Al-Hussaini *et. al.,* Babcock Univ. Med. J.2026 9(1):54-65
https://doi.org/10.38029/babcockuniv.med.j..v9i1.1089
eISSN: 2756-4657

RESEARCH ARTICLE OPEN ACCESS

In vitro antifungal activity of ZnO, CuO, and SiO_2 nanoparticles against Lecanicillium lecanii isolated from honeybee (Apis mellifera)

Al-Hussaini HRK[1]ID, Al Shibly MKA[1]ID

[1]*Department of Biology, College of Education, University of Al-Qadisiyah, Iraq*

Submitted: 2nd August 2025
Accepted: 23rd September 2025
Published: 31st March 2026
ID: Orcid ID

Abstract

Background: The entomopathogen fungus *Lecanicillium lecanii* threatens the health of the honeybee (*Apis mellifera*). Due to the increasing resistance of fungi to conventional antifungal agents, environmental concerns have led to a focus on nanoparticle-based alternatives. This study aimed to evaluate the antifungal activity of zinc oxides (ZnO) and copper oxides (CuO), and silica (SiO_2) nanoparticles (NPs) against *L. lecanii*.

Methods: The fungal isolate was identified using molecular techniques targeting the internal transcribed spacer (ITS) region, sequencing and phylogenetic tree analysis. In vitro antifungal activity was determined by the broth microdilution at 0, 10, 100, and 500 µg/mL for each nanoparticle. In the same way, the sensitivity of *L. lecanii* to Amphotericin B, Nystatin, Fluconazole, and Griseofulvin (four common antifungal agents) was also tested.

Results: ZnO nanoparticles exhibited the highest mycelial growth inhibition, followed by CuO and SiO_2 NPs. The effect of ZnO was significant at higher concentrations. Amphotericin B and Nystatin were highly effective drugs, Fluconazole had moderate activity, and Griseofulvin had little effect. The prominent antifungal activity of ZnO-NPs might be attributed to their ability to produce reactive oxygen species (ROS), causing cell damage.

Conclusion: ZnO-NPs demonstrated antifungal activity comparable to some conventional antifungals, suggesting their applicability as a safe and environmentally sustainable option for managing fungal infection in honey bees. Further in vivo and molecular studies are needed to evaluate their safety and mechanisms of action.

Keywords: Honeybee; *Apis mellifera*; Antifungal activity; Nanoparticles; *Lecanicillium lecanii*

Plain English Summary

Honeybees play a vital role in food production because they pollinate many of the plants that provide fruits and vegetables for human consumption. However, honeybee populations are under threat from several diseases, including fungal infections. One of these harmful fungi, Lecanicillium lecanii, can infect and kill honeybees, leading to the loss of entire colonies. This study explored new, environmentally friendly ways to control such fungal infections without harming the bees or the environment.

Researchers collected honeybee samples from different apiaries in Iraq and identified L. lecanii using laboratory and genetic methods. They then tested three types of nanoparticles—zinc oxide (ZnO), copper oxide (CuO), and silicon dioxide (SiO_2)—to see how well they could stop the fungus from growing in the laboratory. Nanoparticles are extremely tiny materials that can interact with microbes in unique ways, sometimes killing them more effectively than conventional chemicals.

Correspondence:
Al-Hussaini Hani RK
Department of Biology, College of Education
University of Al-Qadisiyah
Iraq
+9647816404772, hanialhussainy@gmail.com

The results showed that ZnO nanoparticles were the most effective at reducing fungal growth, especially at higher concentrations. CuO and SiO_2 nanoparticles showed only moderate effects. When compared to commonly used antifungal drugs, such as Amphotericin B and Nystatin, the performance of ZnO nanoparticles was found to be quite similar. The antifungal effect of ZnO is thought to result from its ability to produce reactive oxygen species—tiny reactive molecules that can damage fungal cells.
These findings suggest that ZnO nanoparticles could be a promising alternative to chemical antifungal agents for managing fungal infections in honeybee colonies. They could help protect bee health and support pollination without contributing to chemical resistance or environmental pollution. However, since this research was conducted in laboratory conditions, further studies in real hives and natural environments are needed to confirm their safety and effectiveness for bees and the ecosystem.

Introduction:
Honeybees (*Apis mellifera*) are well-known as important social insects, especially because of their critical role in pollination. Pollination is an important ecosystem service that maintains plant biodiversity and improves the quality of fruit and vegetables (1). *A. mellifera* plays an important role in ecosystem processes and pollinates essential reproductive structures for many flowering plants. This ecosystem service is necessary for global food security (2, 3). Honey bees are widely recognised in agriculture and horticulture as the most important pollinator for many crops (4). Honeybees are threatened by severe fungi, including Chalkbrood caused by *Ascosphaera apis* and Nosemosis (*Nosema apis*, *N. ceranae*), which severely cause damage to colony health and production (5, 6). Also, infection with *L. lecanii* causes death in bee colonies (7).
L. lecanii is a highly effective entomopathogenic fungus with a broad host range, which is pathogenic to all developmental stages of sap-sucking insects, such as aphids, whiteflies, scale insects, thrips, and mealybugs (8, 9). Furthermore, *L. lecanii* infects many insects across multiple orders (e.g., Orthoptera, Hemiptera, Lepidoptera, Thysanoptera, and Coleoptera), but its potential undesired effects on non-target insects have been questioned (10).
Nanotechnology is a scientific field that studies and uses materials on the nanoscale, which is a measurement of 1 - 100 nanometers. Materials at the nanoscale have different properties that make them more effective, and they can be used in many fields such as agriculture, biotechnology, engineering, and medicine (11). These materials exhibit unique physicochemical properties, including high surface area, enhanced chemical reactivity, and the ability to penetrate cell membranes of microorganisms, rendering them superior to conventional materials in numerous applications (11, 12, 13). They are associated with having a high surface area and higher chemical behaviour, gaining new solutions to more complex problems; improving energy efficiency and disease treatment (13). Different applications have utilised these properties in many areas, including medicine, biotechnology, agriculture, and environmental management. In particular, nanomaterials have been advocated as alternatives to chemical pesticides and antibiotics, which, when overused in apiculture, can lead to pathogen resistance and residues in honey and other bee products, which can affect bee health and environmental sustainability (14, 15). Different nanoparticles exhibit significant antifungal activity against *L. lecanii* and other phytopathogens. For example, cobalt and nickel ferrite nanoparticles (16), copper oxide nanoparticles (especially in combination with fungicides) (17), silver (Ag-SiO_2) nanoparticles (18), Zinc oxide nanoparticles have reduced fungal growth, presumably via the release of ions or the generation of reactive oxygen species generation (19).
Therefore, the present study aimed to assess the antifungal activity of zinc oxides (ZnO) and copper oxides (CuO), and silica (SiO_2) nanoparticles (NPs) against *L. lecanii* isolated from honeybees (*Apis mellifera*).

Material and Methods:
Samples collection
A total of 225 samples were collected from three sources (bee bodies, comb frames, and honey; 75 for each) in different apiaries of Al-Diwaniyah Governorate (Al-Diwaniyah City Centre, Sumer District, Afak District, Al-Shamiya District, and Al-Hamza District) from October 8, 2024, to January 8, 2025. Every apiary was inspected regularly, three times each month. For the viability of isolates, comb frames and honey were sampled using transport media swabs. In the case of bee body samples, 10 live worker bees were taken from each hive and introduced into sterile containers (Viamed Ltd., UK) with 0.85% normal saline (Pioneer Pharmaceutical Co., Iraq) to minimise contamination and to ensure that the microbial community remained as close as possible to that in the gut before the isolation of the bacteria (20). The swabs were subsequently taken to the Advanced Microbiology Laboratory, Department of Biology, College of Education, University of Al-Qadisiyah, for further investigation.

Isolation and identification
The samples were cultured on Sabouraud Dextrose Agar (SDA) (HiMedia Laboratories Private Limited, India) and incubated at 25–30 °C for 5–7 days. The fungal isolates were characterised morphologically and microscopically, and identification was confirmed using standard taxonomic keys and recognised fungal atlases (21, 22). Colonial morphology, hyphal structures and conidial characteristics were compared with standard descriptions.

Molecular Identification
Genomic DNA of *L. lecanii* isolates was extracted using the FavorPrep™ Fungi/Yeast Genomic DNA Extraction Mini Kit. The internal transcribed spacer (ITS) region of the rDNA gene was amplified with universal primers ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) (23). PCR products were subjected to agarose gel electrophoresis and sequenced for a selection of amplicons. The sequences obtained were then submitted to the NCBI GenBank database and compared with the available entries using BLAST to confirm the identity of the isolates.

Antifungal sensitivity tests
Four antifungal agents, Amphotericin B, Fluconazole, Nystatin, and Griseofulvin, were assessed at concentrations of 100, 200, and 400 µg/mL. Antifungal sensitivity testing was conducted using the broth microdilution method according to Clinical and Laboratory Standards Institute (CLSI M61) guidelines. The assay was performed using a 96-well microtiter plate (24).

Nanoparticle Preparation and Characterisation
Powders of zinc oxide (ZnO), copper oxide (CuO), and silicon dioxide (SiO_2) nanoparticles were obtained from US Research Nanomaterials Inc. (Houston, TX, USA). All nanoparticles were of ≥ 99% purity and ≤100 nm in size. Stock suspensions (1000 µg/mL) were obtained by dispersing 10 mg of each nanoparticle powder in 10 mL sterile distilled water, followed by sonication for 30 minutes to stabilise the dispersion.
A Field Emission Scanning Electron Microscope (FESEM) was used to study the morphological structure and surface characteristics of the nanoparticles. Elemental composition was verified using Energy-Dispersive X-ray Spectroscopy (EDX). Due to limited funding and availability of resources, other advanced methods - like transmission electron microscopy (TEM), X-ray diffraction (XRD), or Fourier-transform infrared spectroscopy (FTIR) - were not used. However, the results of the FESEM and EDX analyses offered enough preliminary information about particle morphology, size distribution, and elemental composition that were suitable for the goals of the study.

Antifungal activity of nanoparticles
Antifungal activity of nanoparticles was assessed by the broth microdilution method in a 96-well microtiter plate following the methodology proposed by Wiegand *et al.* (2008). 100 µL of Sabouraud Dextrose Broth (SDB) (HiMedia Laboratories Private Limited, India), 100 µL of nanoparticle concentration (0, 10, 100, and 500 µg/mL) and 100 µL of fungal suspension were added in each well, giving a final volume of 300 µL with three replicates. Positive controls consisted of SDB, fungal suspension, whereas Carbendazim (50%), and the negative controls comprised SDB. The plates were sealed and incubated at 37°C for 48–72 h. Fungal growth inhibition was determined based on absorbance readings in an ELISA reader.

Statistical analysis
The statistical analysis of the experimental data was carried out using the Statistical Package for the Social Sciences (SPSS) (version 27). Results are presented as the mean values and standard error (Mean ± SE) of three replicates per treatment. Statistical analysis was performed using Two-way ANOVA to analyse the differences among the treatments. The Least Significant Difference (LSD) test was used to compare means. Significance of differences was set at $p < 0.05$.

Results
Isolation and identification
100 fungal isolates were obtained from a total of 225 samples. 15% of the total isolates were *L. lecanii* (Figure 1).

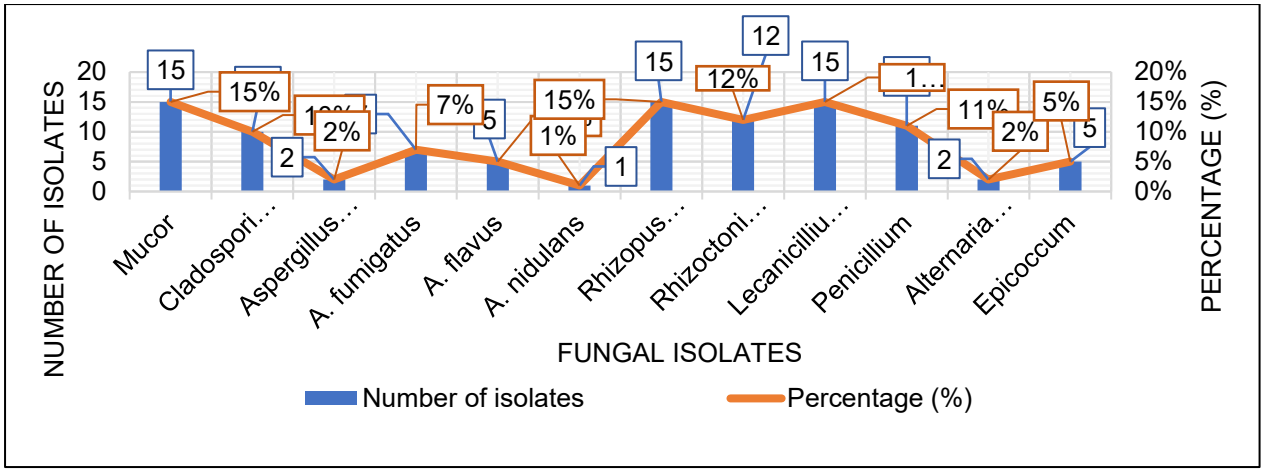


Figure 1: Distribution of fungal isolates by number and percentage

L. lecanii isolate colonies on SDA were round, soft, and cottony. They were bright white at first, but when they became older, they turned cream yellow. The reverse side remained smooth and pale yellow. No pigment diffusion was observed in the medium, indicating the absence of the extracellular pigment and secondary metabolites production in this medium. Microscopically, *L. lecanii* isolates were stained with methylene blue. The hyphae were hyaline and filamentous with transverse septation; conidiophores were typically delicate and unbranched, producing single, ellipsoidal conidia or short chains of conidia (Figure 2).

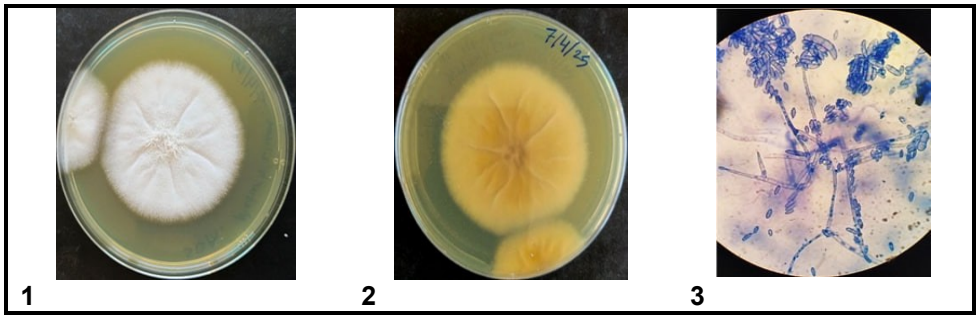


Figure 2: Morphological characteristics of *L. lecanii* isolate: (1) colony on PDA (upper surface), (2) colony reverse, and (3) microscopic structures

Molecular Identification

Molecular identification of the fungal isolates was carried out by amplifying the Internal Transcribed Spacer (ITS) region using universal primers ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3'). PCR products were electrophoresed on a 1.5% agarose gel stained with ethidium bromide (100 V, 80 mA, 90 min). A single clear band of 600–700 bp was observed for each isolate, confirming successful amplification of the ITS region, as shown in Figure 3.

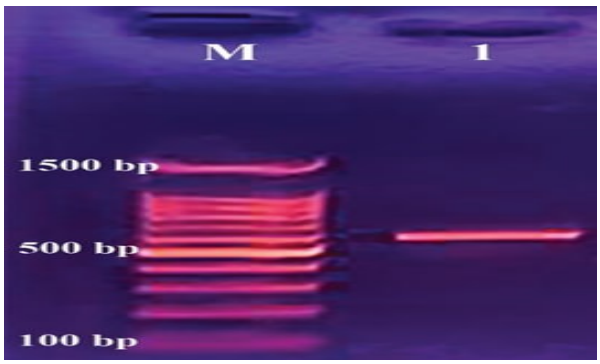


Figure 3: PCR amplification of the ITS1 region in *L. lecanii* was resolved on agarose gel electrophoresis. Lane 1 shows a distinct band of approximately 600–700 bp, and M represents the DNA ladder (100–1500 bp)

Sequence analysis of the ITS region of the ribosomal DNA (rDNA) of L. lecanii

The BLAST algorithm in the NCBI database was employed to analyse the sequence of the *L. lecanii* isolate (as illustrated in Figure 4). The high similarity of this sequence confirmed the accurate molecular identification of the fungal isolate. Alignment of the obtained sequence revealed 97.3% identity with the reference sequence GU598131.1, encompassing the ITS1 and ITS2 regions, the 5.8S rRNA gene and a part of the 28S rRNA gene. This sequence similarity, together with a very low E-value (3e-134) provides strong confirmation that the isolate belongs to *L. lecanii*.

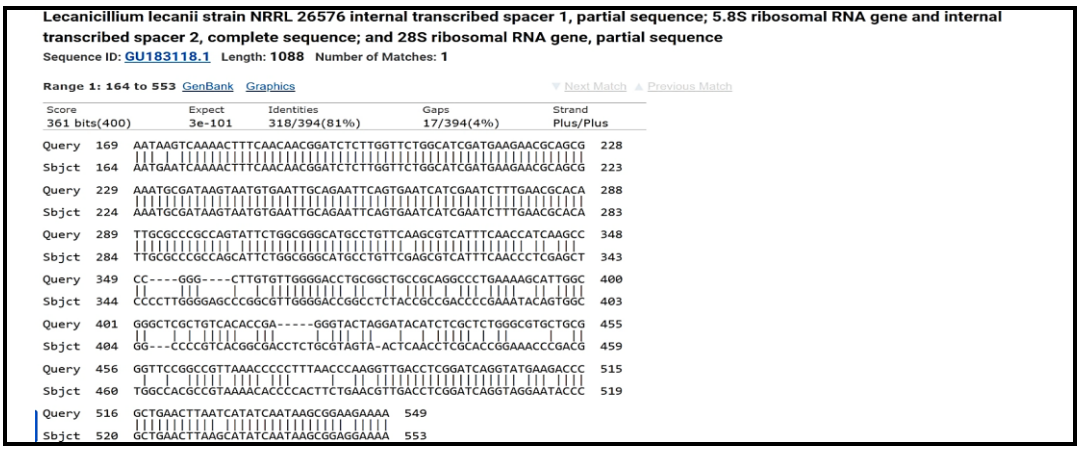


Figure 4: of the ITS region (ITS1, 5.8S, ITS2) of rDNA from *L. lecanii* isolate with reference sequence GU183181.1 from GenBank

Phylogenetic Tree Analysis of Fungal Isolates

The molecular identification of the fungi isolated from honeybees revealed two major groups among the isolated fungi, Ascomycota and Basidiomycota, according to internal transcribed spacer (ITS) sequencing of rDNA. The phylogenetic tree (Figure 5) showed multiple clustered isolates in the Ascomycetes group, reflecting a predominant detection of the species, *Akanthomyces lecanii*. Phylogenetically closely related isolates and isolates of uncertain affiliation were included in "unknown and ascomycete fungi". Comparison of the ITS sequence of one of the isolates and the reference sequence GU183118. 1 (NCBI) showed 81% similarity to *L. lecanii* with 4% sequence gaps.

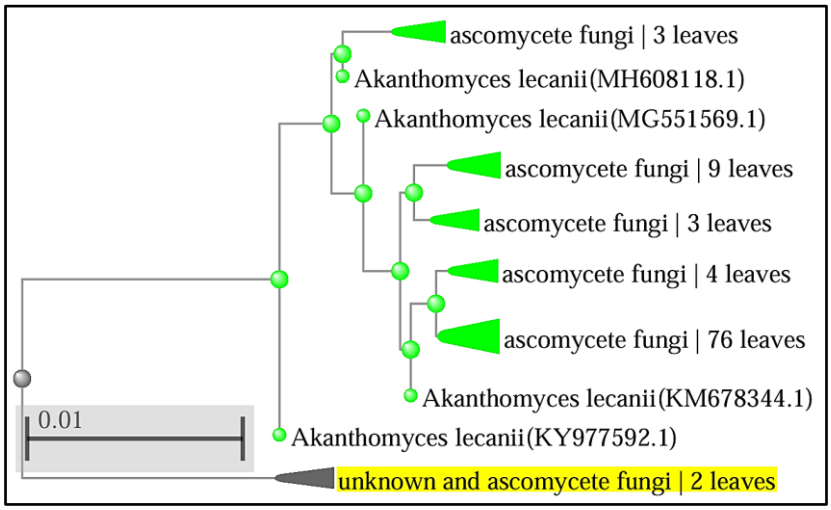


Figure 5: Phylogenetic tree illustrating the evolutionary placement of *Akanthomyces lecanii* (formerly *L. lecanii*) among ascomycete fungi. The yellow-highlighted clade includes two unclassified fungal isolates, one of which corresponds to *A. lecanii*

Antifungal sensitivity tests

As described in Table 1 and Figure 6, *L. lecanii* exhibited varying responses to the four antifungals studied. Amphotericin B was the most effective, with a decrease in absorbance of 0.343±0.003 in the control and 0.093±0.005, which was statistically significant at the highest concentration (400 µg/mL). Nystatin

demonstrated a strong inhibitory effect, with an absorbance of 0.103±0.001 at 400 µg/mL. Similarly, Fluconazole was only moderately effective, with an absorbance of 0.224±0.009 at the highest concentration. Griseofulvin was the least active drug, with an absorbance of 0.402±0.004 at 400 µg/mL. Statistical analysis indicated that the treatment effect was highly significant (P < 0.0001), with LSD 0.008 confirming the significance of the observed differences.

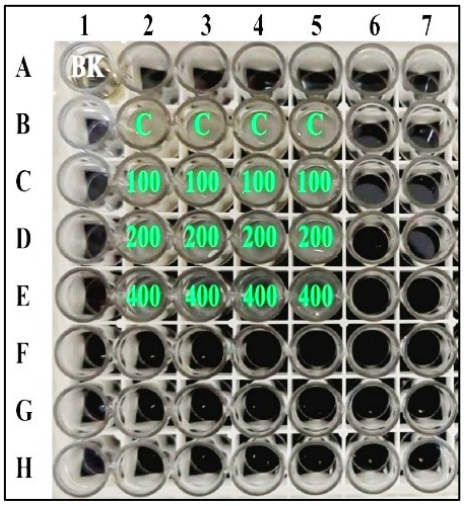


Figure 6: Microdilution assay showing antifungal susceptibility of *L. lecanii* to Amphotericin B, Fluconazole, Nystatin, and Griseofulvin

BK: blank (Background absorbance); C: Control. The concentrations of the antifungal agents are indicated for rows C, D, and E as follows: Column 2: Amphotericin B Concentrations; Column 3: Concentrations of Fluconazole; Column 4: Concentrations of Nystatin; Column 5: Concentrations of Griseofulvin

Table 1: Effect of Different Concentrations of Four Antifungal Agents on the Growth of *L. lecanii* Measured by Optical Density at 530 nm

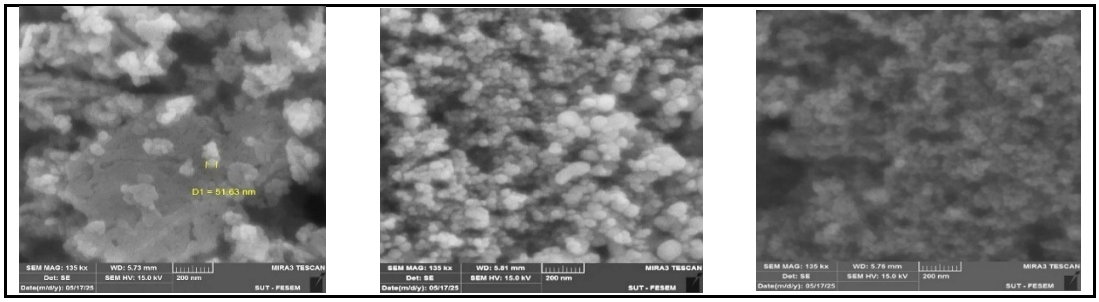
Concentrations	Antifungal agent concentrations (µg/mL) (Mean ± SE)			
Antifungal agents	**0**	**100**	**200**	**400**
Amphotericin B	0.343±0.003 Aa	0.292±0.004 Ab	0.205±0.003 Ac	0.093±0.005 Ad
Fluconazole	0.335±0.002 ABa	0.325±0.001 Bb	0.296±0.002 Bc	0.224±0.009 Bd
Nystatin	0.327±0.002 Ba	0.302±0.002 Cb	0.296±0.004 Bb	0.103±0.001 Cc
Griseofulvin	0.366±0.004 Ca	0.359±0.002 Da	0.358±0.003 Ca	0.402±0.004 Db
P-value	< 0.0001			
LSD	0.008			

Capital letters in the same column indicate significant differences between means, while lowercase letters in the same row indicate significant differences within means

Characterisation of Nanoparticles

Surface Characterisation using Field Emission Scanning Electron Microscope (FESEM)

FESEM images of ZnO-NPs, CuO-NPs, and SiO_2-NPs (Figure 7) exhibited different morphological and surface features. The ZnO nanoparticles had a uniform spherical shape with smooth surfaces, clear edges, and a particle size between 30 and 70 nm, and no obvious cracks or agglomeration were observed. CuO nanoparticles exhibited a spherical to near spherical shape and were dispersed in an agglomerated and aggregated manner with smooth and defect-free surfaces. In contrast, SiO_2 nanoparticles showed large differences in sizes and shapes, varying from under 50 nm to around 300 nm in diameter. The majority of particles were spherical in shape with a certain degree of roughness, accompanied by some agglomeration and small projections on the surface with an irregular edge.

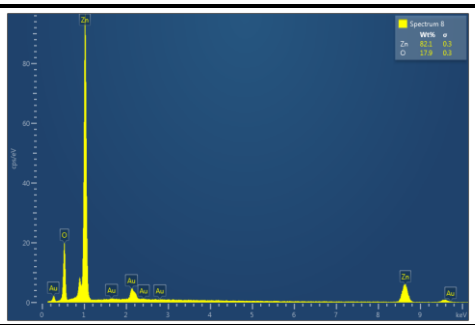


1 2 3

Figures 7: Field Emission Scanning Electron Microscopy (FESEM) images of ZnO (1), CuO (2), and SiO_2 (3) nanoparticles showing the morphological and surface characteristics of each type

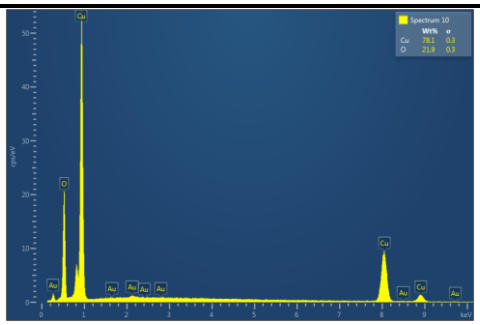
Analysing the structure of nanoparticles using Energy Dispersive X-ray Spectroscopy (EDX)

The EDX analysis confirmed the elements and purity present in the ZnO, CuO, and SiO_2 NPs (Figure 8). For ZnO-NPs, zinc and oxygen were present at weight percentages of 82.13% and 17.87%, with atomic percentages of 52.95% and 47.05%, respectively. CuO-NPs contained 78.07 wt% Cu and 21.93 wt% O, and atomic ratios of 47.27% and 52.73%. The weight percentages of Si and O for SiO_2-NPs were 45.42% and 54.58%; atomic percentages were 32.16% and 67.84%. Minor Au peaks could be observed in all the spectra as a result of the gold coating for imagery to improve the conductivity, which were removed when analysing the doped particles.



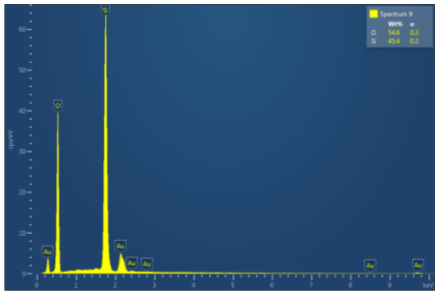
Elements	Weight percentage %	Atomic percentages %
Zn	82.13	52.95
O	17.87	47.05

1



Elements	Weight percentage %	Atomic percentages %
Cu	78.07	47.27
O	21.93	52.73

2



Elements	Weight percentage %	Atomic percentages %
Si	45.42	32.16
O	54.58	67.84

3

Figure 8: EDX spectra of ZnO (1), CuO (2), and SiO_2 (3) nanoparticles, with the corresponding elemental composition presented as weight and atomic percentages

Antifungal activity of nanoparticles

Antifungal activity was determined by the Broth Microdilution Method with a 96-well microtiter plate. As presented in Table 2, the results showed significant differences among all treatments in their suppressive effects on *L. lecanii* with a very

highly significant *P-value* (P<0.0001). Least Significant Difference (LSD) at the 0.05 level of significance was 0.264, meaning that the differences among applied concentrations were statistically acceptable and reliable.

Zinc oxide nanoparticles (ZnO-NPs), the highest antifungal activity was observed against *Lecanicillium lecanii,* where the mean value of growth was reduced significantly from 1.453±0.13 at 0µg/mL to 0.736±0.03 at 500µg/mL of Zn concentration. Copper oxide nanoparticles (CuO-NPs) showed moderate antifungal activity since the average growth at 500µg/mL (1.219±0.11) was still similar to the control (there was no statistical difference in mean growth at different concentrations). Silicon dioxide nanoparticles (SiO_2-NPs) showed moderate concentration-dependent effects on growth (1.047±0.01 at 100µg/mL, decrease to 0.989±0.44 at 500µg/mL), but less effectively than ZnO-NPs.

Thyme oil in combination with 95% ethanol reduced fungal growth to 0.298 ± 0.006 at 500µg/mL, whereas cinnamon oil with ethanol reduced fungal growth to 0.236±0.01 at 500µg/mL. The solvent control (95% ethanol) demonstrated a minimal inhibitory action with only a slight reduction from 0.07±0.005 to 0.028±0.002 at 500µg/mL. At 0µg/mL, the negative control (NC) had a growth rate of 0.139±0.07, whereas the positive control (PC), Carbendazim 50%, had the maximum inhibition, which was the lowest growth value among all, which was 0.075±0.001 at 500µg/mL.

Statistical analysis indicated that there were significant differences between treatments (P< 0.0001) with an LSD value of 0.264.

Table 2: Treatment concentrations and their effect on the growth of *L. lecanii*

Concentration	Concentrations (µg\mL) (mean ±SE)			
Substance	0	10	100	500
ZnO	1.453±0.13Aa	1.04±0.03Ab	0.855±0.05ABbc	0.736±0.03Ac
CuO	1.019±0.01Bab	0.999±0.09Aab	0.808±0.03Aa	1.219±0.11Bb
SiO2	0.702±0.11Ca	0.992±0.05Ab	1.047±0.01Bb	0.989±0.44Ab
Thymol oil + Ethanol 95%	0.745±0.01Ca	0.596±0.01Bab	0.447±0.009Cbc	0.298±0.006Cc
Cinnamon oil + Ethanol 95%	0.589±0.02Ca	0.471±0.02Bab	0.353±0.01Cbc	0.236±0.01Cac
Only Ethanol 95%	0.07±0.005Da	0.056±0.004Ca	0.042±0.02Da	0.028±0.002Ca
NC	0.139±0.07Da	0.111±0.05Ca	0.083±0.04Da	0.056±0.02Ca
PC	0.188±0.004Da	0.15±0.003Ca	0.113±0.002Da	0.075±0.001Ca
P-value	<0.0001			
LSD	0.264			

Capital letters in the same column indicate significant differences between means, while lowercase letters in the same row indicate significant differences within means

Discussion

The present study demonstrated that ZnO-NPs exerted a significant inhibitory effect on the growth of *L. lecanii*, as the inhibition of fungal growth was significantly reduced with the increasing concentration of the nanoparticles, reaching the highest rate at 500 µg/mL. This is consistent with the findings of Raghupathi *et al.* (25) They demonstrated that the efficacy of ZnO-NPs is attributed to their ability to generate reactive oxygen species (ROS), which directly damage the cell wall and membrane of fungal cells, leading to cell death. Furthermore, the smaller dimensions of ZnO-NPs permit their infiltration through the fungal cell walls, resulting in intracellular accumulation and therefore interaction with nucleic acids and proteins, generating oxidative stress and inducing apoptosis-like cell death (26, 27). The generation of ROS, release of ions, high surface reactivity, and cellular internalisation may explain the efficacy of ZnO-NPs as a potent antifungal agent in the present study, and demonstrate the potential utility of ZnO-NPs as a new alternative to conventional antifungal agents.

CuO-NPs and SiO_2-NPs exhibited only a moderate inhibitory effect on fungi at the concentrations tested. These differences in efficacy underscore the importance of nanoparticle physicochemical properties, including particle size, surface charge, and ion release, in their antifungal efficacy (28, 29). This may indicate that the fungus possesses defence mechanisms that reduce the effect of nanoparticles, such as the production of antioxidants or biofilm formation, that hinder the penetration of the active particles into the cell (17).

This finding is supported by Azam *et al.* (2012), who reported that CuO-NPs are less effective than ZnO-NPs in ROS induction and antifungal activity. Previous studies, such as Rabiee *et al.* (30), which have reported CuO-NPs effectiveness against other fungi, including *Alternaria* spp. and *Fusarium* spp. however, this does not mean that they have the same degree of response, as differential levels of response

were dependent on the fungi and their cellular structures.

A major strength of this study is that the antifungal effectiveness of three nanoparticles was compared under identical experimental conditions to determine relative effectiveness. Using the standard broth microdilution assay as described by Wiegand *et al.* (31), with appropriate replicates, enhanced the validity and reliability of the results obtained. The use of serial dilution and the revised experiment conducted in replicate treatments further confirmed the reproducibility of the findings. However, all the results presented in this study are limited to laboratory levels, and if generalising to the natural environment, the limited effects of these particles on honeybees should be made with caution because the effects of these nanoparticles on honeybees have not been researched in the field (32). Further, the study was limited in focusing on a single species of fungus, limiting the application of the results.

SiO_2-NPs exhibited an intermediate effect, a growth reduction for increasing concentration; however, they were less effective than ZnO-NPs. This is consistent with the findings of Sirelkhatim *et al.* (33) who observed that SiO_2 may serve more effectively as a carrier, rather than as an antifungal.

Compared to CuO and SiO_2 nanoparticles, ZnO nanoparticles exhibited improved antifungal activity against *L. lecanii*. The reason for improved activity could be related to the smaller size of the ZnO nanoparticles, which facilitated a larger surface area for interaction, a positive charge on the surface of the ZnO enhanced electrostatic attraction leading to binding to negatively charged fungal cell membranes, and finally, higher chemical reactivity leading to effective generation of reactive oxygen species that cause oxidative damage to fungal cells. CuO nanoparticles that are larger and have different surface chemistry exhibited moderate antifungal activity, while chemically inert SiO_2 nanoparticles only exhibited minimal efficacy from physical interactions. The cumulative physicochemical properties explain the increase in inhibition of fungal growth with ZnO nanoparticles in the current study (19, 34, 35).

Although the effects of nanoparticles on *L. lecanii* appear to be promising from an antifungal perspective, it should be noted that this was an *in vitro* study, and any potential toxicity of nanoparticles to non-target organisms must be noted. Future investigations of these nanoparticles under field conditions will help to assess their impact on the environment and practical use. Future studies should incorporate these environmental assessments and long-term field experiments if the results of using nanoparticles in the suppression of fungal pathogens are going to be safely implemented.

Conclusion

The research recommends conducting field trials to evaluate the effects of ZnO-NPs on honeybee colonies, as well as molecular studies to determine their antifungal mechanisms. In addition, investigations into potential synergies with natural products such as essential oils or plant extracts would be of interest. These results ultimately represent progress toward reinforcing honeybee immunity and reducing economic losses due to fungal diseases through safer, environmentally friendly measures of treatment.

List of Abbreviations

ITS: Internal Transcribed Spacer
NPs: Nanoparticles
ZnO: Zinc Oxide
CuO: Copper Oxide
SiO_2: Silicon Dioxide (Silica)
ROS: Reactive Oxygen Species
SDA: Sabouraud Dextrose Agar
DNA: Deoxyribonucleic Acid
PCR: Polymerase Chain Reaction
NCBI: National Center for Biotechnology Information
BLAST: Basic Local Alignment Search Tool
CLSI: Clinical and Laboratory Standards Institute
FESEM: Field Emission Scanning Electron Microscope
EDX: Energy-Dispersive X-ray Spectroscopy
TEM: Transmission Electron Microscopy
XRD: X-ray Diffraction
FTIR: Fourier-Transform Infrared Spectroscopy
SDB: Sabouraud Dextrose Broth
SPSS: Statistical Package for the Social Sciences
ANOVA: Analysis of Variance
LSD: Least Significant Difference
SE: Standard Error
rDNA: Ribosomal DNA
NCBI: National Centre for Biotechnology Information
PDA: Potato Dextrose Agar
NC: Negative Control
PC: Positive Control

Declarations

Ethical approval and consent to participate

Not required.

Consent for publication

Availability of data and materials
The data and materials associated with this review will be made available by the corresponding author upon reasonable request.

Conflict of interest
The authors declare that there is no conflict of interest.

Funding
None

Author's contributions
All authors contributed to the study design, data collection, analysis, and manuscript preparation. All authors have read and approved the final manuscript.

Acknowledgment
Nil.

References

1. Papa G, Maier R, Durazzo A, Lucarini M, Karabagias IK, Plutino M, Bianchetto E, Aromolo R, Pignatti G, Ambrogio A, Pellecchia M. The honey bee Apis mellifera: An insect at the interface between human and ecosystem health. Biology. 2022 Feb 1;11(2):233. https://doi.org/10.3390/biology11020233
2. Kim DY, Maeng S, Cho SJ, Park HJ, Kim K, Lee JK, Srinivasan S. The Ascosphaera apis infection (Chalkbrood disease) alters the gut bacteriome composition of the honeybee. Pathogens. 2023 May 19;12(5):734. https://doi.org/10.3390/pathogens12050734
3. Jovanovic NM, Glavinic U, Stevanovic J, Ristanic M, Vejnovic B, Dolasevic S, Stanimirovic Z. A field trial to demonstrate the potential of a vitamin B diet supplement in reducing oxidative stress and improving hygienic and grooming behaviors in honey bees. Insects. 2025 Jan 2;16(1):36. https://doi.org/10.3390/insects16010036
4. Bankova V, Bertelli D, Borba R, Conti BJ, da Silva Cunha IB, Danert C, Eberlin MN, I Falcão S, Isla MI, Moreno MI, Papotti G. Standard methods for Apis mellifera propolis research. Journal of Apicultural Research. 2019 Mar 15;58(2):1-49. https://doi.org/10.1080/00218839.2016.1222661
5. Iorizzo M, Letizia F, Ganassi S, Testa B, Petrarca S, Albanese G, Di Criscio D, De Cristofaro A. Recent advances in the biocontrol of nosemosis in honey bees (Apis mellifera L.). Journal of Fungi. 2022 Apr 20;8(5):424. https://doi.org/10.3390/jof8050424
6. El-Sayed AS, Fathy NA, Labib M, El-Baz AF, El-Sheikh AA, Moustafa AH. Biological control of nosemosis in Apis mellifera L. with Acacia nilotica extract. Scientific Reports. 2024 Nov 16;14(1):28340. https://doi.org/10.1038/s41598-024-78874-6
7. Akkoç S, Karaca İ, Karaca G. Effects of some entomopathogen fungi on Apis mellifera L. and Bombus terrestris L. Süleyman Demirel University Journal of the Institute of Natural and Applied Sciences. 2019 Aug 25; 23(2):433-9. https://doi.org/10.19113/sdufenbed.477889
8. Xie T, Jiang L, Li J, Hong B, Wang X, Jia Y. Effects of Lecanicillium lecanii strain JMC-01 on the physiology, biochemistry, and mortality of Bemisia tabaci Q-biotype nymphs. PeerJ. 2019 Sep 16;7:e7690. https://doi.org/10.7717/peerj.7690
9. Nikhade PB, Bhalkare SK, Patil YB, Ingle YV, Satpute NS, Undirwade DB. Compatibility and Toxicity of Lecanicillium lecanii with Insecticides against Cotton Aphids In vitro. Journal of Advances in Biology & Biotechnology. 2024 Sep 18;27(10):47-54. https://doi.org/10.9734/jabb/2024/v27i101428
10. Hadi MS, Taufiqurrahman AF, Choliq FA, Istiqomah I, Karindah S. Pathogenicity of Entomophatogenic Fungi Lecanicillium lecanii Against Predator Insect Menochilus Sexmaculatus. Planta Tropika. 2020 Nov 28;8(2):63-8. https://doi.org/10.18196/pt.2020.115.63-68
11. Witharana S, Napagoda MT, editors. Nanotechnology in modern medicine. Springer; 2023. https://doi.org/10.1007/978-981-19-8050-3
12. Rai M, Ingle A. Role of nanotechnology in agriculture with special reference to management of insect pests. Applied microbiology and biotechnology. 2012 Apr;94(2):287-93. https://doi.org/10.1007/s00253-012-3969-4
13. Anjum A, Das M, Garg R. Introduction to Nanotechnology: Transformative Frontier. InSmart and Sustainable Applications of Nanocomposites 2024 (pp. 1-35). IGI Global Scientific Publishing. https://doi.org/10.4018/979-8-3693-1094-6.ch001
14. Goulson D, Nicholls E, Botías C, Rotheray EL. Bee declines driven by combined stress from parasites, pesticides, and lack of flowers. Science. 2015 Mar 27;347(6229):1255957. https://doi.org/10.1126/science.1255957
15. Raymann K, Shaffer Z, Moran NA. Antibiotic exposure perturbs the gut microbiota and elevates mortality in honeybees. PLoS

biology. 2017 Mar 14;15(3):e2001861. https://doi.org/10.1371/journal.pbio.2001861
16. Sharma P, Sharma A, Sharma M, Bhalla N, Estrela P, Jain A, Thakur P, Thakur A. Nanomaterial fungicides: in vitro and in vivo antimycotic activity of cobalt and nickel nanoferrites on phytopathogenic fungi. Global Challenges. 2017 Dec;1(9):1700041. https://doi.org/10.1002/gch2.201700041
17. Parada J, Tortella G, Seabra AB, Fincheira P, Rubilar O. Potential antifungal effect of copper oxide nanoparticles combined with fungicides against botrytis cinerea and fusarium oxysporum. Antibiotics. 2024 Feb 26;13(3):215. https://doi.org/10.3390/antibiotics13030215
18. Zheng LP, Zhang Z, Zhang B, Wang JW. Antifungal properties of Ag-SiO2 core-shell nanoparticles against phytopathogenic fungi. Advanced Materials Research. 2012 Mar 28;476:814-8. https://doi.org/10.4028/www.scientific.net/AMR.476-478.814
19. Mosquera-Sánchez LP, Arciniegas-Grijalba PA, Patiño-Portela MC, Guerra–Sierra BE, Muñoz-Florez JE, Rodríguez-Páez JE. Antifungal effect of zinc oxide nanoparticles (ZnO-NPs) on Colletotrichum sp., causal agent of anthracnose in coffee crops. Biocatalysis and Agricultural Biotechnology. 2020 May 1;25:101579. https://doi.org/10.1016/j.bcab.2020.101579
20. Alberoni D, Gaggìa F, Baffoni L, Di Gioia D. Beneficial microorganisms for honey bees: problems and progresses. Applied microbiology and biotechnology. 2016 Nov;100(22):9469-82. https://doi.org/10.1007/s00253-016-7870-4
21. Watanabe T. Pictorial atlas of soil and seed fungi: morphologies of cultured fungi and key to species. CRC press; 2002 Apr 18. https://doi.org/10.1201/9781420040821
22. Samson RA, Evans HC, Latgé JP. Atlas of entomopathogenic fungi. Springer Science & Business Media; 2013 Mar 9.
23. White TJ, Bruns T, Lee SJ, Taylor J. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. PCR protocols: a guide to methods and applications. 1990 Jan 1;18(1):315-22. https://doi.org/10.1016/B978-0-12-372180-8.50042-1
24. Procop GW. Performance standards for antifungal susceptibility testing of yeasts. Clinical and Laboratory Standards Institute; 2022.
25. Raghupathi KR, Koodali RT, Manna AC. Size-dependent bacterial growth inhibition and mechanism of antibacterial activity of zinc oxide nanoparticles. Langmuir. 2011 Apr 5;27(7):4020-8. https://doi.org/10.1021/la104825u
26. He L, Liu Y, Mustapha A, Lin M. Antifungal activity of zinc oxide nanoparticles against Botrytis cinerea and Penicillium expansum. Microbiological research. 2011 Mar 20;166(3):207-15. https://doi.org/10.1016/j.micres.2010.03.003
27. Abdelaziz AM, Salem SS, Khalil AM, El-Wakil DA, Fouda HM, Hashem AH. Potential of biosynthesized zinc oxide nanoparticles to control Fusarium wilt disease in eggplant (Solanum melongena) and promote plant growth. BioMetals. 2022 Jun;35(3):601-16. https://doi.org/10.1007/s10534-022-00391-8
28. Rasmussen JW, Martinez E, Louka P, Wingett DG. Zinc oxide nanoparticles for selective destruction of tumor cells and potential for drug delivery applications. Expert opinion on drug delivery. 2010 Sep 1;7(9):1063-77. https://doi.org/10.1517/17425247.2010.502560
29. He L, Liu Y, Mustapha A, Lin M. Antifungal activity of zinc oxide nanoparticles against Botrytis cinerea and Penicillium expansum. Microbiological research. 2011 Mar 20;166(3):207-15. https://doi.org/10.1016/j.micres.2010.03.003
30. Rabiee N, Bagherzadeh M, Kiani M, Ghadiri AM, Etessamifar F, Jaberizadeh AH, Shakeri A. Biosynthesis of copper oxide nanoparticles with potential biomedical applications. International Journal of Nanomedicine. 2020 Jun 9:3983-99. https://doi.org/10.2147/IJN.S255398
31. Wiegand I, Hilpert K, Hancock RE. Agar and broth dilution methods to determine the minimal inhibitory concentration (MIC) of antimicrobial substances. Nature protocols. 2008 Feb;3(2):163-75. https://doi.org/10.1038/nprot.2007.521
32. Nel A, Xia T, Madler L, Li N. Toxic potential of materials at the nanolevel. science. 2006 Feb 3;311(5761):622-7. https://doi.org/10.1126/science.1114397
33. Sirelkhatim A, Mahmud S, Seeni A, Kaus NH, Ann LC, Bakhori SK, Hasan H, Mohamad D. Review on zinc oxide nanoparticles: antibacterial activity and toxicity mechanism. Nano-micro letters. 2015 Jul;7(3):219-42. https://doi.org/10.1007/s40820-015-0040-x
34. Elshafie HS, Osman A, El-Saber MM, Camele I, Abbas E. Antifungal activity of green and chemically synthesized ZnO nanoparticles against Alternaria citri, the causal agent citrus black rot. The Plant Pathology Journal. 2023 Jun 1;39(3):265. https://doi.org/10.5423/PPJ.OA.02.2023.0035

35. Sharma I, Sharma MV, Haque MA, Simal-Gandara J. Antifungal action and targeted mechanism of Bio fabricated zinc oxide (ZnO) nanoparticles against Ascochytafabae. Heliyon. 2023 Sep 1;9(9). https://doi.org/10.1016/j.heliyon.2023.e19179