Nature-Driven Nanotechnology Phoenix dactylifera-Mediated Synthesis of Nickel Oxide Nanoparticles for Bio-Insecticidal and Antibacterial Applications.

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Abstract- Recently, nanoparticle (NP) synthesis has evolved into a green nanotechnology field, requiring more methods for the eco-synthesis of nanoparticles due to the high costs of other chemical–physical methods. Among the most commonly used nanomaterials, NiO-NPs are highly valuable due to their specific, thermal, optical, and electronic features. Thus, the main objective of this work was to investigate the green synthesis of NiO-NPs employing the *Phoenix dactylifera L*., extract, which is rich in phytochemicals, as a reducing agent. In this way, the effect of the concentration of nickel oxide nanoparticle as an efficient termiticidal was evaluated. The NPs were characterized and compared through Ultra Violet Visible Spectroscopy (UV) Additionally, we evaluated the antibacterial properties of these nanoparticles against Gram-positive *Staphylococcus aureus (S. aureus), Bacillus cereus* (*B. cereus*) and *Shigella flexneri (S. flexneri)* and Gram-negative *Escherichia coli (E. coli)* pathogenic strains. The results showed that NiO-NPs using a green reducing agent *phoenix datylifera* extract has shown an efficient termiticidal activity and greater antibacterial property against gram negative bacteria i.e. *E.coli*. In addition, this synthesis highlighted the termiticidal and antibacterial activities of these nanoparticles. In conclusion, this method could be a suitable substitute for typical toxic methods for the synthesis of metallic nanoparticles and its applications as an efficient termiticidal and antimicrobial materials.

Keywords: Anti-termite activity, Antibacterial activity, Green synthesis, Nickel oxide nanoparticles.

Introduction

Certain termite species have the ability to degrade wood, which would cost money in lost building materials. The Formosan subterranean termite is the most significant wood pest in the world, causing significant damage in Asian nations (Oramahi et al., 2022; Kuswanto et al., 2015). Plant components have been used to make insecticides, insect repellents, antifeedants, and growth and development regulators (Adfa et al., 2020; Arsyad et al., 2020; Nkogo et al., 2022; El-Wakeil, 2013; Hadi et al., 2020; Isman, 2020; Oramahi et al., 2022).

Phytochemicals or crude plant extracts are used in a variety of ways, including suppression of calling behavior (Lamy et al., 2017), growth retardation (Anshul et al., 2014), feeding inhibition, toxicity, oviposition deterrence (Abdullah et al., 2015; Sinthusiri and Soonwera, 2014), and reduced fecundity to destroy insects (Schmutterer, 2019).

Termites present a significant risk to the viability of plants and structures, making them a prominent pest in global forestry and agriculture industries. (Yang et al., 2021). Research indicates that there are over 2800 known species of termites globally, with 185 of these species recognized as detrimental pests (Verma et al., 2009), particularly, termites from the Hodotermitidae, Kalotermitidae, Rhinotermitidae, and Termitidae families are of significant concern. Among these, Odontotermes formosanus Shiraki, a subterranean termite, is recognized as one of the most damaging species globally (Soleymaninejadian et al., 2014). In addition to the significant harm inflicted by workers of the termite species *O. formosanus* through their consumption of a diverse array of vegetation, including plants, shrubs, and weeds, they also pose a substantial threat to infrastructure such as buildings and dams, leading to substantial financial losses amounting to billions of dollars (Vidkjær et al., 2021). Prior research has indicated that the global annual spending on termite management has reached $22 billion, with a yearly growth rate of 50% (Su, 2002). In the United States, the annual expenditure for termite control amounts to $175 million (Govorushko, 2019). Certainly, one of the most widely used and effective methods for managing termite infestations is the utilization of insecticides containing chemical compounds (Ahmad et al., 2021). Excessive utilization of pesticides has led to numerous challenges, such as the development of biological resistance, disruptions in ecological equilibrium, and detrimental impacts on the environment (Govindarajan et al., 2012). In the past twenty years, nanotechnology has emerged as a prominent and dynamic field of research within the realms of science and technology (Frima et al., 2012). Metal oxide and natural polymer nanoparticles offer advantages including reduced harmful impacts and superior stability, chemical characteristics, and toxicity in comparison to alternative metal nanoparticles (Fouda et al., 2020). These benefits have led to their preferential incorporation in a variety of applications within the fields of biomedicine and biotechnology (Tso et al., 2010). Nanomaterials have been utilized in agriculture to effectively control various plant pathogens and pests, while also playing a significant role in enhancing overall plant health (Kamle et al., 2020). Nevertheless, the application of nanomaterials for termite management to mitigate the detrimental impacts of termites has not been thoroughly investigated. Nanomaterials can be synthesized through different techniques such as physical, chemical, and biological methods. Nonetheless, the physical and chemical approaches are resource-intensive, time-consuming, and result in the emission of hazardous compounds into the surroundings (Gopinath et al., 2015). On the contrary, the biological approach is characterized by its simplicity, efficiency, cost-effectiveness, and environmental friendliness in comparison to chemical and physical methodologies (Dobrucka and Długaszewska, 2016; Eid et al., 2021; Velgosova et al., 2020). Hence, scholars have focused their attention on exploring the utilization of diverse substances in biosynthesis. This includes investigating plant extracts derived from different parts of plants such as fruits, stems, flowers, leaves, and roots, as well as exploring the potential of metabolites produced by various microbial species like fungi, algae, yeast, bacteria, and phages. These natural sources are being considered as environmentally safe alternatives for various applications (Clarance et al., 2020; Velgosova et al., 2021).

Materials and Methods

Preparation of aqueous extract

Approximately 20g of ground leaves of *Phoenix dactylifera* were 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 Nickel oxide nanoparticles (NiO-NPs)

The production of Nickel oxide nanoparticles (NiO-NPs) was conducted through a microwave-assisted environmentally friendly approach (Otari et al., 2012).

In this process, a 50mL Erlenmeyer flask containing 2mM $Ni(NO_3)_2$ was reacted with a 10mL aqueous leaf extract of *Phoenix dactylifera* at room temperature.

To stabilize the pH of the mixture, a 1M NaOH solution was introduced to a 20mL extract of *Phoenix dactylifera,* which also contained a 1M precursor solution of Nickel nitrate Ni $(NO_3)_2$. 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 nickel 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 gathered 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.

Leaf collection:

Selected leaves of plant was gathered from the Botanical Garden of the University of the Punjab, Lahore. They were air-dried in the shade for three days and then stored in polyethylene packets for experimental use.

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, Staphylococcus aureus, 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 NiO-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.

$$\text{Percent termite lethality} = \frac{\text{No. of dead termites} \times 100}{\text{Total no. of termites}}$$

Characterizations

NiO-NPs synthesized through biogenic methods were subjected to continuous examination using a UVD-3500 UV-Visible spectrophotometer. Furthermore, the biosynthesized nickel oxide nanoparticles were further analyzed using Fourier-transform infrared spectroscopy is to characterize specific biomolecules existing in the extract which facilitate 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.

Results and discussions

Ultra Violet-Visible spectroscopy of NiO-NPs

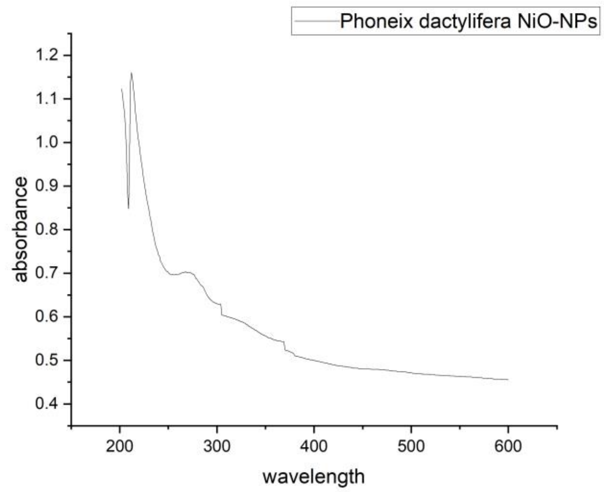

Figure 1: UV-Visible absorbance spectrum of NiO-NPs synthesized using *Phoenix dactylifera* leaf extracts

One way ANOVA of *Phoenix dactylifera* extract NiO-NPs used for termiticidal activity against *H.indicola.*

In this study, we employ one-way ANOVA by using "Minitab" to analyze the synthesis of NiO-NPs, exploring how variations in experimental conditions or precursor materials impact the properties of the synthesized nanoparticles. Through the use of ANOVA analysis, our objective is to identify any notable variations in the average properties of Nickel oxide nanoparticles derived the leaves extracts of *Phoenix dactylifera*. This investigation will offer 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: ANOVA for mortality of *H.indicola* treated with leaves extracts based NiO-NP at different exposure periods.

Plant NPs	Test products	
	Green synthesized NiO	**Leave extract**
	Mean ±S.E	Mean ±S.E
P. dactylifera	40.33±0.333 [a]	17.00±0.577[b]

Means (within the same column) having similar letters are not significantly different.

The ANOVA results demonstrated that the mortality of *H. indicola* is significantly affected by the exposure period to NiO-NPs. A p-value < 0.05 indicated that the differences in mortality among exposure periods are statistically significant. Longer exposure period likely result in higher mortality rates, showcasing the efficacy of NiO-NPs synthesized from leaf extract.

Table 2: Percentage corrected mortality (Mean±SE) of *H. indicola* caused by different concentrations of NiO-NPs *P. dactylifera* and *P. dactylifera* leaf extracts.

Means (within the same column) having similar letters are not significantly different.

Source of Variation	Sum of Squares (SS)	DF	Mean square	F-value	Sig.
Between Groups	1882.96	7	268.994	47.82	0.000
Error	90				
total	1972.96				

Comparison among Plant Extract with NiO-NPs

P. dactylifera extracts synthesized NiO-NPs that resulted in the highest corrected mortality (40.33%). *P. dactylifera* leaves extract resulted in a slightly lower mortality (33.67%).

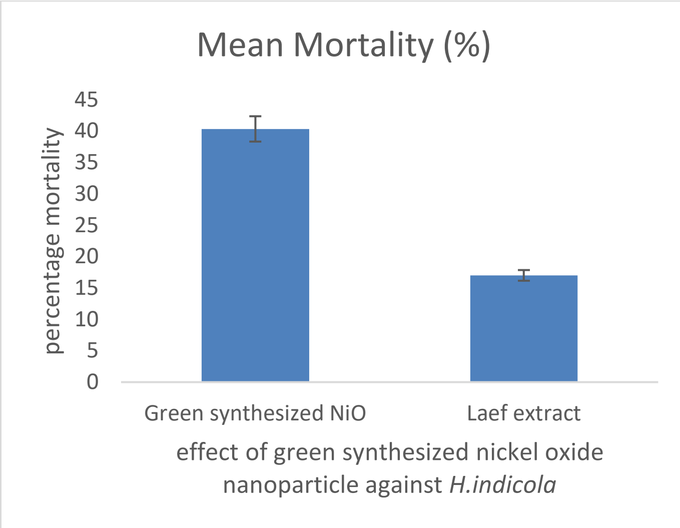

Figure 2: Percentage corrected mortality of *H. indicola* caused by different concentrations of NiO-NPs *P. dactylifera* and *P. dactylifera* leaf extracts.

Green synthesized NiO-NPs that showed in the highest mortality. Whereas, *P. dactylifera* leaves extract resulted in a slightly lower mortality.

Table 3 Phytochemical screening of n-hexane extracts of *P. dactylifera*

Plant name	***P. dactylifera***		
Test	**Stem**	**Leaves**	**fruit**
Fehling's test	-	+	+
Benedict's test	+	+	+
Molisch test	+	+	+
Iodine test	+	-	+
Froth formation test (Saponin)	-	-	-
Steroid test	-	+	+
Ferric chloride test	+	-	-
Alkaline reagent test	+	+	-
Shindola test	-	+	+

Key: (+): Presence (-): Absence

The table 3 indicated the phytochemical diversity in the stem, leaves, and fruit extracts of *Phoenix dactylifera* based on the results of various tests: Benedict's and Molisch tests confirmed the presence of carbohydrates in all parts, while Fehling's test showed reducing sugars in the leaves and fruit only. The iodine test is positive for stem and fruit, indicating the presence of starch, but absent in leaves (Khaliq et al., 2023).

Ferric chloride test showed phenolics only in the stem, with none in the leaves or fruit. Steroids were present in the leaves and fruit (positive steroid test). Alkaloids (Shindola test) observed in leaves and fruit but not in the stem. The froth formation test reveals no presence of saponins in any part of the plant (Shoaib et al., 2023).

Table 4: One way ANOVA of NiO-NPs plant extract against bacterial strains

Source Variation	Sum of Squares	DF	Mean square	F-value	Sig.
Bacterial strains	980.81	15	65.388	39.73	0.000
Error	52.67	32	1.646		
Total	1033.48	47			

The table 4 presented the results of a one-way ANOVA that examined the effects of NiO-NPs plant extract on different bacterial strains.

The analysis revealed a statistically significant effect of NiO-NPs on bacterial strains. The variability in inhibition zones is primarily due to differences among bacterial strains, as indicated by the high F-value and significant p-value. This suggested that NiO-NPs synthesized from plant extract exhibit strain-specific antibacterial activity.

Table 5: Zone of inhibition (mm) of green synthesized NiO-NPs against different bacterial strains using well diffusion method.

Sample	*E. coli* (mm)	*S. aureus* (mm)	*S. flexneri* (mm)	*B. cereus* (mm)
Phoenix dactylifera NiO-NPs	16 ± $0.577^{a,b}$	11 ± 0.577^{a}	14 ± 0.577^{b}	13.33 ± $0.882^{a,b}$
Negative control	0	0	0	0

Figure 3: Zone of inhibition (mm) of green synthesized NiO-NPs against different bacterial strains

Highest inhibition zone was observed in *E. coli* (16 mm), moderate zone of inhibition was observed in *S. flexneri* (14 mm) and *B. cereus* (13.33 mm) and lesser sensitivity was observed in *S. aureus* (11 mm). Negative control indicated no antimicrobial activity is nickel oxide nanoparticles.

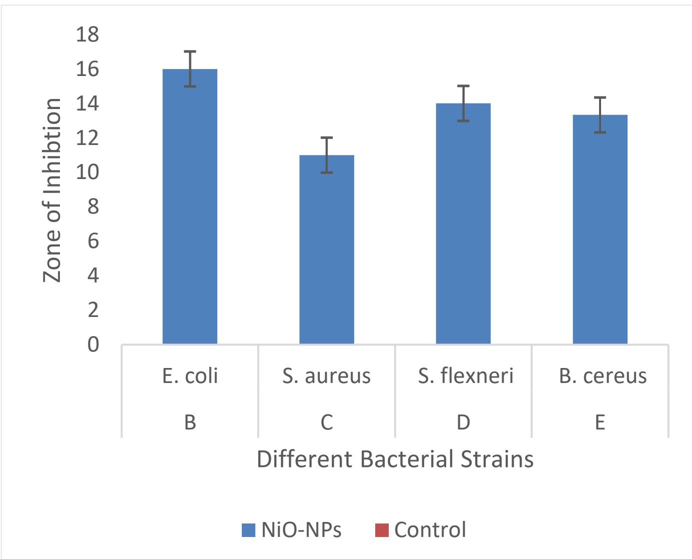

Figure 4: Zone of inhibition (mm) of green synthesized NiO-NPs against different bacterial strains

Figure 4 illustrated that *Escherichia coli* showed highest average inhibition. *Staphylococcus aureus* showed lowest average inhibition, suggesting moderate resistance. *Shigella flexneri* and *Bacillus cereus* showed intermediate inhibition zones. Sterilized water acts as negative control showed no inhibition (0 mm), confirming that antibacterial activity arises solely from NiO-NPs.

Conclusion

Green synthesized *Phoenix dactylifera* nickel oxide nanoparticle showed great mortality of *H. indicola.* The green synthesis of nickel oxide nanoparticles (NiO-NPs) using *Phoenix dactylifera* leaf extracts offers an effective, sustainable, and environmentally friendly approach for producing antibacterial and termiticidal materials. The biogenic synthesis of NiO-NPs enhances their biological activity, showing significant potential for use in pest control and as antimicrobial agents. This method not only provides an eco-friendly alternative to synthetic chemicals but also contributes to the growing field of green nanotechnology, promising broader applications in both agricultural and medical fields. Studies on the efficacy of nanoparticle-based pesticides have shown time-dependent mortality in pest species. (Ali et al., 2023) reported that exposure time is a critical determinant of nanoparticle toxicity, with longer periods significantly enhancing mortality. (Mahmood et al., 2024) noted that biogenic nanoparticles disrupt cellular processes in pests, with effects intensifying over time. These results align with the findings here, confirming the potential of NiO-NPs as eco-friendly and efficient termiticidal agents. Green-synthesized nanoparticles exhibited strong antibacterial activity due to their unique surface properties and ability to disrupt bacterial membranes (Ahmad et al., 2022; Khan et al., 2023). These findings align with studies highlighting the potential of biogenic metal oxide nanoparticles. And UV spectra confirm the formation of green synthesized nickel oxide nanoparticles.

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