Zoo Botanica
ISSN: 3007-2050 (E), 3007-2042 (P)

Research Article

Eco-friendly synthesis of copper oxide nanoparticles using Moringa oleifera and Syzygium cumini: termiticidal and antibacterial comparisons

Asma Iqbal[1], Ayesha Aihetasham[1], Muhammad Imran Din[2] Zaib-un-Nisa Hussain[2], Maham Mahnoor Iqbal Cheema[3]
[1]Institute of Zoology, University of the Punjab, Lahore 54590, Pakistan.
[2]School of Chemistry, University of the Punjab, Lahore 54590, Pakistan.
[3]Govt. Graduate College, Satellite Town, Gujranwala 52520, Pakistan.

Correspondence
Asma Iqbal
asmacheema856@gmail.com

Article History
Received: Mar 21, 2025
Accepted: July 30, 2025
Published: August 31, 2025

ABSTRACT

Medicinal plants are extensively utilized in various traditional medicinal systems owing to their wide range of pharmacological properties and minimal adverse reactions in living organisms. The study was aim to assesses the green synthesis and characterization of Copper oxide nanoparticle against *Hetrotermes indicola* and antibacterial activity. Utilizing plant extracts for the production of metal nanoparticles shows great potential as an environmentally sustainable synthesis approach. In particular, green synthesis of metal-based nanoparticles using medicinal plants represents a promising alternative to conventional chemical and physical methods. The study at hand amalgamated the medicinal attributes of *Moringa oleifera* and *Syzygium cumini* leaves, known for their termiticidal and antibacterial qualities to synthesize copper oxide nanoparticles (CuO-NPs), thereby creating a value-added, multifunctional, inorganic material. The preference for biological pathways in nanoparticle fabrication is steadily increasing due to factors such as cost-effectiveness, scalability, and environmental sustainability. In this context, a green synthesis method utilizing aqueous leaf extracts has been successfully employed to fabricate CuO-NPs. Additionally, the termiticidal efficacy of the CuO-NPs was evaluated through a bioassay approach. A comparison between two groups revealed a comparatively lower termiticidal impact with the CuO-NPs derived from *S. cumini*, whereas a significantly higher efficacy was observed with those from *M. oleifera*. This suggests that the phytochemicals present in *M. oleifera* may contribute more effectively to the synthesis of bioactive CuO-NPs with termiticidal properties. Green synthesized CuO-NPs from *S. cumini* have the largest zone of inhibition against *Escherichia coli*, indicating they are particularly effective against this bacterium while CuO-NPs from *M. oleifera* showed broad spectrum antibacterial activity with the highest inhibition against *Bacillus cercus*. These results highlight the selective efficacy of each extract-derived nanoparticle type against specific microbial strains. This study underscores the significant potential of green-synthesized CuO-NPs as effective termiticidal and antibacterial agents. These nanoparticles are capable of mitigating the harmful effects caused by *H. indicola* and exhibit antibacterial properties against various pathogenic strains. Overall, the findings advocate for the utilization of plant-based green synthesis methods in developing biofunctional nanomaterials for pest and microbial control.

Keywords: Nanotechnology; termiticidal activity; H. indicola; M. oleifera; copper oxide; nanoparticles; antibacterial activity.

INTRODUCTION

More than 3,000 species of termites have been documented across humid, subtropical, and moderate regions (Krishna et al., 2013; Shah et al., 2024; Su et al., 2016). In contrast, a total of 183 destructive species of termite species (Hassan & Morrell, 2021; Nasser et al., 2024). The process of organic waste decomposition is

https://doi.org/10.55627/zoobotanica.003.02.1246

attributed to the synergistic relationship between termites, bacteria, and fungi, facilitating food digestion (Su et al., 2016; Zhou et al., 2019). Termites, identified as one of the critical deleterious pests in tropical areas, inflict considerable harm on wood structure, agriculture, as well as to residential structures (Ahmed et al., 2016; Shah et al., 2024). The termite population is characterized by a diverse assemblage of species. (Akpan et al., 2020). Within tropical and subtropical regions worldwide exhibit notable detrimental effects on structures, furniture, and various timber goods. Among this extensive selection of species, a mere 3.2% are recognized as pest species accountable for causing substantial damage to human possessions Among the extensive diversity of termite species, only about 3.2% are recognized as pests responsible for substantial damage to human property (Austin et al., 2002). These termites attack cultivated fruits, ornamental plants, forest nurseries, and a wide range of wooden structures, including buildings, bridges, furniture, storage facilities, poles, and railway sleepers (Donovan et al., 2007; Rouland-Lefèvre, 2011). In Pakistan, the agricultural sector faces significant economic losses from infestations by fungus-growing termites (Ahmed et al., 2011; Aljedani, 2023). The severity of termite damage to woodlands is influenced by their feeding preferences, nesting behavior, and colony size (Rouland-Lefèvre, 2011). Subterranean termites, in particular, consume a broad spectrum of fibrous materials due to their varied diet (Muhammad Afzal et al., 2017; Rasib et al., 2014). On a worldwide scale, subterranean termites are accountable for as much as 90% of financial losses and 70% of structural damage in construction (Kuswanto et al., 2015). There are number of techniques are used for the control of termites but one of them is chemical control. The most commonly used chemically formulated pesticides are organophosphate, pyrethroid are applied in soil and agroecosystem. As well as other non-repellent pesticides such as imidacloprid and fipronil are being used in dry and wet form into the soil (Faruq et al., 2015). Research worldwide has shown that numerous plant-derived compounds, such as those from *M oleifera* and *S. cumini*, exhibit strong termiticidal activity due to their toxic effects on termites. These natural extracts offer an eco-friendly and sustainable alternative for termite control, highlighting the potential of plant-based solutions in pest management (Aljedani, 2023). Currently, industries are making novel green nanotechnology techniques to reduce the adverse effects of non-green technology. Currently, industries are making novel green nanotechnology techniques to reduce the adverse effects of non-green technology (Al-Rawashdeh et al., 2013).

Nanotechnology has made significant strides in various domains, including biomedical applications, drug delivery, and biotechnology. Among the various nanomaterials, metal oxide nanoparticles have attracted considerable attention due to their unique physicochemical properties and wide range of functionalities. The major value of green synthesis is the use of harmless chemicals, ecologically safe solvents, and biodegradable products, which make it environment friendly and sustainable approach. (Raveendran et al., 2003). Copper oxide is widely used in engineering biological, environmental, and medicinal fields. Specifically, CuO-NPs have numerous applications in medicine and biology, for example in anticancer treatments (Gnanavel et al., 2017), antibacterial efficacy (Khan & Lee, 2020; Usman et al., 2013), anti-oxidative capability (Muthuvel et al., 2020), fungicidal efficacy (Khatami et al., 2019; Shammout & Awwad, 2021), and antimicrobial activity (Begum et al., 2019; Ebrahımı et al., 2017; Gowri et al., 2019; Ramzan et al., 2021). In addition, CuO-NPs synthesized through green methods often exhibit enhanced biocompatibility and reduced toxicity, further supporting their suitability for biomedical applications.

Green synthesis is particularly appealing due to its environmental friendliness and the potential for large-scale production. *M. oleifera*, a member of the Mceae family, has gathered substantial interest in present times because of its versatile applications in addition to numerous advantages for both agriculture and industry (Ashfaq & Ashfaq, 2012). These compounds are known for their many beneficial effects, including antioxidant, antihypertensive, and anti-inflammatory properties (Vongsak et al., 2013). This study presents innovative approach to synthesize CuO-NPs using aqueous extracts from *M. oleifera* and *S. cumini* leaves. The unique aspect of this approach is the combination of both plant leaves extracts, which serve as stabilizing and reducing agents, resulting to more efficient and stable nanoparticles. This green synthesis method not only aligns with sustainable chemistry principles but also takes advantage of the bioactive compounds found in these plant species, potentially giving the CuO-NPs distinct physicochemical properties. Copper nanoparticles (CuNPs) exhibit toxic effects on insects, making them a potential tool for biological pest control. This scientific approach offers a way to reduce reliance on chemical pesticides, lowering associated costs, while also providing an environmentally friendly, biodegradable alternative (Tahir et al., 2022).

The nanoparticles were thoroughly characterized using infrared and UV-visible spectroscopy, providing a comprehensive understanding of their structure and functionality. Additionally, the study goes beyond traditional analysis by evaluating the termiticidal and antibacterial activities of the synthesized CuO-NPs, specifically against *H. indicola*. This dual focus on pest and bacterial control demonstrates the broad-spectrum potential of these green-synthesized nanoparticles, making them relevant for both agricultural and medical applications. The combination of *M. oleifera* and *S. cumini* extracts in nanoparticle synthesis, along with the multifaceted evaluation of their biological activities, highlights the originality and practical significance of this research.

MATERIALS AND METHODS

Leaf Collection

Leaves from selected *M. oleifera* and *S. cumini* were collected from the Botanical Garden at the University of the Punjab, Lahore. They were shade-dried for three days and subsequently stored in polyethylene bags for experimental purposes.

Preparation of Aqueous Extract

Approximately 20g of ground leaves of *M. oleifera* was combined with 200 milliliters of distilled water and heated a duration of 2 hours at a temperature ranging from 80 to 90 degree centigrade. During this procedure, a solution of light brown hue was produced. Following this, the solution was allowed to cool to ambient temperature, passed through a Whatmann no. 1 filter paper, and then preserved in a refrigerator for future utilization.

Green Synthesis of Copper Oxide Nanoparticles (CuO-NPs)

The production of copper oxide nanoparticles (CuO-NPs) was conducted through a microwave-assisted environmentally friendly approach (Otari et al., 2012). In this process, a 50mL Erlenmeyer flask containing 2mM Cu(NO3)2 was reacted with a 10mL aqueous leaf extract of *M. oleifera* and *S. cumini* at room temperature.

To stabilize the pH of the mixture, a 1M NaOH solution was introduced to a 20mL extract of *M. oleifera* and *S. cumini*, which also contained a 1M precursor solution of copper nitrate Cu (NO3)2. Subsequently, the blend underwent magnetic stirring on a heated surface at a temperature of 60ºC for a duration of 30 minutes, leading to a discernible alteration in color that signified the successful environmentally sustainable production of copper oxide nanoparticles. Synthesis was confirmed using Ultra Violet-Visible spectroscopy, and the nanoparticles were separated from the supernatant by centrifugation. The nanoparticles were subsequently dehydrated in an oven at 70ºC for 42 hours.

Collection of *H. indicola*

The *H. indicola* workers and soldiers were collected from old *Populous euramericiana* trees in Lahore. These termites were placed on filter paper soaked in water and 5g of dehydrated soil in each petri dish for a minimum of one week.

Collection of Test Pathogens

All the bacterial cultures were obtained from Microbial Biotechnology Lab at the Institute of Zoology, University of the Punjab, Lahore. The test pathogens designated for this investigation are Gram-positive bacteria: *Bacillus cereus*, *Shigella flexneri*. Gram-negative bacteria: *Escherichia coli*.

Experimental Termiticidal Assay

A termiticide assay was conducted prior to the contact toxicity bioassay (Ahmad et al., 2011). Clean and sterilized filter paper pieces were cut in the shape of a petri dish. A stock solution of 2mg/mL concentration was prepared using n-hexane of analytical grade. The filter paper was carefully placed at the base of the petri dish, the stock solution of prepared CuO-NPs was evenly spread on it and then left undisturbed at room temperature for vaporization. Following solvent evaporation, 50 healthy termites were introduced into the test petri dishes, all of which were incubated at 28°C using desiccators for maintaining constant relative humidity.

Lethality was assessed by observing the termites after every 24 hours up to complete recording the number of deceased termites. The mortality rate was calculated by applying the subsequent formula.

$$\text{Mortality}(\%) = \frac{\text{No.of dead termites}}{\text{Total no.of termites}} \times 100$$

Characterization of CuO-NPs

CuO-NPs synthesized through biogenic methods were subjected to continuous examination using a UVD-3500 UV-Visible spectrophotometer. Furthermore, the biosynthesized copper oxide nanoparticles were further analyzed using Fourier-transform infrared spectroscopy is to characterize specific biomolecules existing in the extract, which facilitate the transformation of metallic salt into metallic nanoparticles. This comprehensive examination offers significant insights into the synthesis mechanism and the involvement of biomolecules in the reduction reaction. Fourier transform infrared (FTIR) Spectroscopy is a non-invasive analytical method that allows for the immediate monitoring of chemical characteristics linked to a variety of organic and inorganic bonding structures. Fourier-transform infrared spectroscopy is a valuable process for analyzing material composition, particularly in the context of biomolecules utilized in the production of bio-reduced metal nanoparticles with plant extracts. The characterization of functional groups was carried out utilizing an equipment Cary 630 Fourier Transform Infrared (FTIR) spectrophotometer, where samples were analyzed across a wavenumber spectrum of 4,000 to 650 cm-1 by averaging 20 scans at a resolution of 4 cm-1 (Din et al., 2022). X-ray Diffraction (XRD) analysis was used to identify the phase composition and crystalline size of Al2O3 NPs by using XRD diffractometer DS Advance, Bruker Corporation, USA operating at 30kV, 20mA, and Cu-Kα radiations having 1.54 Å. Scanning electron microscopy (SEM) analysis: (Hitachi S-4700) was performed for the determination of morphological structural of synthesized CuO NPs, operation at 25kV.

RESULTS AND DISCUSSION

Ultraviolet-Visible Spectroscopy of CuO-NPs

CuO nanoparticles display an excitation absorption band that enables the movement of an electron from the valence band to the conduction band (Elseman et al., 2016; Muthuvel et al., 2020). When copper oxide nanoparticles (CuO-NPs) are exposed to a photon, an electron is elevated to the conduction band, resulting in the formation of a gap in the valence band. The excitation results in a strong Columbic attraction between the hole and the excited electron due to their similar properties, leading to additional stabilizing energy. Nevertheless, in unstable nanomaterials, there is a possibility of recombination where the electron returns to its original state.

The successful production of CuO-NPs was demonstrated through a noticeable change in the color of the reaction solution, shifting from a pale blue tint to a deeper hue. This color modification signifies the conversion of copper ions into CuO-NPs with the assistance of the bioactive compounds present in *M. oleifera* extract. Subsequent validation of the nanoparticle formation was conducted using UV-Vis spectroscopy, revealing a distinct peak at 395 nm corresponding to the CuO-NPs (Figure 1) (Sundaramurthy & Parthiban, 2015). In this study, it is likely that the peak of the synthesized CuO-NPs corresponds to the peak reported in existing literature for CuO-NPs (Din et al., 2017). The study investigated the optical characteristics of copper oxide nanoparticles (CuO-NPs) by varying reaction parameters including concentration, pH, temperature, incubation time, and other variables, employing a double beam UV-Vis spectrophotometer for analysis (Akintelu et al., 2020). In this research, the pH level of the reaction solution significantly influences both the structure of the produced nanoparticles and the intensity of absorption spectra peaks. The absorbance peak ratio for CuO-NPs ranges between 409-417 nm. The advancement of copper ion reduction was observed by analyzing the UV-Vis spectrum of the reaction mixture, conducted five hours after diluting a small portion of the sample in deionized water. The UV-Vis spectral assessment was executed utilizing a double-beam UV Visible spectrophotometer.

Absorbance peaks of *M. oleifera* copper oxide nanoparticles are observed to be greater in magnitude when compared to those of S. cumini CuO-NPs, particularly within the wavelength range of 300 nm to 500 nm. Conversely, *S. cumini* CuO-NPs display a wider absorption band with lower peak absorbance levels in contrast to M. oleifera CuO-NPs. Both spectra display multiple peaks, indicating the presence of various size distributions and possibly different shapes or crystallinity of CuO-NPs. The absorbance decreases gradually after 500 nm, indicating the tailing effect, which is common in nanoparticles due to their size distribution.

The peak positions can be attributed to the specific electronic transitions within the CuO-NPs. For example, the peaks observed in the ultraviolet region (approximately 340-360 nm) may be indicative of the band gap transitions exhibited by the CuO-NPs. The discrepancies observed in the peak positions and intensities indicate potential differences in the particle size, morphology, and potentially the surface characteristics of the nanoparticles produced through the utilization of distinct plant extracts. Higher absorbance in *M. oleifera* CuO-NPs indicates that these nanoparticles might have a higher concentration or more significant light interaction due to their size or morphology. *M. oleifera* CuO-NPs exhibit stronger absorption, indicating potentially smaller or more uniformly distributed nanoparticles compared to *S. cumini* CuO-NPs. The distinct peaks suggested different synthesis mechanisms or stabilizing compounds from the plant extracts affecting the nanoparticle formation. This analysis offers valuable information on the impact of various biological extracts on the characteristics of produced nanoparticles, a critical aspect for customizing their utility across diverse sectors including biomedicine, catalysis, termite control, antimicrobial applications, and environmental cleanup.

The synthesis of copper oxide nanoparticles was confirmed by conducting UV-Vis spectrophotometer analysis across the wavelength spectrum of 200 to 800 nanometers, as depicted in Figure 1. The UV-Visible spectra displayed a prominent absorbance peak at 236 nanometers, indicating the formation of cuprous oxide nanoparticles (Renuga et al., 2020). This discovery aligns with findings documented by multiple researchers, as well as previous studies (Abel et al., 2021; Gebremedhn et al., 2019; Hazaa et al., 2021). The precise antimicrobial mechanism of copper oxide nanoparticles remains incompletely understood and remains a subject of ongoing debate within the scientific community (Prabhu & Poulose, 2012). It is widely recognized that compounds containing copper ions in the +1 and +2 oxidation states demonstrate strong antimicrobial characteristics. There is a growing interest among researchers to explore the antibacterial capabilities of various other inorganic nanoparticles. (Jeeva et al., 2014). CuO NPs can adhere to bacterial cell walls, causing disruption by altering membrane permeability and inhibiting cell respiration.

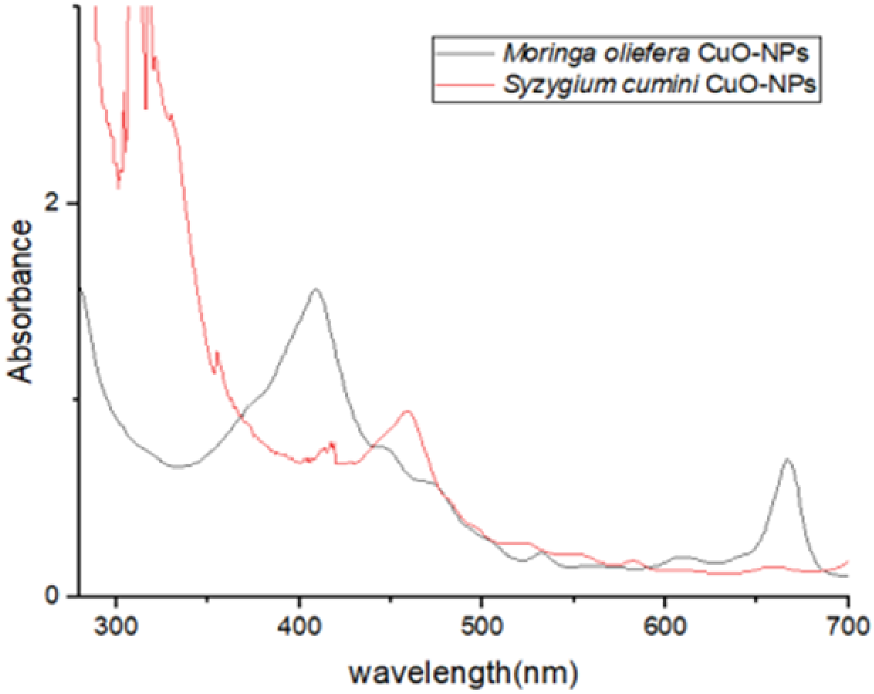

Figure 1. UV-Vis spectrum of *M. oleifera* and *S. cumini* Copper Oxide nanoparticles

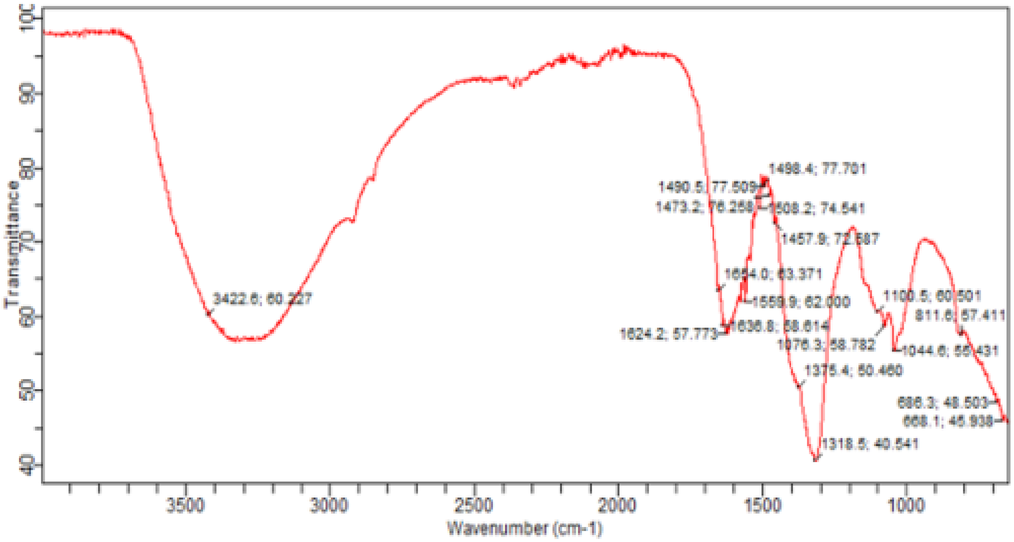

Figure 2. FTIR spectra of copper oxide nanoparticles synthesized using *M. oleifera.*

FTIR Analysis

Fourier-Transform Infrared (FT-IR) spectrum represents (Figure 2) the functional groups present in *M oleifera* mediated CuO-NPs. Broad O-H Stretching (3422.6 cm^{-1}): This indicated the presence of hydroxyl groups, which may come from water or hydroxylated compounds in the *M oleifera* extract used for synthesizing the nanoparticles C=O Stretching (1638.6 cm^{-1}): This peak suggested the presence of carbonyl groups, likely from organic compounds such as proteins or other biomolecules in the plant extract. C-H Bending (1498.4 cm^{-1}, 1473.2 cm^{-1}): These peaks indicated the presence of aliphatic or aromatic compounds. C-O Stretching (1100.5 cm^{-1}, 1044.6 cm^{-1}): The presence of these peaks indicates the presence of alcohols, ethers, or carboxylic acids.

The spectrum obtained from FTIR of *M. oleifera* mediated copper oxide nanoparticles revealed various functional groups that originate from the biomolecules in the plant extract used for nanoparticle synthesis. These functional groups play a role in stabilizing and capping the nanoparticles, preventing agglomeration and providing biological activity. The complexity of the plant extract is demonstrated by the existence of hydroxyl, carbonyl, and various other organic functional groups, which play a role in reducing and stabilizing CuO-NPs.

FT-IR spectrum represented (Figure 3) the functional groups present in *S. cumini* mediated CuO-NPs. Broad O-H Stretching (3422.8 cm^{-1}) this indicated the presence of hydroxyl groups, which may come from water or hydroxylated compounds in the *S. cumini* extract used for synthesizing the nanoparticles. N-H Bending/C=C Stretching (1559.9 cm^{-1}) this peak suggested the presence of amines or aromatic compounds. C-O and C-O-C Stretching (1189.0 cm^{-1}, 1027.8 cm^{-1}) these peaks indicated the presence of alcohols, ethers, or carboxylic acids. C-H Bending (1319.9 cm^{-1}) indicated aliphatic or aromatic compounds. Metal-Oxygen Stretching (620.9 cm-1) the presence of this peak confirms the formation of CuO-NPs.

Comparing this spectrum with the one from *M. oleifera* mediated CuO-NPs, both spectra exhibited similar functional groups, indicating that both plant extracts contribute similar types of biomolecules for nanoparticle synthesis. However, slight differences in peak positions and intensities can be observed, reflecting the unique composition of each plant extract.

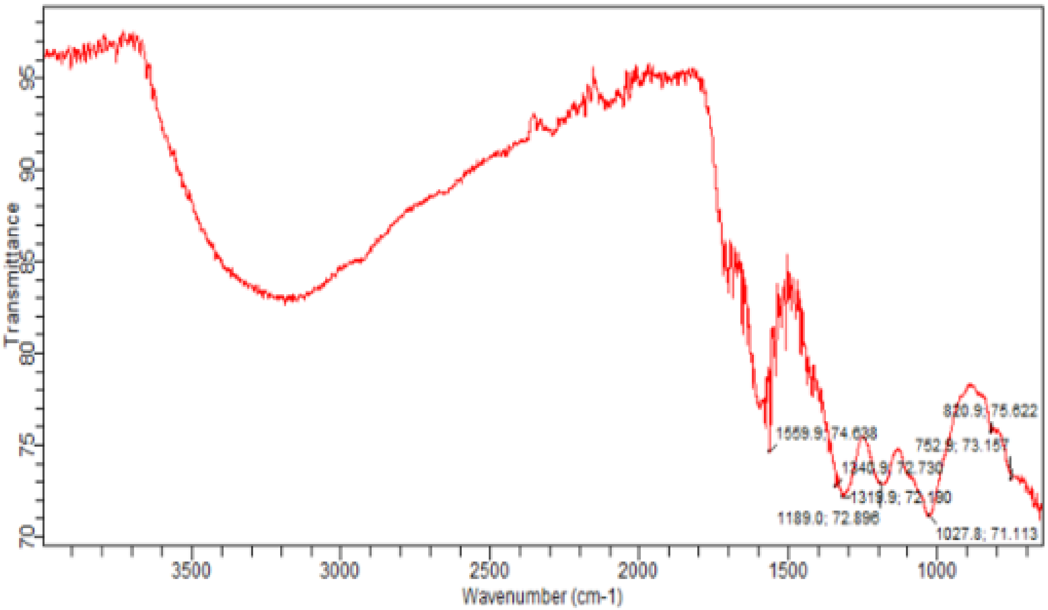

Figure 3. FTIR spectra of *S. cumini* mediated copper oxide nanoparticles.

Scanning Electron Microscopy

Scanning Electron Microscopy (SEM) are employed for the analysis of morphology at the nanometer and micrometer scales. The figure (4) showed SEM image of synthesized CuO-NPs in the presence of *M. oleifera* and *S. cumini* leaves extract. The SEM image of both NPs prepared through different extracts showed uniform and defined surface morphology. The less aggregation was found due to the presence of plant extract that act as strong stabilizing and capping agent.

One way ANOVA of Different Plants Extracts CuO-NPs Used for Termiticidal Activity Against *H.indicola*

In this study, we employed one-way ANOVA by using “Minitab” to analyze the synthesis of CuO-NPs, exploring how variations in experimental conditions or precursor materials impact the properties of the synthesized nanoparticles. With ANOVA analysis, our objective is to identify any notable variations in the average properties of copper oxide nanopaticles derived the leaves extracts of *M. oleifera* and *S. cumini*. This investigation offers valuable perspectives on the synthesis procedures and the potential utility of these nanoparticles in diverse sectors such as catalysis, electronics, biomedicine, termite control, and antimicrobial applications.

Table (1) showed that *M. oleifera* CuO-NPs appear to be more effective than *S. cumini* CuO-NPs against *H. indicola* based on the LC50 values, the high p-values indicate that the observed differences are not statistically significant.

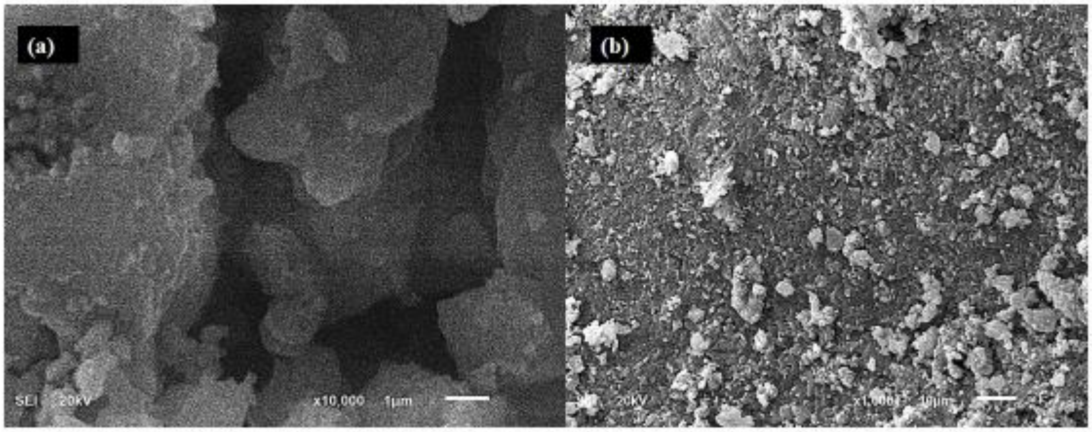

Figure 4. SEM analysis of CuO NPs prepared through a)*M. oleifera* and b)*S. cumini* leaves.

Additionally, the confidence intervals suggested there is considerable variability in the effectiveness of *S. cumini* CuO-NPs. This means the results may be due to random chance rather than a significant effect of the CuO-NPs.
Figure (5) showed the plot suggested that the response (e.g., termite mortality) to *M. oleifera* CuO-NPs followed a normal distribution, with an LC50 value around 115.368 ppm. The variability in the data is moderate, as indicated by the standard deviation and IQR.

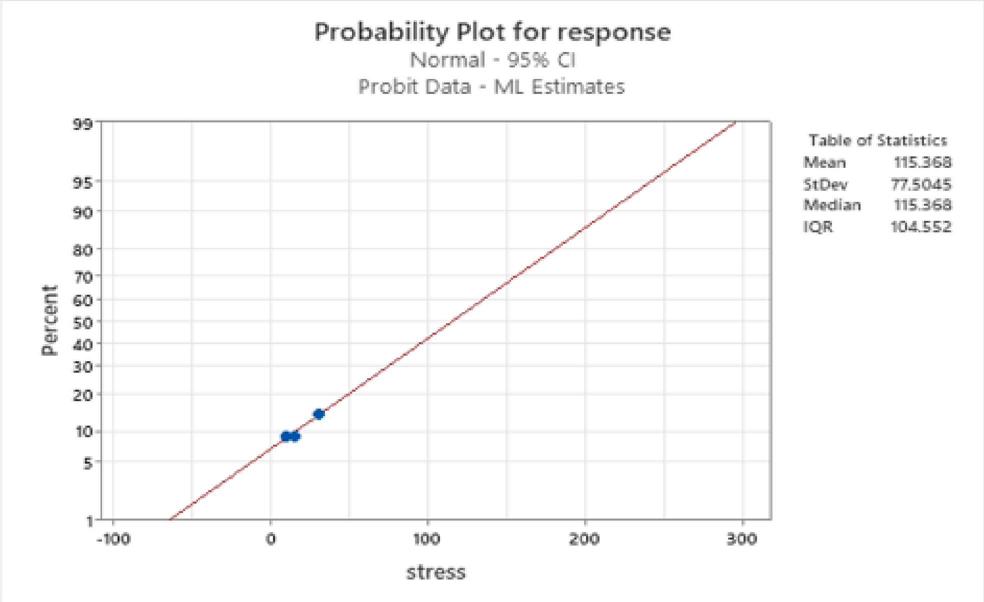

Figure 5. Probit analysis of *M oleifera* CuO-NPs.

Figure (6) illustrated that the probability plot and the associated statistics indicated that the stress response data from the *S. cumini* CuO-NPs have a mean and median of 295.110, suggesting symmetry. The high standard deviation and interquartile range highlight significant variability in the stress values. The single data point plotted fits well with the expected normal distribution line, but additional data points would be needed for a more comprehensive assessment. Table (2) demonstrated that conditions sharing the same letter are not statistically significantly distinct from one another. Each condition has a mean value with a grouping letter (a, b) assigned using the Tukey method with a 95% confidence level. The grouping letters indicate whether the means are statistically significantly different from each other. *S. cumini* produces CuO-NPs with a mean particle size that is not significantly different from the larger particles of *M. oleifera* either.

Table 1. LC_{50} values of green synthesized Copper oxide nanoparticles for their effectiveness in termiticidal activity against *H.indicola.*

Plants name	LC50(ppm)	(Upper confidence limit - Lower confidence limit) 95%CI	DF	P-value
M. oleifera CuO-NPs	115.368	278.758-48.0224	1	0.444
S. cumini CuO-NPs	295. 110	2379.32-1789.10	1	0.785

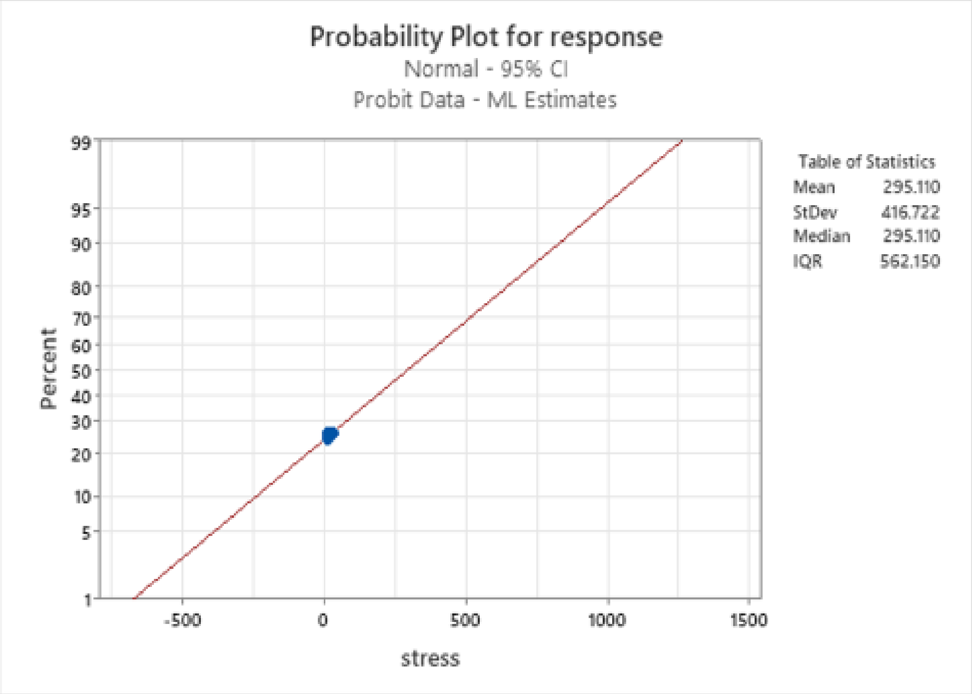

Figure 6. Probit analysis of *S. cumini* CuO-NPs.

Plant NPs	CuO-NPs particle size (Mean ±S.E)µL/L
M. oleifera	40.33±1.20^{a}
S. cumini	33.667±0.882$^{a\ b}$

Table 2. Average particle size of green synthesized copper Oxide nanoparticles

Table 3. Mean particle size by using different plant extracts.

Plants	Mean ±S.E	Significant
M. oleifera	23±1.15^{a}	Yes
S. cumini	18±0.577^{b}	No

Table (3) displayed the mean particle size obtained by using different plant extracts for termiticidal activity. By using *M. oleifera* extract, the mean particle size is 23 nanometers with a standard error of 1.15 nanometers. The notation "a" indicated that this result is statistically significant. By using *S. cumini* extract, the mean particle size is 18 nanometers with a standard error of 0.577 nanometers. The notation "b" suggested that there is no statistically significant difference compared to other results. Overall the table (2) showed that Group 1 (Mean = 23.00) is significantly different from Groups 2 (Mean = 18.00).

M. oleifera CuO-NPs exhibited the highest mortality rate, followed by *S. cumini* CuO-NPs. The *M. oleifera* extract showed a lower mortality rate compared to its CuO-NPs, while the *S. cumini* extract demonstrated (Figure 7 & 8) the lowest mortality rate.

Antibacterial Activity of Copper Oxide Nanoparticles

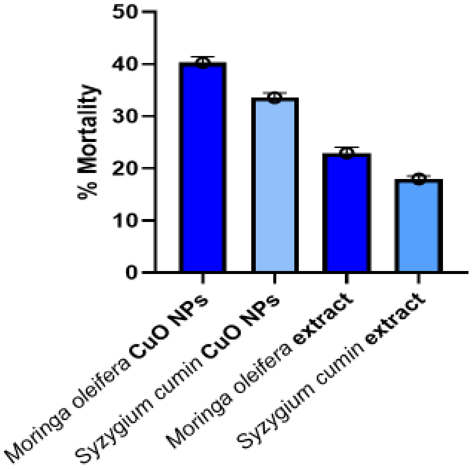

Figure 7. Impact of different plants derived extract and plant based CuO-NPs on mortality.

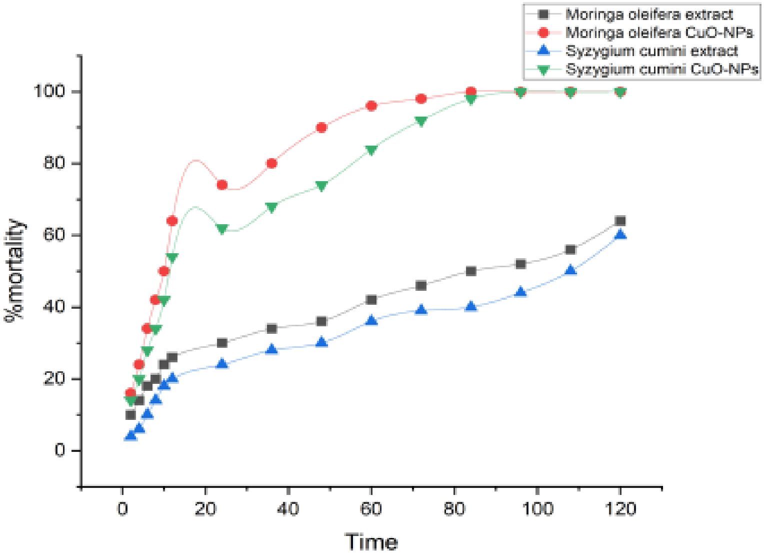

Figure 8. Impact of various plant extracts and plant-derived copper oxide nanoparticles on mortality rates.

The antimicrobial efficacy of copper oxide nanoparticles (CuO-NPs) produced via green synthesis was assessed using the well diffusion technique against three pathogenic bacteria, namely *E. coli, B. cereus,* and *S. flexneri*. The glassware and media were sterilized using an autoclave operating at a temperature of 121°C for a duration of 20 minutes. A bacterial suspension containing 108 colony-forming units per milliliter (CFU mL-1) was evenly spread across the nutrient agar (NA) surface. The nutrient agar (NA) medium was composed of peptone (5.0 g), beef extract (1.0 g), yeast extract (2.0 g), and agar (15.0 g), dissolved in distilled water as described by Atlas in 1995. Petri dishes were filled with 20 mL of nutrient agar for each bacterial sample. The bacterial cultures were then evenly distributed on the solidified agar surface. After the agar solidified, wells with a diameter of 6 millimeters were made using a sterilized stainless steel cork borer. Subsequently, 20 microliters of copper oxide nanoparticles (CuO-NPs) were added to individual wells. The plates were allowed to remain undisturbed for a 30-minute period to promote diffusion, after which they were transferred to an incubator set at a temperature of 28 ºC for a duration of 48 hours. Distilled water was employed as a control in the experimental protocol. Subsequently, the plates were inspected to detect inhibition zones, which were characterized by clear areas surrounding the wells. The size of these inhibition zones was measured using a meter ruler, and the mean value for each bacterium was recorded in millimeters. A comparison was conducted between the inhibition zones of the experimental group and those of the control group. The experiment was repeated three times to ensure reliability and consistency of results.

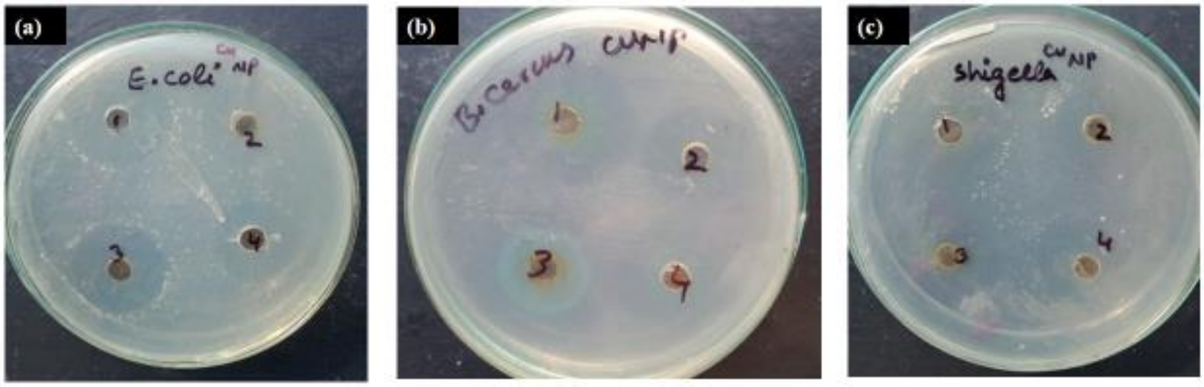

Figure 9. Showed antimicrobial efficacy of CuO NPs a) *E. coli* ZOI b) *B. cereus* ZOI c) *S. flexneri* ZOI.

Table 5. Antibacterial effect of CuO-NPs using well diffusion method.

Sample	Inhibition zone (mm)		
	E.coli.	*B. cereus*	*S. flexneri*
CuO-NPs of *M. Oleifera*	25±0.3	26±0.5	23±0.5
CuO-NPs of *S. cumini*	29±0.577	17.667±0.882	24±0.577
Negative control (sterilized water)	00	00	00

Table (5) displayed the antimicrobial properties of copper oxide nanoparticles (CuO-NPs) produced from *M. oleifera* extract and *S. cumini* extract using a green synthesis method. The assessment was conducted utilizing the well diffusion technique. The antimicrobial activity results were assessed after 24 hours by measuring the zone of inhibition (ZOI) in millimeters using a scale (Figure 9). A larger ZOI radius indicates the NPs are effective against bacterial strains, while a smaller ZOI radius suggests the NPs inhibit bacterial growth.

CuO-NPs derived from *M. oleifera* and *S. cumini* exhibited antibacterial properties against *E. coli, B. cereus, and S. flexneri*. CuO-NPs sourced from *S. cumini* displayed the largest inhibition zone against *E. coli* at 29 mm, indicating strong effectiveness against this bacterium (Figure 10). CuO-NPs obtained from *M. oleifera* consistently demonstrated antibacterial activity against all three bacteria, with the highest inhibition observed against *B. cereus*. The findings confirmed the potential of CuO-NPs from these plants as effective antibacterial agents.

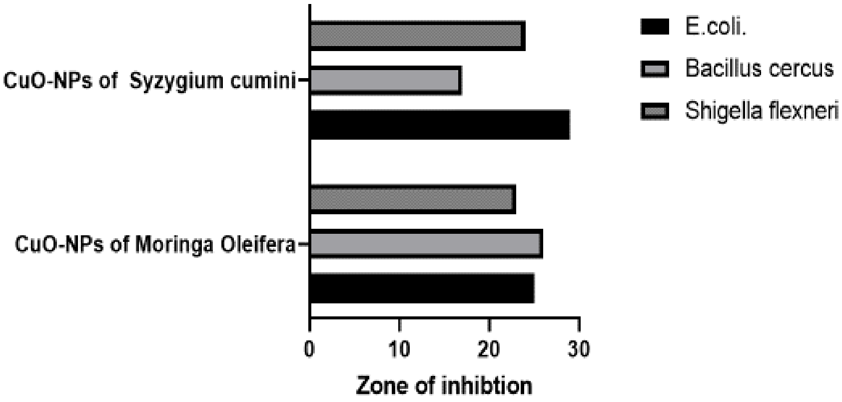

Figure 10. Antibacterial characteristics of copper oxide nanoparticles synthesized through a green method.

The measurement of the diameter of the inhibition zone in millimeters (mm) is utilized as an indicator of the efficacy of nanoparticles in inhibiting the proliferation of different bacterial strains, specifically *E. coli, B. cereus, and S. flexneri*.

The findings demonstrated notable antibacterial efficacy against *E. coli* (with an inhibition zone diameter of 25mm), *B. cereus* (with an inhibition zone diameter of 26mm), and *S. flexneri* (with an inhibition zone diameter of 23mm). On the other hand, the antibacterial efficacy was less significant against *S. flexneri*, with no noticeable effect observed on this bacterium compared to the control group (Fig 10). This study highlighted the considerable promise of the produced CuO-NPs as potent antibacterial agents for the treatment of diverse bacterial infections.

On the other hand, the antibacterial efficacy was less significant against *S. flexneri*, with no noticeable effect observed on this bacterium compared to the control group. This study highlighted the considerable promise of the produced CuO-NPs as potent antibacterial agents for the treatment of diverse bacterial infections (Preethi & Philominal, 2022). The mechanism by which copper oxide nanoparticles function as antimicrobial agents remains incompletely elucidated and is a subject of ongoing scholarly discourse (Prabhu & Poulose, 2012). Researchers have verified that compounds containing Cu+ and Cu- ions exhibited potent antimicrobial characteristics. Numerous scientists are intrigued by the potential of investigating alternative inorganic nanoparticles for their antibacterial properties (Jeeva et al., 2014). This study highlighted the significant promise of the produced CuO-NPs as potent antibacterial agents for the treatment of diverse bacterial infections associated with pathogenic diseases.

CONCLUSION

The research illustrated the biological production of CuO-NPs using leaves extracts derived from *M. oleifera and S. cumini* in the presence of copper nitrate. The findings suggested that *M oleifera* and *S. cumini* possess potential as effective agents for reducing and capping CuO-NPs. The results indicated significant termiticidal activity against *H. indicola* by using CuO-NPs of *M. oleifera* and *S. cumini.* When compared to both groups, the termiticidal effect was less pronounced by using the CuO-NPs of *S. cumini* and discernible impact was observed by using the CuO-NPs of *M oleifera*. This study highlighted the significant promise of the produced CuO-NPs as efficient termiticidal agents for mitigating the diverse severe impacts induced by *H. indicola*. Copper oxide nanoparticles can be efficiently and securely produced through the utilization of *M oleifera* leaf extract as a reducing and capping agent under ambient conditions. *M oleifera* generally produces larger and more potent nanoparticles, while both extracts provide valuable insights for customizing CuO-NPs for diverse applications in biomedicine, catalysis, and environmental remediation. The differences in synthesis mechanisms and stabilizing compounds from each plant extract significantly influence the properties and effectiveness of the nanoparticles, underscoring the importance of selecting suitable biological sources for termiticidal, antimicrobial, antifungal, and anti-cancer applications. The UV-visible spectrometer was utilized to analyze and characterize the CuO-NPs that were synthesized. These particles demonstrated significant inhibitory properties against *H. indicola*. The findings collectively underscore the cost-effectiveness, non-toxic nature, and ease of production of CuO-NPs. These nanoparticles possess diverse applications in areas such as antimicrobial, antifungal, and anti-cancer activities.

AUTHOR’S CONTRIBUTION

AI executed the experiments, AA led the study's design, supervision, and critical manuscript evaluation, MID assisted in experiments and data analysis. ZN handled literature review and MMIC final proofreading.

FUNDING

No funding was received for the present study.

AVAILABILITY OF DATA AND MATERIAL

The datasets supporting this study are included in the article. Extended methodological details and raw data can be accessed by contacting the corresponding author, subject to ethical and institutional guidelines.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This study did not involve human subjects or animal models. In accordance with institutional guidelines, ethical approval and informed consent requirements were formally waived for this research.

CONSENT FOR PUBLICATION

I, the undersigned, consent to the publication of my identifiable information.

CONFLICT OF INTERESTS

The authors declare there is no conflict of interest.

ACKNOWLEDGEMENT

We are grateful for the facilities provided by the Institute of Zoology, University of the Punjab. Lahore, Pakistan

REFERENCES

Abel, S., Tesfaye, J. L., Shanmugam, R., Dwarampudi, L. P., Lamessa, G., Nagaprasad, N., Benti, M., & Krishnaraj, R. (2021). Green synthesis and characterizations of zinc oxide (ZnO) nanoparticles using aqueous leaf extracts of coffee (*Coffea arabica*) and its application in environmental toxicity reduction. Journal of Nanomaterials, 2021(1), 3413350.

Afzal, M., Rasib, K., & Hussain, I. (2017). Resistance of Commercial and Non-commercial Woods against *Heterotermes indicola* Wasmann Blattodea. *Pak J Zool*, *49*, 785-792.

Ahmed, N., Huma, Z., Rehman, S.-U., Ullah, M., & Ahmed, S. (2016). Effect of different plant extracts on termite species (*Heterotermis indicola*). Journal of Bioresource Management, 3(2), 2.

Ahmed, S., Zafar, M. I., Hussain, A., Riaz, M. A., & Shahid, M. (2011). Evaluation of plant extracts on mortality and tunneling activities of subterranean termites in Pakistan. Pesticides in the Modern World-Pests Control and Pesticides Exposure and Toxicity Assessment, 1(1), 39-64.

Akintelu, S. A., Folorunso, A. S., Folorunso, F. A., & Oyebamiji, A. K. (2020). Green synthesis of copper oxide nanoparticles for biomedical application and environmental remediation. Heliyon, 6(7).

Akpan, A. U., Ojianwuna, C., Ubulom, P., Yaro, C., & Oboho, D. (2020). Effect of physico-chemical parameters on the abundance and diversity of termites and other arthropods in termite mounds in Uyo, Akwa Ibom State, Nigeria. FUDMA Journal of Sciences, 4(2), 92-100.

Al-Rawashdeh, M. m., Alfeeli, B., Rawashdeh, A. M., & Hessel, V. (2013). Development highlights of micro-nano technologies in the MENA region and pathways for initiatives to support and network. Green Processing and Synthesis, 2(2), 91-100.

Aljedani, D. M. (2023). Evaluation of Some Plant Extracts Effectiveness on the Termites Reticulitermes spp.(Isoptera: Rhinotermitidae). Polish Journal of Environmental Studies, 32(4).

Ashfaq, M., & Ashfaq, U. (2012). Evaluation of mosquitocidal activity of water extract of *M oleifera* seeds against Culex quinquefasciatus (Diptera: Culicidae) in Pakistan. Pak Entomol, 34(1), 21-26.

Austin, J. W., Szalanski, A. L., Uva, P., Bagnères, A.-G., & Kence, A. (2002). A comparative genetic analysis of the subterranean termite genus Reticulitermes (Isoptera: Rhinotermitidae). Annals of the Entomological Society of America, 95(6), 753-760.

Begum, S. N., Esakkiraja, A., Asan, S. M., Muthumari, M., & Raj, G. V. (2019). Green synthesis of copper oxide nanoparticles using *Catharanthus roseus* leaf extract and their antibacterial activity. Int. J. Sci. Res. in Multidisciplinary Studies, 5, 8.

Din, M. I., Arshad, F., Rani, A., Aihetasham, A., Mukhtar, M., & Mehmood, H. (2017). Single step green synthesis of stable copper oxide nanoparticles as efficient photo catalyst material. Biomed Mater, 9, 41-48.

Din, M. I., Yamin, A., Hussain, Z., Khalid, R., & Arshad, M. (2022). Investigation of biologically synthesized stable copper oxide nanoparticles using *Allium sativum* extract by photocatalysis of methylene blue. Inorganic and Nano-Metal Chemistry, 1-8.

Donovan, S. E., Griffiths, G. J., Homathevi, R., & Winder, L. (2007). The spatial pattern of soil-dwelling termites in primary and logged forest in Sabah, Malaysia. Ecological Entomology, 32(1), 1-10.

Ebrahımı, K., Shıravand, S., & Mahmoudvand, H. (2017). Biosynthesis of copper nanoparticles using aqueous extract of *Capparis spinosa* fruit and investigation of its antibacterial activity. Marmara Pharmaceutical Journal, 21(4), 866-871.

Elseman, A. M., Rayan, D., & Rashad, M. (2016). Structure, optical and magnetic behavior of nanocrystalline CuO nanopowders synthesized via a new technique using Schiff base complex. Journal of Materials Science: Materials in Electronics, 27, 2652-2661.

Faruq, A., Amin, M., Islam, M., Islam, M., & Alam, M. (2015). Evaluation of some selected seed treatments against leaf blast, brown spot and narrow brown leaf spot diseases of hybrid rice. Advance in Agriculture and Biology, 4(1), 8-15.

Gebremedhn, K., Kahsay, M. H., & Aklilu, M. (2019). Green synthesis of CuO nanoparticles using leaf extract of *Catha edulis* and its antibacterial activity. Journal of Pharmacy and Pharmacology, 7(6), 2328-2150.

Gnanavel, V., Palanichamy, V., & Roopan, S. M. (2017). Biosynthesis and characterization of copper oxide nanoparticles and its anticancer activity on human colon cancer cell lines (HCT-116). Journal of Photochemistry and Photobiology B: Biology, 171, 133-138.

Gowri, M., Latha, N., & Rajan, M. (2019). Copper oxide nanoparticles synthesized using *Eupatorium odoratum*, *Acanthospermum hispidum* leaf extracts, and its antibacterial effects against pathogens: a comparative study. Bio Nano Science, 9, 545-552.

Hassan, B., & Morrell, J. J. (2021). Termite testing methods: A global review. Journal of Testing and Evaluation, 49(6), 4607-4636.

Hazaa, M., Alm-Eldin, M., Ibrahim, A.-E., Elbarky, N., Salama, M., Sayed, R., & Sayed, W. (2021). Biosynthesis of Silver Nanoparticles using *Borago officinslis* leaf extract, characterization and larvicidal activity against cotton leaf worm, *Spodoptera littoralis* (Bosid). International Journal of Tropical Insect Science, 41, 145-156.

Jeeva, K., Thiyagarajan, M., Elangovan, V., Geetha, N., & Venkatachalam, P. (2014). *Caesalpinia coriaria* leaf extracts mediated biosynthesis of metallic silver nanoparticles and their antibacterial activity against clinically isolated pathogens. Industrial Crops and Products, 52, 714-720.

Khan, S. A., & Lee, C.-S. (2020). Green biological synthesis of nanoparticles and their biomedical applications. Applications of nanotechnology for green synthesis, 247-280.

Khatami, M., Varma, R. S., Heydari, M., Peydayesh, M., Sedighi, A., Agha Askari, H., Rohani, M., Baniasadi, M., Arkia, S., & Seyedi, F. (2019). Copper oxide nanoparticles greener synthesis using tea and its antifungal efficiency on *Fusarium solani*. Geomicrobiology Journal, 36(9), 777-781.

Krishna, K., Grimaldi, D. A., Krishna, V., & Engel, M. S. (2013). Treatise on the Isoptera of the world: Termitidae (part one). Bulletin of the American Museum of Natural History, 2013(377), 973-1495.

Kuswanto, E., Ahmad, I., & Dungani, R. (2015). Threat of subterranean termites attack in the Asian countries and their control: A review. Asian Journal of Applied Sciences, 8(4), 227-239.

Muthuvel, A., Jothibas, M., & Manoharan, C. (2020). Synthesis of copper oxide nanoparticles by chemical and biogenic methods: photocatalytic degradation and in vitro antioxidant activity. Nanotechnology for Environmental Engineering, 5(2), 14.

Nasser, R., Ibrahim, E., Fouad, H., Ahmad, F., Li, W., Zhou, Q., Yu, T., Chidwala, N., & Mo, J. (2024). Termiticidal Effects and Morpho-Histological Alterations in the Subterranean Termite (*Odontotermes formosanus*) Induced by Biosynthesized Zinc Oxide, Titanium Dioxide, and Chitosan Nanoparticles. Nanomaterials, 14(11), 927.

Otari, S., Patil, R., Nadaf, N., Ghosh, S., & Pawar, S. (2012). Green biosynthesis of silver nanoparticles from an actinobacteria Rhodococcus sp. Materials Letters, 72, 92-94.

Prabhu, S., & Poulose, E. K. (2012). Silver nanoparticles: mechanism of antimicrobial action, synthesis, medical applications, and toxicity effects. International Nano letters, 2, 1-10.

Preethi, D. R. A., & Philominal, A. (2022). Antimicrobial and antiurolithiatic activities of pure and silver doped copper oxide nanoparticles using *M Oleifera* leaf extract on struvite urinary stones. Applied Surface Science Advances, 12, 100351.

Ramzan, M., Obodo, R. M., Mukhtar, S., Ilyas, S., Aziz, F., & Thovhogi, N. (2021). Green synthesis of copper oxide nanoparticles using *Cedrus deodara* aqueous extract for antibacterial activity. Materials Today: Proceedings, 36, 576-581.

Rasib, K. Z., Ashraf, H., & Afzal, M. (2014). Feeding preferences of *Odontotermes obesus* (Rambur)(Isoptera: Termitidae) on different commercial and non-commercial woods from Lahore, Pakistan, under laboratory and field conditions. Zoology and Ecology, 24(4), 369-379.

Raveendran, P., Fu, J., & Wallen, S. L. (2003). Completely “green” synthesis and stabilization of metal nanoparticles. Journal of the American Chemical Society, 125(46), 13940-13941.

Renuga, D., Jeyasundari, J., Athithan, A. S., & Jacob, Y. B. A. (2020). Synthesis and characterization of copper oxide nanoparticles using *Brassica oleracea* var. italic extract for its antifungal application. Materials Research Express, 7(4), 045007.

Rouland-Lefèvre, C. (2011). Termites as pests of agriculture. Biology of termites: a modern synthesis, 499-517.

Shah, S. S. A. A., Aihetasham, A., & Rasib, K. Z. (2024). Effect of Drying Temperature on Resistance of Commercial and Non-Commercial Woods against *Microtermes obesi* (Holmgren) under Laboratory and Field Conditions. *Pakistan Journal of Zoology*, *57*(3), 1381.

Shammout, M., & Awwad, A. (2021). A novel route for the synthesis of copper oxide nanoparticles using Bougainvillea plant flowers extract and antifungal activity evaluation. MW Shammout and AM Awwad. A novel route for the synthesis of copper oxide nanoparticles using Bougainvillea plant flowers extract and antifungal activity evaluation. Chemistry International, 7(1), 71-78.

Su, L., Yang, L., Huang, S., Su, X., Li, Y., Wang, F., Wang, E., Kang, N., Xu, J., & Song, A. (2016). Comparative gut microbiomes of four species representing the higher and the lower termites. Journal of Insect Science, 16(1), 97.

Sundaramurthy, N., & Parthiban, C. (2015). Biosynthesis of copper oxide nanoparticles using *Pyrus pyrifolia* leaf extract and evolve the catalytic activity. Int Res J Eng Technol, 2(6), 332-338.

Tahir, A., Quispe, C., Herrera-Bravo, J., Iqbal, H., Anum, F., Javed, Z., Sehar, A., & Sharifi-Rad, J. (2022). Green synthesis, characterization and antibacterial, antifungal, larvicidal and anti-termite activities of copper nanoparticles derived from *Grewia asiatica L*. Bulletin of the National Research Centre, 46(1), 1-11.

Usman, M. S., Zowalaty, M. E. E., Shameli, K., Zainuddin, N., Salama, M., & Ibrahim, N. A. (2013). Synthesis, characterization, and antimicrobial properties of copper nanoparticles. International journal of nanomedicine, 4467-4479.

Vongsak, B., Sithisarn, P., & Gritsanapan, W. (2013). Bioactive contents and free radical scavenging activity of *M oleifera* leaf extract under different storage conditions. Industrial Crops and Products, 49, 419-421.

Zhou, J., Duan, J., Gao, M., Wang, Y., Wang, X., & Zhao, K. (2019). Diversity, roles, and biotechnological applications of symbiotic microorganisms in the gut of termite. Current microbiology, 76, 755-761.