide Nanoparticles

Morphological, optical and structural studies of these nanoparticles were carried out using Ultraviolet–visible spectroscopy (UV_vis), scanning electron microscope (SEM), X-ray diffraction (XRD), Transmission electron microscope (TEM) and Atomic force microscopy (AFM) of the nanoparticles.

Insecticidal Effect of Synthesized ZnO NPs

Twenty grams of Limu variety maize grains were added to 3 glass jars of 500 *mL* and 3 levels of ZnO NPs (0.2, 0.4 and 0.6 *g*) were added to each jar and mixed following Ibrahim *et al.* (2022) procedures. To each jar, 10 unsexed adult maize weevils were introduced. The experiment was replicated thrice with a completely randomized design and kept under 23±3 °C and 64.8±9% RH laboratory conditions for 14 days.

Effect of ZnO NPs on F1 Progeny Emergence and Seed Germination

Following Hiruy and Getu (2018) procedures, mortality count of the test insect was carried out at 2, 7 and 14 days after treatment application. After 14 days, all dead and live maize weevils were removed and the jars containing the grains were kept for an additional 14 days for the F1 progeny emergence. After the F1 progeny emergence count, the seed germination test was carried out by taking 5 maize seeds randomly from each jar and placed on moistened filter paper with 10 mL of distilled water in a petri dish of 9 cm diameter that was replicated 3 times. Germinated seeds were counted after four days.

Data Analysis

Obtained data on maize weevil mortality, F1 progeny emergence and seed germination were analyzed using one-way ANOVA

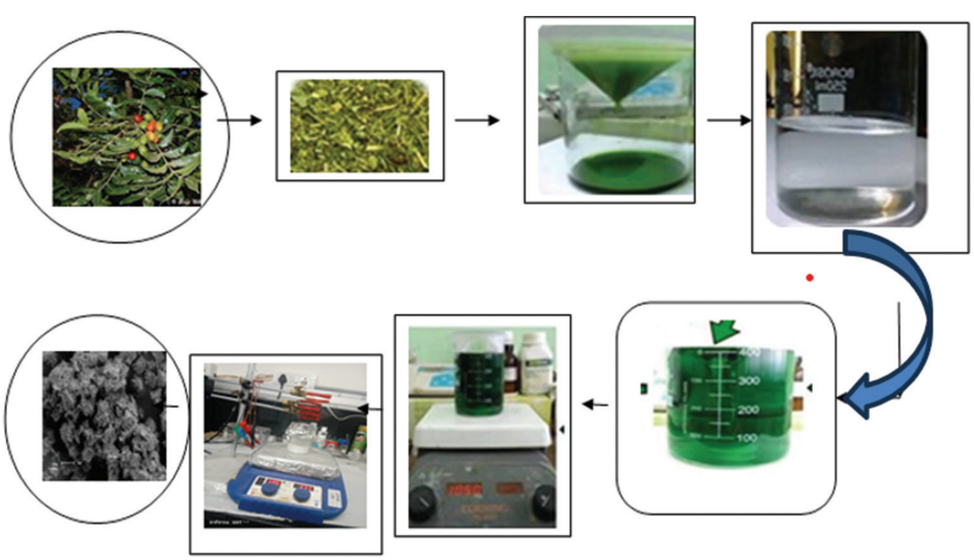

Figure 1: Diagram representation green synthesis of ZnO nanoparticle

and the significance level was set at $P<0.05$. The data was analyzed using IBM SPSS Statistics for Windows, version 25 (IBM Corp., Armonk, N.Y., USA). The data obtained from the characterization of ZnO NPs were analyzed using ORIGINPRO Graphing and Analysis 2022.

RESULTS AND DISCUSSIONS

UV-Vis Spectroscopic Study

The optical property of synthesized ZnO NPs was studied by UV-vis absorptions spectroscopies (Halo DB-20 UV-vis double beam). The reactions mixtures were perused in the wave length (λ) ranges between 200 and 800 nm (Varadavenkatesan *et al.*, 2019). Green Synthesized ZnO NPs displayed an absorption swelling at 280 nm because of the absorptions of a photon (hv) and the excitations of electrons from the valences bands to the electrons or holes pair producing by the conductions bands as shown Figure 2a. Then, the energy band gap was valued by the band gap Tauc plots calculation and it showed 2.93 eV. Normally, the optical energy band gap decline when the absorptions edges alterations towards a lengthier wavelength. Nevertheless, the energy band gap decreases, while relatively increase the better conductivities. The present result agrees with results reported by (Sunny & Shanmugam, 2021). The energy band gap was

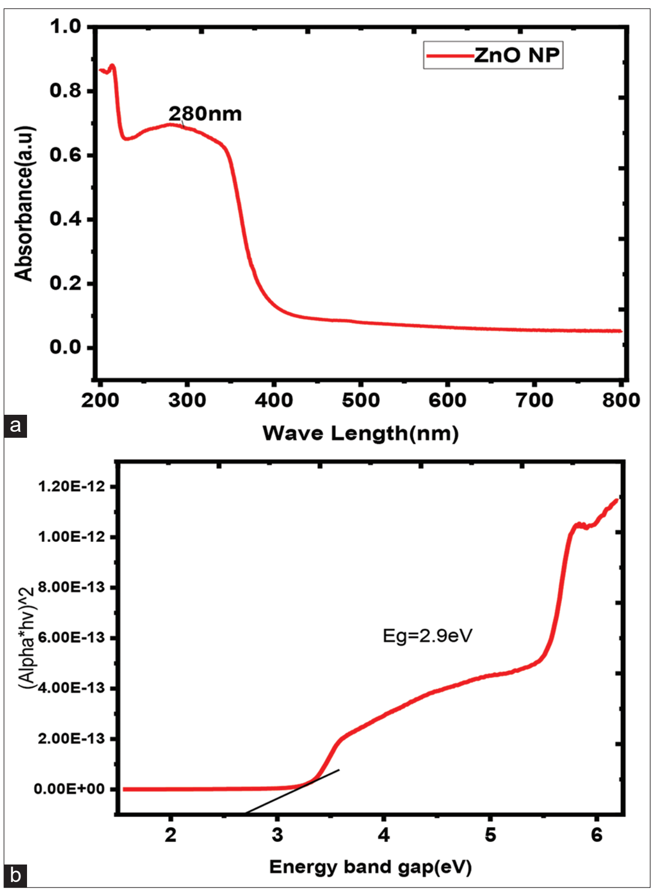

Figure 2: a) UV-visible spectrum of green synthesized ZnO NPs from *Clausena anisata* leaf extract and b)The energy band gap of green synthesized ZnO NP calculated using Tauc relation

dogged from the UV-visible spectroscopy's data. By the next equation (1), the optical energy band gaps of the ZnO NPs was obtained to be 2.93 eV, is very much suitable for application of antimicrobial and insecticidal as shown in Figure 2b.

$$hv = A(hv - Eg)^n \tag{1}$$

where α is the absorptions coefficients, hv represent the energies of the photons, A shows proportionality constant and varies with the material, and n represents the indexes.

Structural Study

X-Ray Diffractions (XRDs) were performed to analyze the components of the samples (Model-D8 Advance, Germany). As seen from the XRD patterns in Figure 3, there are peaks at 2Thetta=27.53114, 27.83267, 42.69071, 45.50552, 53.82441, 53.82441, 66.16616 and 72.89006, respectively, that match to the (111) (101) (110) (112) (123) (100), (100), and (101) crystal planes of Zinc Oxide hexagonal crystal geometries affording to JCPSD card no. 01-007-2551 (Muhammad *et al.*, 2019). The characteristic peaks observed confirm the hexagonal -wurtzites structure of Zinc Oxide, signifying integrity of the Zinc Oxide lattices. The biosynthesis of ZnO NPs was approved by XRD analysis. These spectrums show extremely strong peaks as well as natures of ZnO NPs. The average crystal size of green synthesized ZnO NPs was calculated by Debye- Scherer's formula (Yekkaluri *et al.*, 2022).

$$D(nm) = \frac{K\lambda}{\beta Cos\theta} \tag{2}$$

Where

D –is denoted for the crystal sizes, whereas λ= 1.5406) for CuKa, λ – the wave length of the x-ray radiations, K- usually taken as 0.9, β- the line Full width at half- maximum height (FWHM) and stands for peak position.

X-ray diffraction analysis shows crystalline structures peaks as well as the average crystalline size of nanoparticles around 6.9 nm. Table 1 shows crystalline parameters gained from XRD

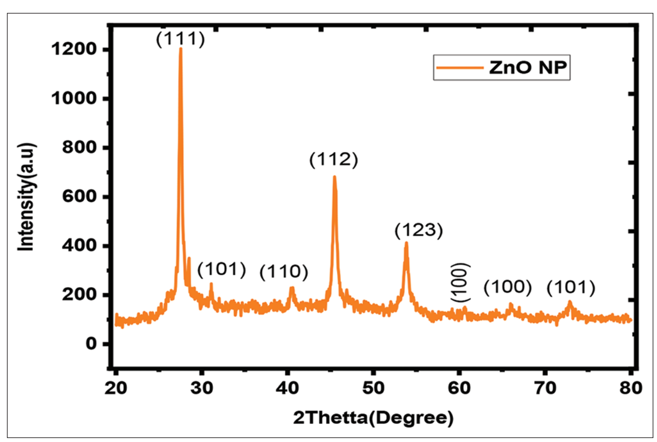

Figure 3: XRD graphs of ZnO NPs synthesized from leaf extract of *Clausena anisata*

data and Scherer formula. These results agreed with recently published works and it is promising alternative for different applications like antimicrobials and insecticidal activity (Xie *et al.*, 2022; Jothibas *et al.*, 2023).

Morphological Analysis of Synthesized ZnO NPs

The surface morphology and particle sizes distribution of the ZnO NPs were also studied through scanning electron microscope (SEM), Transmission electron microscope (TEM) and Atomic Force Microscopy, as shown in Figures 4a, b and c. From the SEM and TEM results, the shapes of the particles can be inferred as being crooked flower and sphere-like structure. As can be seen, the spherical particles appear to be quite distinct and uniform, and the size of the particles ranges from 5 to 25 nm (Nguyen *et al.*, 2020).

A SEM and TEM images of nanoparticles prepared by green synthesized method is shown. X-Ray Diffraction outcomes are approved by the joint studies of these images. It also displays that a grid formation of the zinc oxide nanoparticle has occurred which evidently specifies that agglomerations has taken places. The obtained outcome is very much suitable for insecticidal applications and antimicrobial (Kamaraj *et al.*, 2022). This result agree with recently reports of Zafar *et al.* (2019), Muhammad *et al.* (2019), Naseer *et al.* (2020) and Jeyabharathi *et al.* (2022).

Figure 4c displays the AFM (3D) image of ZnO nanoparticle. AFM image proves that the grains are dispersed evenly within the scanning area (1518x1514) nm. The average diameter of ZnO is measured from AFM investigation using software and is found to be about (26) nm (Aysa & Salman, 2016).

Table 1: Crystalline parameters calculated from XRD data and Scherer formula

2θ(Degree)	FWHM(Radian)	D(nm)	Average Crystal Size(nm)
26.53114	0.3704	20.82371	6.875892
27.83267	3.12275	2.468371	
44.69071	25.29121	0.292452	
45.50552	0.5858	12.50125	
54.82441	0.75806	9.341177	
56.82441	673.9922	0.010506	
66.16616	1.4361	4.633221	

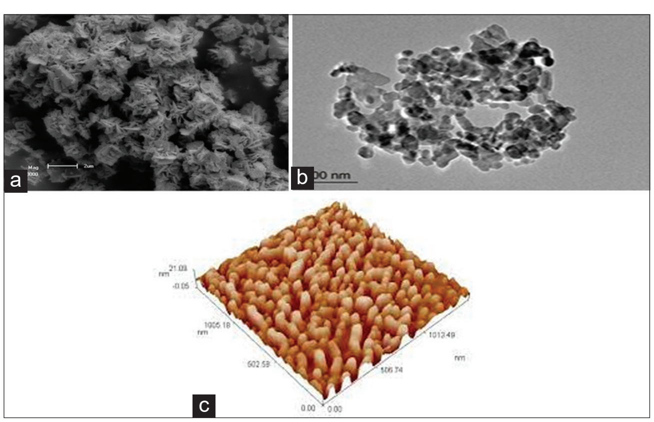

Figure 4: Morphological and topological Analysis ZnO NP images of a) SEM, b) TEM and c) AFM

Insecticidal Effect of Synthesized ZnO NPs

The toxicity experiment's results, which are shown in Table 2, revealed that mortality of *S. zeamais* adults treated with green synthesized ZnO NPs was consistently higher than that of untreated controls at all concentrations, with no statistically significantly differences ($P>0.05$) between the three concentrations. Synthesized ZnO nanoparticles were effective in killing adult maize weevils. When *S. zeamais* was observed under a dissecting microscope at a magnification of 20X, it was found that the applied ZnO NPs dust stuck to the insect's body (Figure 5). In contrast to untreated treatment, the photos obtained showed significant nanoparticle coverage on the dorsal and ventral surfaces of the insects. According to Debnath *et al.* (2011) and Rumbos *et al.* (2016), the mode of action of inert dusts is dependent on both the absorption and the abrasion the particles induce. As a result, when the water barrier is compromised, insects start to lose water, which causes them to die from desiccation (Ebeling, 1971). Due to their physical mechanism of action, such inert dusts and nanocides are well suited for use as dust protectants against many storage insect pests.

Influences of ZnO NPs on F1 progeny emergence and seed germination

Influences of ZnO NPs on F1 progeny emergence

F1 *S. zeamais* progeny count was considerably decreased ($P <0.05$) after exposure to 3 dosages of ZnO NPs compared to the untreated control (Table 3). With administration of the three dosages, there was no appreciable difference in the average number of emerging adults ($P>0.05$). This conclusion is consistent with that found by Salem *et al.* (2015), who found that *T. castaneum* progeny were dramatically reduced when ZnO and Al_2O_3 nanoparticles were sprayed to wheat grains. Additionally, Ibrahim *et al.* (2022) observed that green ZnO NPs reduced the adult progeny emergence of *S. oryzae* and *S. cerealella*.

Influences of ZnO NPs on seed germination

The results in Table 4 show maize seed germination after insecticidal effect of ZnO NPs. Seed germination after

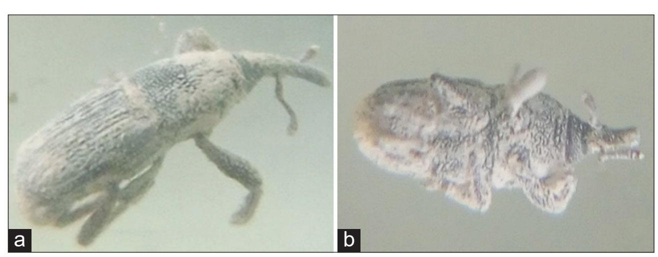

Figure 5: Adult S. zeamais under dissecting microscope a) dorsal view b) ventral view

Table 2: Mortality effect of synthesized ZnO NPs using leaf extract of *C. anisata* after 14 days of exposure

	Cumulative mean mortality after treatment application			
Dosage/ 20 g maize	0.6 g	0.4 g	0.2 g	Untreated control
Percent mortality (Mean±SD)	100±0.00[a]	99.65±0.61[a]	99.65±0.61[a]	0.00+0.00[b]

Means followed by the same letter across the row are not significantly different at P<0.05

Table 3: Number of F1 progeny emergence after green synthesized ZnO NPs using *Clausena anisata* leaf extract

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

Means followed by the same letter across the row are not significantly different at P<0.05

Table 4: Effect of green synthesized ZnO NPs using *C. anisata* leaf extract on seed germination

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

Means followed by the same letter across the row are not significantly different at P<0.05

treatment application was significantly different (P<0.05) compared to untreated check but no significant difference (p>0.05) among the 3 dosages of ZnO NPs application. Due to insecticidal effect of green synthesized ZnO NPs, treated maize seeds were not damaged by insects and also Itroutwar *et al.* (2020) reported in their result that ZnO NPs enhanced maize seed germination.

CONCLUSION

This study was conducted to synthesize ZnO NPs by green method. Leaf extract from C. *anisata* 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 NPs were synthesized with an average crystal size of 6.87 nm. The energy band gap for the synthesized ZnO NPs was 2.9 eV. Investigated was the insecticidal potential of ZnO NPs as an efficient, environmentally benign control agent against *S. zeamais*, one of the most significant pests of stored grains. In this study, the insecticidal impact of ZnO NPs against *S. zeamais* was observed with high insect pest mortality rates when ZnO NPs 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 small quantity, however, they were highly poisonous to *S. zeamais*. Future research on the possible hazardous consequences of nanoparticles on human health, other beneficial (non-target) insects and the environment is necessary. Detailed experiment on the ZnO NPs mode of action is also recommended for future research.

AUTHOR'S CONTRIBUTION

All authors have contributed equally.

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