Effect of zinc oxide nanoparticles synthesized from *Ocimum basilicum* (basil) leaves extract on sugarcane pests

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ABSTRACT
Background: Injudicious pesticide use can hinder sustainable agriculture practices by causing several ecological and environmental safety issues. Nanotechnology has enabled the development of nano-sized pesticides, including improved plant absorption and conduction with increased efficacy, lower dosage, delayed resistance, reduced residues, and protection from beneficial insects and natural predators. Assessing the insecticidal qualities of zinc oxide nanoparticles (ZnO-NPs) made from *Ocimum basilicum* (basil) leaves extract against *Diatraea saccaralis, Spodoptera frugiperda*, and *Cavelerius excavates* that damage sugarcane crops was the aim of this study. **Methodology:** Preparation of leaf extract and salt solution was done for the synthesis of zinc oxide nanoparticles. Characterisation of ZnO-NPs were done by using different techniques such as UV visible spectroscopy, X-ray diffraction (XRD), Fourier Transmission Infrared spectroscopy (FT-IR), and Scanning Electron Microscopy (SEM). Insects were exposed to various concentrations of ZnO-NPs (i.e. 100, 200, 300, and 500 ppm) to evaluate their insecticidal activity**.** One-way (ANOVA) was used to analyse mortality data. **Result:** Results revealed that mortality against ZnO-NPs was concentration-dependent, with greater concentrations leading to higher mortality rates. **Conclusion:** It is concluded from the study that ZnO-NPs can be used effectively against sugarcane pests in a pest management program. However, to fully understand the ecological implications of using ZnO-NPs as pesticides, more research is required.

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INTRODUCTION

The branch of technology concerned with materials having dimensions less than 100 nm, and the manipulation of these materials, such as atoms and molecules, is known as Nanotechnology (Bhat, 2023). The unique qualities and capabilities of nanotechnology have positioned it as an emerging technology that holds immense promise for scientific advancements across various sectors, including nutrition delivery, target specificity, crop protection, medications, electronics, material sciences, environmental remediation, water purification, textiles, clothing, and other areas (Gupta et al., 2024). Nanotechnology involves manipulating the materials at the nano scale level to alter their properties, leading to unique features that are utilised for specific purposes (Sarwar et al., 2024).

Inorganic nanoparticles encompass a variety of metals and metal oxides including metals like silver (Ag), molybdenum (Mo), tin (Sn), titanium (Ti), gold (Au), copper (Cu), indium (In), tungsten (W), aluminum (Al), iron (Fe), nickel (Ni), Bismuth (Bi), zinc (Zn), cobalt (Co) as well as metal oxides such as copper oxides (CuO), cerium oxides (CeO_2), indium oxide (In_2O_3), aluminum oxide (Al_2O_3), magnesium oxides (MgO), lanthanum oxide (La_2O_3), nickel oxide (NiO), tin oxide (SnO_2), titanium oxides (TiO_2), zirconium oxides (ZrO_2), and quantum dots and organic nanoparticles includes fullerenes and carbon nanotubes (Shukla, et al., 2024).

Zinc oxide nanoparticles (ZnO-NPs) are extremely valued for their exceptional chemical and physical characteristics, making them an important and versatile organic compound among metal oxide nanomaterials

(Islam et al., 2022). They have excellent chemical stability, a strong electrochemical coupling coefficient, a wide radiation absorption spectrum, and exceptional photo stability (Zhou et al., 2023). ZnO-NPs have found extensive applications in a wide range of additives and commercial products. These include cosmetics, ceramics, fire retardants, cement, ferrite, plastics, glass, batteries, pigments, ointments, sealants, adhesives, sunscreens, and even as an alternative source of zinc nutrients (Zafar & Iqbal, 2024).

Sugarcane (*Saccharum officinarum*) plays a significant role in sugar production, bioenergy production, and has economic importance, environmental benefits, and diversification opportunities, which hold crucial importance in the global economy (Mirajkar et al., 2019). Conventional techniques of pest management, including the application of chemical pesticides, have achieved successful outcomes however, they have several limitations, such as harmful impacts on non-targeted species, environmental pollution, and the emergence of insect populations that have pesticide resistance (Ahmed et al., 2021). The speedy advancement of nanotechnology with its unconventional methodologies such as green agriculture intended to manufacture nanomaterials having the least environmental persistence and high insecticidal activity, causing less harm to the environment and human health (Dangi & Verma, 2021; Abel & Scott, 2020). Different nanoparticles have emerged in the last few years, such as Ag (Raguvaran et al., 2021), CuO (Rahman et al., 2022), MgO (Abdelfattah et al., 2023), and ZnO (Thakur et al., 2022), which exhibit insecticidal action against insects of many orders, either by itself or in combination with other medications (Ishwarya et al., 2018).

The physical impact of nanoparticles is noteworthy, as they have the potential to engage in physical interactions with pests, resulting in mechanical harm, disturbance of the insect cuticle, or obstruction of respiratory structures (Pittarate et al., 2021). The generation of reactive oxygen species (ROS) by nanoparticles has been observed to cause oxidative stress in pests, resulting in damage to cellular components. This phenomenon has been documented in studies conducted by Kumar et al. (2019). In addition, it has been discovered that nanoparticles can impede the biological processes of pests through the interference of enzymatic activities, modification of hormone regulation, or disruption of cellular signalling pathways (Iqbal et al., 2022).

The Labiatae family includes the genus *Ocimum L*., or basil. *Ocimum* is a vital herb for both commerce and medicine (Shahrajabian et al., 2020). It is also a recognised source of additive agents and essential oils that have antimicrobial and anti-oxidative activities (Naji-Tabasi & Razavi, 2017). It is an odorous herb that has been broadly utilised in gastronomic applications (Li & Chang, 2016). Terpenes and phenylpropanoids are the major components of basil essential oil, followed by aldehydes and alcohols (da Silva et al., 2022). Recent studies have looked into the possibility of using extracts from basil leaves to create ZnO-NPs with enhanced pest management capabilities in a variety of agricultural settings (Lucic et al., 2022).

MATERIALS AND METHODS

The study was conducted at the zoology lab, University of Education, Lahore, Faisalabad Campus.

Preparation of leaf extract

After being collected from Gatwala Park in Faisalabad, Punjab, the *Ocimum basilicum* leaves were cleaned with distilled water and allowed to dry in the shade. The Geepas 1000W Mixer Grinder was then used to grind the leaves into a fine powder. For the preparation of aqueous leaf extract of *O. basilicum*, using a magnetic stirrer, 5 g of finely ground leaves were continuously stirred for 30 minutes while boiling in 100 millilitres of purified water. The extract was cooled to room temperature, filtered through Whatman No. 1 filter paper, and stored at 4°C for use in subsequent studies.

Preparation of the salt solution

To prepare the 0.5M stock solution, 100 millilitres of distilled water were used to dissolve 1.749 grams of zinc nitrate hexahydrate, or $\{Zn(NO_3)_2 . 6H_2O\}$.

Synthesis of zinc oxide nanoparticles (ZnO-NPs)

ZnO-NPs were synthesised by the titration method. For the synthesis of the ZnO-NPs, 50 ml solution was taken in a beaker. Fifty millilitres (50 ml) of salt (0.5 M of $Zn(NO_3)_2 . 6H_2O$) solution was taken in a beaker. After that, the *O. bascilium* extract was taken in beuarte and added drop by drop into zinc nitrate hexahydrate solution until the mixture's colour shifts from pale to brown. The mixture was heated at 80^0C with continuous stirring during the reaction. After that mixture was centrifuged in a Hettich centrifuge (D-78532) at 1000rpm for 15 minutes. The pellets of nanoparticles were separated, and the supernatants were disposed of. After three rounds of washing with distilled water, ZnO-NPs were dried in a Memmert hot air oven at 600ºC. The ZnO-NPs were obtained in powder form and stored for insect bioassay and additional characterisation.

Characterisation of ZnO-NPs

To determine the size, structure, and optical characteristics of ZnO-NPs, they were characterised by UV-visible spectroscopy, X-ray Diffraction (XRD), Fourier Transmission Infrared Spectroscopy (FT-IR), and Scanning Electron Microscopy (SEM).

a. ***UV visible spectroscopy:*** For the analysis of ZnO-NPs by UV-visible spectrophotometer, one gram of ZnO-NPs powder was suspended in 10ml of distilled water, homogenised and subjected to a UV-spectrophotometer. The absorption spectrum was recorded at 200-800 nm.

b. ***X-ray diffraction (XRD):*** The XRD (D8 Advance Bruker Germany) of ZnO-NPs was performed at Nano-Materials and Bio-Sensing Research Centre (NBRC), Department of Physics, Government College University, Gurunanakpura, Faisalabad, Pakistan. Using Debye's Scherrer Equation, the Silver nanoparticles' average crystalline size (D) was determined.

$$D = K\lambda / \beta \cos\theta$$

In this equation:
D=crystalline size
λ=the used X-ray radiation's wavelength
θ= Bragg's angle derived from the pattern of X-ray diffraction
β= diffraction peak's full width at half maximum (FWHM)

c. ***Scanning Electron Microscopy:*** At the Nano-Materials and Bio-Sensing Research Centre (NBRC), Department of Physics, Government College University, Gurunanakpura, Faisalabad, Pakistan, ZnO-NPs were examined using scanning electron microscopy (SEM). SEM was used to determining the shape of ZnO-NPs. SEM is a powerful analytical technique that is used for imaging the smaller particles (Nanoparticles) at high magnification and resolution.

Collection of insects

Insects such as *Diatraea saccharalis, Spodoptera frugiperda,* and *Cavelerius excavates* were collected from sugarcane production area of Ayub Agricultural Research Institute, Faisalabad. Samples were taken in the early morning. Samples were transported to the Zoology Lab in plastic jars covered with mesh clothes after being collected using standard instruments such as forceps, aerial nets, sweep nets, and hand picking. In the lab standard feed was provided to the insects and were kept at 27°C temperature and 70% humidity with 12 hours light and dark period.

Insecticidal activity of ZnO-NPs

The insecticidal activity of ZnO-NPs against *D. saccharalis, S. frugiperda* and *C. excavates* was assessed by residual bioassay. To evaluate the performance of ZnO-NPs, various treatments were designed. Each jar contained 100, 200, 300, or 500 ppm of ZnO-NPs, which were utilized to treat insects of each kind (i.e., T1, T2, T3, and T4, respectively). Distilled water was utilised as the negative control, while the manufacturer's suggested field dose of lambda-cyhalothrin was employed as the positive control. The bioassay filter paper was lined on the bottom and walls of each individual jar after submerging and air drying in each treatment independently. Following the release of twenty insects in each jar for an hour, mortality was measured at specific intervals, such as six, twelve, eighteen, twenty-four, thirty, and forty-eight hours following treatment. The mortality rate was then determined using the following formula. Every bioassay was carried out three times.

$$\text{Mortality rate} = \frac{\textit{Number of dead insects}}{\textit{total number of insects}} \times 100$$

Statistical analysis

One-way ANOVA was performed by SPSS 16 to analyse the mortality data. Results were expressed as Mean mortality rate (± Standard Error).

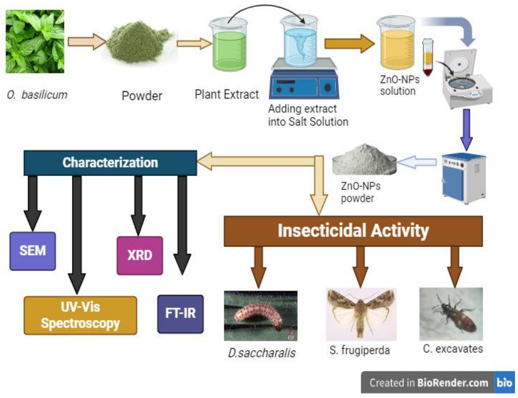

Figure 1: Graphical summary of methodology.

RESULTS

UV visible spectroscopy

The ZnO-NPs UV visible absorption spectrum is shown in figure 2, with the absorption peak appearing at 221 nm.

XRD analysis

An important source of information about the level of crystallinity shown by nanoparticles in a sample is provided by XRD spectra. The XRD spectra of the ZnO-NPs produced using the leaf extract from *O. basilicum* are shown in Figure 3. There are four unique peaks at 28.81°, 32.45°, 38.27°, and 47.54°, respectively. ZnO-NPs crystalline size, as determined by the equation, was 14 nm.

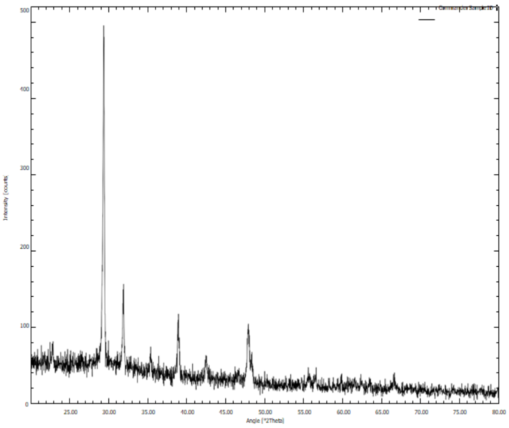

Figure 2: XRD Patterns of ZnO-NPs.

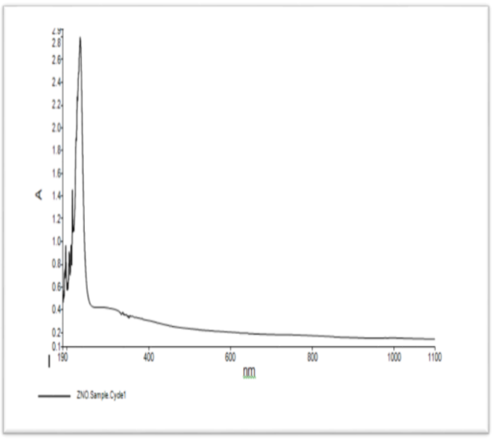

Figure 3: Absorption spectrum of ZnO-NPs produced by UV-Vis Spectrophotometer.

FT-IR analysis of ZnO-NPs

Figure 4 shows FTIR spectra for zinc oxide nanoparticles. FTIR spectra displayed a variety of peaks, including 666, 818, 1002, 1334, 1610, 2433, and 3526. These peaks represent a variety of *O. basilicum* biomolecules that were involved in the generation of zinc oxide nanoparticles. During the creation of the nanoparticles, these compounds acted as reducing, stabilising and capping agents.

Scanning Electron Microscope (SEM) analysis

SEM examination was used to evaluate the ZnO-NPs shape and elemental makeup, as shown in figures 5. A, B, and C. According to the micrographs, ZnO-NPs were found to have a uniform dispersion, a spherical morphology, and diameters in the nanoscale range.

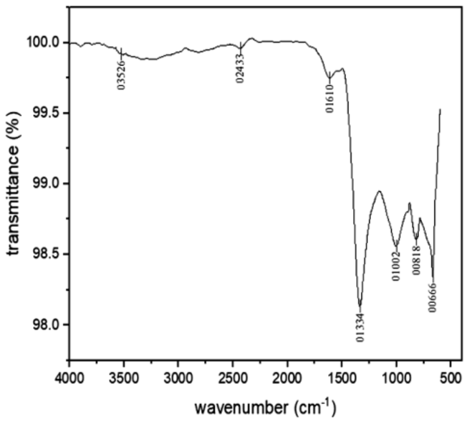

Figure 4: FTIR analysis of zinc oxide nanoparticles

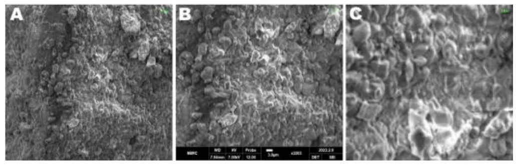

Figure 5: SEM Micrograph of Zinc oxide nanoparticles at 5 μm (A), 3 μm (B), and 1 μm (C).

Insecticidal effect of ZnO-NPs on sugarcane pests

Mortality rate of Diatraea saccharalis: The effects of increasing ZnO-NP dosages on *D. saccharalis* mortality rates are displayed in table 1.

The mortality rate in this trial was 6±1.67 for the negative control group and 95 ±2.89 for the positive control group. Death rates of *D. saccharalis* increased as ZnO-NPs concentrations were raised. The death rate was 15±2.84 at 100 ppm, 18.33±1.67 at 200 ppm 45±5.78 at 300 ppm, and 51.67±1.67 at 500 ppm (Table 1).

Mortality rate of Spodoptera frugiperda: The mortality rates of *S. frugiperda* insects subjected to varying concentrations of ZnO-NPs are also shown in Table 1.

At 100 ppm, the mean mortality was 16.67±1.67, which increased to 36±2.84 at 200 ppm. The death rates increased as the concentration increased further, reaching 38.33±1.67 at 300 ppm and 68.33 ±1.67 at 500 ppm. The positive control group had a mortality rate of 87.67±6. The low mortality rate for the negative control group was 1.67 ±1.67.

Mortality rate of Cavelerius excavates: The positive control group had a mortality rate of 93.33±4.41. The minimal mortality rate for the negative control group, which was given simply distilled water, was 6.67±1.67. As ZnO-NPs concentration grew, so did the death rates of *C. excavates*. The average death rate was 10±2.89 at 100 ppm and 16.67±1.67 at 200 ppm. When the concentration reached 300 parts per million, the average death rate was 28.33±1.67. The maximum mortality rate, 48.33±4.41, was seen at the highest quantity tested, 500 ppm. According to these investigations, ZnO-NP significantly inhibits *C. excavates*, and higher concentrations result in a higher mortality rate.

Table 1: Comparison of mortality rate in sugarcane Insect pests due to insecticidal effect of ZnO-NPs

Concentration (ppm)	*Diatraea saccharalis*	*Spodoptera frugiperda*	*Cavelerius excavates*
Positive Control	95^{c} ±2.89	86.67^{e} ±6	93.33^{d} ±4.41
Negative Control	6^{a} ±1.67	1.67^{a} ±1.67	6.67^{a} ±1.67
T1 (100 ppm)	15^{a} ±2.84	16.67^{b} ±1.67	10^{a} ±2.89
T2 (200 ppm)	18.33^{a} ±1.67	36^{bc} ±2.84	16.67^{ab} ±1.67
T3 (300 ppm)	45^{b} ±5.78	38.33^{c} ±1.67	28.33^{b} ±1.67
T4 (500 ppm)	51.67^{b} ±1.67	68.33^{d} ±1.67	48.33^{c} ±4.41
P-Value	<0.05	<0.05	<0.05
Df	5,12	5,12	5,12

DISCUSSION

In recent years, nanoparticles have attracted a lot of interest in the agricultural sector due to their ability to control diseases (Elek et al., 2010; Guan et al., 2008). The findings from the research suggest that using nanoparticles to control sugarcane pests might represent a practical approach. The results of our study show a strong relationship between pesticide concentrations and insect mortality, which is consistent with other researchers' prior investigations (Rouhani et al., 2011; Samih et al., 2011), One benefit of using them is that resistance is less likely to develop with continued use. The current study investigated how ZnO-NPs, which were made from *Ocimum basilicum* (Basil) leaf extract, affected the mortality rates of various insect pests that prey on sugarcane crops. The findings show that exposure to ZnO-NPs and insect mortality for all investigated insect species are concentration-dependent.

These results are in line with prior investigations examining how ZnO-NPs affect insect mortality. In a study conducted by Thumar et al. (2020), Aphid mortality from ZnO-NPs was shown to be concentration-dependent, with greater concentrations leading to higher mortality rates. Similarly, Khooshe-Bast et al. (2016) reported concentration-dependent mortality in whiteflies exposed to ZnO-NPs. The concept that ZnO-NPs can successfully cause mortality in various insect species is supported by these investigations.

Additionally, our results are consistent with research looking into how nanoparticles affect insect pests in different agricultural settings. As an example, Siddique et al. (2022) showed that exposure to ZnO-NPs had a concentration-dependent effect on the mortality of *Rhyzopertha dominica*. Hemiptera treated with ZnO-NPs showed concentration-dependent death, according to a study reported by Gutiérrez-Ramírez et al. (2021), *C. maculatus* was exposed to 1,000 ppm of 100 nm-sized zinc oxide nanoparticles during the experiment, which resulted in a 100% mortality rate (Mohammed & Aswd, 2019), When tested against the same pest in green grams, zinc oxide nanoparticles at a dosage of 200 ppm produced the lowest egg production and the highest mortality rate of 100%, according to a recent study (Lakshmi et al., 2020).

ZnO-NPs combined with leaf powder from *Citrus paradise*, *Jatropha curcus*, and *Ricinus communis* had a mortality rate of 49.51% for *T. granarium* and 66.32% for *T. casteneum* (Haider et al., 2020). When *R. dominica* was exposed to 600 ppm for 15 days, the use of *E. globulus* extract in the production of ZnO led to an 80.5% mortality rate (Siddique et al., 2022). Similarly, the cumulative mortality in *T. castaneum* after exposure to different concentrations of ZnO-NPs (20.42%, 27.08%, and 33.96%) was recorded for 1, 3, and 5 days post treatment (Hilal et al., 2021). In another study, when combination of chitinase enzyme from *Lactobacillus coryniformis* and ZnONPs was applied, the maize weevil, *Sitophilus zeamais*, died completely, with an average death rate of 2.4 days (Dikbas et al., 2021). These investigations demonstrate ZnO-NPs' potential as a useful tool for managing pests in a range of crop systems.

It is crucial to remember that the recorded death rates are probably due to the lethality of ZnO-NPs. Nanoparticles can greatly increase their toxicity and bioactivity to insects due to their unique physicochemical characteristics. Because of their small size and large surface area, nanoparticles can make more contact with insect tissues, which eventually leads to higher rates of toxicity and death. The generation of Zn^{2+} ions from the ZnO-NPs is part of the toxicity mechanism. In insects, these ions may cause oxidative stress and interfere with physiological functions, both of which may lead to mortality.

Conclusion

The current study makes important advances in expanding our understanding of ZnO-NPs' shape, elemental makeup, and insecticidal effectiveness. There has been evidence of a concentration-dependent relationship between ZnO-NP exposure and insect death, suggesting that ZnO-NPs have the potential to be used as highly effective insecticides. The maximum mortality rate for *Diatraea saccharalis, Spodoptera frugiperda* and *Cavelerius excavates* was seen at the highest quantity tested, 500 ppm.

The results of our investigation suggest that ZnO nanoparticles may be useful tools for implementing pest management programs in sugarcane farming and may also be effective agents for controlling pests. The aforementioned findings make a valuable contribution to the continuous endeavors aimed at devising sustainable and environmentally conscious methods for controlling pests.

In the future, advance research on the effects of ZnO-NPs synthesized from *O. basilicum* leaf extract on sugarcane pests should concentrate on numerous key aspects. These include maximizing the synthesis process to increase the stability and efficacy of nanoparticles, examining the cellular and molecular mechanisms of action to create targeted pest control strategies, assessing various nanoparticle formulations and delivery systems for increased efficacy, carrying out extensive research to determine the long-term environmental impact and potential accumulation of nanoparticles, carrying out extensive field trials to confirm their effectiveness in real-world settings, and investigating the synergistic effects of combining ZnO-NPs with other pest management tactics, like biological control agents and integrated pest management approaches. These opportunities for the future will help create effective and sustainable methods of managing pests in sugarcane farming, which will eventually lessen the need for chemical pesticides while maintaining the best possible crop protection.

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Statement of conflict of interest

No conflict of interest was declared by the authors.

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