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Research article

Green synthesis of *Eucalyptus globulus* zinc nanoparticles and its use in antimicrobial insect repellent paint formulation in bulk industrial production

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A R T I C L E I N F O

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A B S T R A C T

Mitigating climate change can be achieved by opting for sustainable, plant-based materials instead of relying on hazardous chemicals that come with various side effects. Various natural plant extracts find widespread application in synthesizing insect-repellent coatings, particularly in industries such as paint manufacturing. The increasing demand for these coatings has led us to find out the effects of different plant extracts for the efficient preparation of paints with more advanced impacts and low cost. For this purpose, zinc nanoparticles of *Eucalyptus globulus* L. and its extracts were used in this study due to their remarkable biocidal and antimicrobial activities. The extract was prepared by the process of oven-drying and heating followed by their filtration. Then, they were subjected to different phytochemical tests that were performed in which plant material did not contain flavonoids and glycol. The comparison of the size of nanoparticles was visible during the weighing which was found to be 4.451 mg. Advanced characterization techniques like FTIR, UV visible spectroscopy, and particle size analysis were adopted for the analysis of nanoparticles of plant extract. The FTIR analysis of the plant material was reported to lie in the range of 1000–1800 cm^{-1}. On the other hand, the results of UV visible spectroscopy of nanoparticles of plant extract showed absorption peaks around 300 nm. The produced material was integrated into paint formulations to impart insect-repellent and antibacterial characteristics. Painted panels exhibited notable antibacterial efficacy, presenting an inhibition zone of 0.7 cm for *Escherichia coli* and 0.3 cm for *Staphylococcus aureus* when utilizing biocide. Plant nanoparticles yielded inhibition zones of 1 and 1.2, while aqueous extract resulted in zones of 0.2 and 0.5, respectively. A thorough evaluation of the paint's color attributes, including ΔL, Δa, Δb, and ΔE, indicated noteworthy differences. The CMC ΔE values from the trials exceeded 1, indicating a substantial change in shade. The batches of paints containing *E. globulus* extracts and nanoparticles were found to be lighter in color specifically green and yellow colors. Their antimicrobial and insect repellant activity was tested using the mosquitos of *Aedes aegypti* with an age of 4–5 weeks, revealing that formulations with plant extracts exhibited a 61 % effective period,

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greater than the 7 % observed in non-biocidal formulations. The paint responded best towards these mosquitoes in terms of repellency and the ultimate target of this study was achieved.

1. Introduction

Technology has been varying due to some of the rising industries in the cutting-edge world [1]. Addressing climate change is imperative, particularly in tandem with the ongoing industrial revolutions, Industry 4.0 and the forthcoming Industry 5.0 and Industry 6.0 [2]. To usher in a sustainable future, it is paramount that we engage in extensive research on eco-friendly materials facets of manufacturing [3]. This concerted effort is vital to ensure that our products not only meet the demands of modern industrial advancements but also contribute significantly to mitigating the environmental challenges posed by climate change. The integration of sustainable practices into manufacturing processes is not only a responsibility but a necessity for fostering a greener, more resilient planet for generations to come [4,5]. Mitigating our carbon footprint is achievable through the adoption of green technology. By curbing greenhouse gas emissions, we can actively combat global warming, a matter of utmost priority in our collective pursuit to safeguard humanity [6]. Embracing sustainable practices and environmentally friendly technologies is not just a choice; it's an imperative step toward securing a healthier, more sustainable future for our planet and the well-being of present and future generations. Employing natural, plant-based materials proves instrumental in accomplishing this formidable task [7,8]. Introducing a game-changing innovation in the industrial revolutions. Traditional insect repellent and antibacterial solutions have long been a cause for concern due to their non-sustainable nature and the inclusion of harmful chemicals. Not only do these pose risks to human health, but they also pose a grave threat to our fragile environment [9]. To make matters worse, the manufacturing process often involves the use of toxic and hazardous raw materials, exacerbating the problem.

But fear not, for a revolutionary solution has emerged, and it comes in the form of our groundbreaking research. We've tackled numerous issues plaguing the industry, and the results are nothing short of remarkable.

The paint and coatings industry are one of those industries which are intending in the direction of development every day. Some of the marketplace segments like automobiles confronted more than 95 % exchange in the composition of paints applied to them over the past 10 years. The satisfactory way we must adopt inside the improvement of the paint enterprise is to successively improve it by retaining alongside the innovations [10]. For this reason, one needs to keep updated information concerning the industry's protocol. The economic fee of the paint industry is meditated globally as much as greater than 50 billion greenbacks which urges the researchers to have a better perception of the industry. Exclusive plants had been observed to include chemical substances that showcase a high insect-repelling tendency [11]. The chemicals may be used in combination with specific surface coatings like enamel paints, distemper, or oil paints with no effect on their high quality. Therefore, the resulting insect-repellent surface coatings may also showcase precise houses and may be exploited for commercial purposes. Insect-repellent coatings provide safety against mosquitoes spreading illnesses like malaria, dengue, chikungunya, and yellow fever. Bugs such as mosquitoes serve as vectors for some of the infectious sicknesses affecting tens of millions of humans and leading them to death [12]. So, there may be a dire want to introduce a few new strategies for their prevention. One of the strategies is to use insect repellants in floor coatings to cause those to become efficient in opposition to such bugs. One of the principal health issues in this regard is Malaria, living in tropical countries, specifically in sub-Saharan Africa, where almost 90 % of clinical instances mainly occur with at least 7 million deaths consistent with year. In addition, in Sudan, malaria is a primary cause of morbidity and mortality accounting for 7.5 million cases and 35,000 fatalities according to 12 months. The use of indoor residual spraying (IRS), and insecticide-handled bed nets (ITNs) are the number one strategy for lowering malarial vectors in Sudan [13–15]. There are number of techniques for the synthesis of materials that can be used for diverse industrial applications [16–24].

The usage of herbal plant extracts as insect repellants surface coatings is ensured as synthetic repellents are in most cases highly priced and occasionally allergic [25]. *Eucalyptus*, belonging to the family Myrtaceae, is popularly acknowledged to show off antifungal, antibacterial, antioxidative, fumigant, and insecticides sports due to the presence of certain bioactive compounds in it. Maintaining a vital reputation as one of the international's quality trees, *Eucalyptus globulus* L. is commonly known as Tasmanian blue gum and has been reported to expose insecticidal hobby through the approach called nano-encapsulation. Nano-pills organized from their extract have been favored over chemical insecticides as they overcome the impact of toxicity and pollution in comparison to the latter [26,27]. The present study intends to use mosquito repellent from *Eucalyptus globulus* L. in coating.

Inside the nanoscale, the antimicrobial interest of ZnO will increase considerably because it can input the bacterial cells and interacts with its core which could cause the death of the bacterial mobile. A few commercial products including rubber, paint, and floor coatings were observed to utilize zinc oxide nanoparticles (ZnO NPs) for growing their efficiency in insecticide interest. Being a monetary, less toxic, and tremendously biocompatible compound, ZnO NPs have executed a vital reputation in the discipline of biology over the past two decades [28].

After successfully launching this product that manufacturers can market this research in the following way. First, we've connected the power of biodegradable, all-natural ingredients to create an antibacterial and insect repellent formula that's not only highly effective but also eco-friendly. Our products are not only safe for humans but also for the environment. Our research has led to a brilliant eco solution two-in-one formulation for paints. The walls not only look pristine but also being fortified with insect repellent and antibacterial properties. This dual functionality not only enhances your living space but also adds an extra layer of protection with substantial cost savings. Our innovation also carries immense marketing benefits. By choosing our products, you're aligning yourself with sustainability, a value that resonates with today's environmentally conscious consumers. It's a decision that not only makes sense for your business but also for the planet.

The positive impact doesn't end there. Our eco-friendly approach results in a significantly reduced carbon footprint during manufacturing, in stark contrast to synthetic biocides and insect repellent formulations. Our products generate fewer effluents, saving both costs and the environment. Our patented product is a game-changer, offering a multitude of benefits for end-users and paint manufacturers worldwide. Don't just paint your walls; fortify them with the future of industrial products by choosing sustainability, innovation, and a greener world.

2. Materials and methods

2.1. Materials

The plant samples were collected from the botanical garden of GCU Lahore, Pakistan. Chemicals used in this research work include ninhydrin, sulphuric acid, hydrochloric acid, zinc sulfate, sodium hydroxide, distilled water, chloroform, ferric chloride, and ammonia solution, supplied by Coural Associates, Pakistan. The liquid culture of the bacterial strains (*Escherichia coli* and *Staphylococcus aureus*) was collected from Lahore College for Women university, Lahore, Pakistan. All research work was carried out in the Applied Botany Laboratory of the Department of Botany, GC University Lahore, Pakistan under the supervision of Tehreema Iftikhar and the acrylic plastic paint emulsion formulation was prepared in Diamond Paint Industries (Pvt) Ltd, Lahore, Pakistan under the supervision of Hammad Majeed. All the data collection steps, and then subsequent analysis is explained below in detail.

2.2. Methods

2.2.1. Phytochemical analysis

The phytochemical characterization of plant samples was carried out separately to evaluate nutritional aspects like proteins, carbohydrates, flavonoids, alkaloids, tannins, steroids, terpenoids, saponins, wanger's test, and glycoside [29,30].

Protein confirmation (Ninhydrin test): A ninhydrin solution of 1 mL was added to the aqueous extract of each sample and heated. The presence of proteins and amino acids was indicated by the violet color.

Carbohydrates confirmation (Molish test): The extract of each plant is taken 1 mL and a few drops of Molish reagent were dissolved in it and mixed well. Afterward, 1 mL of concentrated sulphuric acid was gently added along the test tube sides. The formation of a violet ring at the interface showed the existence of carbohydrates.

Tannins confirmation: Ferric chloride 1 mL solution was added to 1 mL of each sample of aqueous extract. The blackish blue/ blackish green color showed the existence of tannins.

Flavonoid confirmation: A few drops of 20 % solution of sodium hydroxide were added to 2 mL of each sample of aqueous extract. The presence of flavonoids was revealed by the appearance of a dark yellow color that changed to a colorless solution when diluted HCl was added drop by drop.

Steroids confirmation: For each plant extract of 1 mL, chloroform 2 mL was added. The sulphuric acid concentrate was poured down the sides of the test tubes. The appearance of a reddish color ring confirmed the existence of steroids.

Terpenoids confirmation: In 5 mL of each plant extract, 2 mL chloroform was added. Afterward, 3 mL conc sulphuric acid was added to this solution and mixed gently. The presence of terpenoids was confirmed by the reddish-brown tint.

Alkaloids Confirmation (Wagner's test): Diluted hydrochloric acid (2 mL) was added in each beaker, containing 1 mL of each plant extract, and heated (one by one) in a water bath. In this solution, a few drops of Wagner's reagent were added. The presence of alkaloids was observed in the formation of precipitates.

Anthraquinones Confirmation: Extracted powder (1 g) was taken in a test tube and add 5 mL chloroform in it. The test tube was shaken vigorously for 5 min, and the mixtures were then filtered by using Whatman's filter paper #01. An equal amount of 10 % ammonia was added to it. Anthraquinones were detected by the formation of pink color layered.

Glycosides confirmation (Keller- Killani test): Plant extract (5 mL) was taken in a test tube in which 2 mL of glacial acetic acid was added and then mixed well. Afterward, 5 % of ferric chloride and conc. H_2SO_4 was added to it. Glycosides were detected by the appearance of brown color.

2.2.2. Extract and nanoparticles synthesis

The leaves of the plants were carefully separated and subjected to a thorough washing process involving 3 rinses with distilled water. The leaves were dried in an oven at a temperature range of 60 °C for approximately 7 h. Following the drying phase, the leaves were finely ground into a powder using a grinder. To prepare a solution, 100 g of the dried plant material powder was combined with distilled water and the volume was adjusted to 1000 mL. The resulting solutions were heated 6 times for 30 s each in a microwave oven until a dark coloration became evident, signifying the preparation of a 1:10 solution. The solutions were allowed to cool. The entire extract was then filtered using Whitman's filter paper #01 to obtain the desired extract. Nanoparticles were synthesized through a process involving ultra-sonication and mild alkali hydrolysis. Initially, 1.4 g of zinc sulfate were introduced into 50 mL of distilled water, and the mixture was placed on a magnetic stirrer plate. 40 mL of the plant extract were added drop by drop to the zinc sulfate solution from a burette, while continuously stirring the mixture. Then centrifuge for 30 min at 3000 rpm. During this process, residual material separated and appeared at the top layer in a falcon tube, while settled pellets were collected and transferred to petri plates. These plates were then placed in a drying oven at a temperature of 105 °C for a duration of 4 h. To normalize the resulting product, it was subsequently placed in a desiccator. The obtained pellets were finely powdered using a pestle and mortar. Then it was calcined at

500 °C for 2 h. Then final ready to use powder essentially constituted zinc oxide nanoparticles [31,32].

2.3. FTIR analysis

FTIR analysis was employed to detect the functional groups and diverse phytochemical components engaged in the reduction and stabilization processes of the produced nanoparticles. The FTIR measurements were conducted. The recorded results encompassed the range of 4000–400 cm^{-1} [33].

2.4. UV visible spectroscopic analysis

The UV–Vis spectroscopy of ZnO nanoparticles was examined to assess the bandgap energy and confirm the successful synthesis of the nanoparticles. 0.01 g of ZnO nanoparticles was dispersed in double-distilled water. Subsequently, 2 mL of the dispersion was placed in a quartz cuvette and subjected to analysis across a wavelength scan ranging from 200 to 800 nm [34].

2.5. Particle size analysis

Particle size analysis is good to analyze the properties of the nanoparticles and their behavior in paint formulations.

2.5.1. Antibacterial analysis of plant extract

Escherichia coli and *Staphylococcus aureus* were the selected bacterial strains to compare the antibacterial activity of control and fortified plant sample. Colonies of bacterial strains were obtained by culturing the media on nutrient agar and subsequently incubating it for 24 h 500 mL of LB medium was prepared by dissolving 5 g tryptone, 5 g sodium chloride, 2.5 g yeast extract, and 7.5 g agar in 500 mL of distilled water. Clear the solution by boiling it in a water bath for 5 min. Divided the medium into two flasks each of 250 mL. The medium was then autoclaved at 121 °C for 15 min. The autoclaved material was cooled down and poured into sterile petri plates and subsequently left to purify. The plates were incubated at 37 °C for 24 h to check the sterility of the media. Under an aseptic environment, bacterial colonies of both strains were transferred to 25 mL of double distilled water, and taken in separate falcon tubes, with the help of a sterile inoculation loop. The tubes were vortexed for 10 min to obtain a homogenized mixture. Antibacterial activity of controlled and zinc-fortified samples was evaluated against *Escherichia coli* (gram negative) and *Staphylococcus aureus* (gram positive) by agar well diffusion method. This activity was performed under aseptic conditions to eliminate all possible chances of contamination. A sterile cotton swab was dipped in the liquid inoculum of each bacterial strain and moved over the agar plate to create a uniform bacterial lawn in plates. The lawn of both strains was created in separate plates. A sterile cork borer was used to make wells of 7 mm diameter in each plate. Different concentrations (100–400 μL) of extract were poured into separate wells. The plates were placed in an incubator at 37 °C for 24 h after the zone of inhibition was measured around the well by using a ruler [35,36].

2.5.2. Industrial applications and shade comparison of the paints

At Diamond Paint Industries (Pvt) Ltd, Lahore, Pakistan, acrylic plastic water-based emulsion paint was freshly prepared with their patent recipe in bulk production and put 200 g each paint from the freshly prepared paint in different jars was taken. Put 0.6 g nanoparticles in one jar and 6 g plant extract sample in another jar. After mixing well, color parameters were accessed by using spectrophotometer.

Shade comparison testing: Different batch samples were conducted to observe the resulting shading. Sample # 01 contains 0.6 g plant nanoparticles. Sample #02 contains 6 g plant aqueous extract. ΔL, Δa, Δb, and ΔE in Table 2 will be discussed. If the value of ΔL is positive (+) it is light in color than the control paint. If the value of ΔL is negative (−) it is dark in color than the control paint. If the value of Δa is positive (+) it is redder as compared to the controlled sample. If the value of Δa is negative (−) it is at green side than the control paint. If the value of Δb is positive (+) it is yellower tone than the control paint. If the value of Δb is negative (−) it is bluer in color than the control paint [7,37,38].

Insect repellency test: *Aedes aegypti* species of mosquitoes (in mature form, surviving at 25 °C) were collected from department of zoology, GC University, Lahore, Pakistan. For the repellent activity, there was partition done in a glass chamber (11.5 inches in height, 23.5 inches in length, and 17.5 inches in width) and both samples of paint were examined. Before examining these samples, took small pieces of a hard chart and painted well with all four paint samples (formulated freshly) and put them one by one in a dry oven for 5 min at 60 °C to dry the paint panels. On the next day using cling film completely covered the glass chamber, put the different samples in each glass chamber and released 10 mosquitoes in each part, and observed the number of mosquitoes landing close to the paint sample. Record the number of mosquitoes with the interval of 2 min up to 30 min and using a camera captured the rate, frequency, and the

Table 1
Measurement of Zone of Inhibition of bacterial strains.

Paint with plant sample	*E. coli* zone of Inhibition (cm)	*S. aureus* zone of inhibition
Plant nanoparticles	1 ± 0.02	1.2 ± 0.06
Plant extract	0.2 ± 0.01	0.5 ± 0.01
Paint with biocide	0.7 ± 0.01	0.3 ± 0.01
Paint without biocide	1.1 ± 0.07	1.2 ± 0.04

Table 2
Spectrophotometer results of shade variation in all trials.

Batch #	CIE ΔL	CIE Δa	CIE Δb	CIE ΔC	CIE ΔH	CIE ΔE	CMC ΔE
Standard	−0.04	0.02	−0.15	0.14	0.05	0.15	0.22
1	−2.04	−0.36	2.96	1.82	−2.36	3.61	4.47
2	−1.89	−0.38	4.34	3.19	−2.98	4.75	6.49

number count of mosquitos approaching the sample [39–42].

3. Results

3.1. Phytochemical testing

Plant extract was found to lack flavonoids and glycosides, confirming the presence of other components such as carbohydrates, steroids, tannins, alkaloids, and terpenes. The rationale behind selecting Eucalyptus globulus extracts stemmed from their documented antioxidant, antimicrobial, and protective properties [43–45].

3.2. Green synthesized nanoparticles evaluation

Various analytical techniques, including FTIR, UV visible spectroscopy, and particle size analysis, were employed to comprehensively assess the composition and the extent of influence exerted by these nanoparticles in various aspects.

FTIR of Nanoparticles:

FTIR serves as a confirmation method for nanoparticle formation, providing insights into the vibrational and rotational modes of the molecules involved. This aids in the identification of functional and potential phytochemical molecules participating in the reduction and stabilization of ZnO·NPs. The FTIR spectra of ZnO.NPs synthesized through the green approach reveal a peak at 752.9 cm $^{-1}$, corresponding to the hexagonal ZnO symmetric bending vibration and also attributed to the weak vibration of ZnO. Broad peak observed at 3295 cm^{-1} indicates the presence of OH stretching vibrations of phenols. Smaller peaks representing bond vibrations at 2079.9 cm^{-1} (C–H stretching vibration of alkane), 1565.5 cm^{-1}, and 1476 cm^{-1}, 1423.8 cm^{-1} signify the presence of primary and secondary amines (*N*–H), characteristic of proteins, as well as C–O stretching regions of polysaccharides and phenolic groups and flavonoids. Peaks around 1412 cm-1 suggested the presence of the C═C group in aromatic compounds, and those between 1000 and 1250 cm^{-1} indicated the *C–O*–C group in ethers. This analytical technique aids in confirming the synthesis process and provides valuable information about the molecular composition involved in the green synthesis of ZnO nanoparticles. The FTIR peaks (Fig. 1) observed for *Eucalyptus globulus* fell within the range of 1500–3200 cm^{-1}, which contrasts with the broader range of 400–5000 cm^{-1} documented by Khoshraftar et al. [46]. Similar finding are also observed by Salim et al. [47] and abdelbaky et al. [48].

UV Visible spectrophotometer*:* In terms of UV visible spectroscopy, the absorption peaks (Fig. 2) were between 200 and 300 nm, who reported peaks at 430 nm. The variation observed in these cases could be attributed to the change in metal which impacted the UV visible spectroscopy results. The effective absorption of ZnO-NPs in the UV region suggests its potential use in medical applications, such as sunscreens or antiseptic ointments and the use of zinc nanoparticles in paint formulations is justified with the previous literature [47].

Particle size analysis: Particle size analysis became one of these essential tests because the particle length has a massive impact on the physiochemical properties of paint and may enhance the insect-repellant impact. The nanoparticles exhibited a higher molecular weight (Fig. 3), measuring 4.451 mg. This discrepancy in molecular weight comparison signifies a negative correlation between the particle size of these nanoparticles and their antimicrobial activity [49].

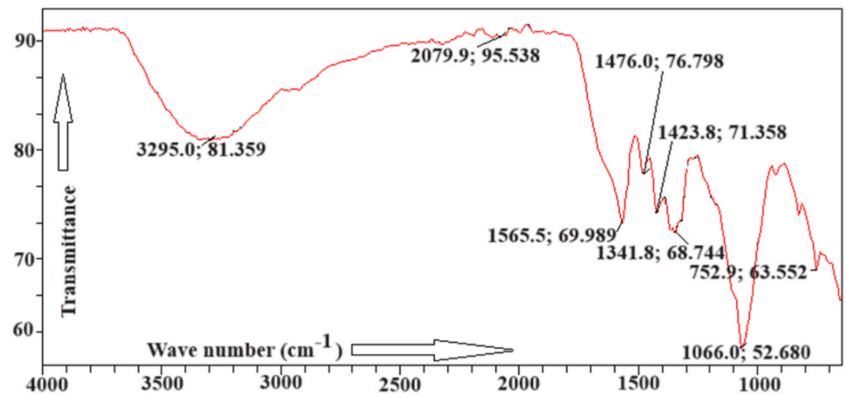

Fig. 1. FTIR of the plant material.

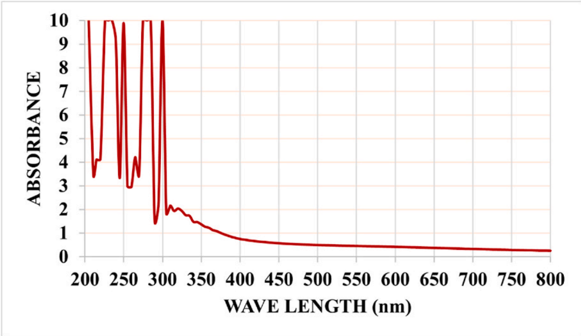

Fig. 2. UV/Visible spectrophotometer.

Antimicrobial Activity of painted panels: The antibacterial efficacy against both bacterial strains was assessed, revealing that the synthetic chemical biocide-based paint formulation exhibited a maximum inhibition zone of 3 cm, whereas the plant-based formulations, devoid of synthetic biocides, only achieved a 1 cm inhibition zone (Table 1). It is noteworthy that this formulation can be further refined to attain equivalent antibacterial activity. The noteworthy aspect is that the plant-based materials demonstrated antibacterial activity, marking an innovative aspect of this study as these formulations are being reported for the first time in a water-based acrylic plastic emulsion. The plant-based extracts were employed both independently and in conjunction with zinc-based nanoparticles. The assessment of the paint samples' antimicrobial properties was conducted using the agar well diffusion method, ensuring the experiment's sterility to eliminate any potential contamination.

3.3. *Paint samples shade evaluation*

These values demonstrate acceptable outcomes regarding the paint's shading, but there is still ample room for improvement in future research. In upcoming research endeavors, our focus will be on achieving more consistent results with minimal shade variation, more compatibility as compared to the current study. The current shade parameters are mentioned in Table 2.

Insect Repellent Activity: The study successfully achieved the primary objective of assessing the antimicrobial and insect-repellent properties of the formulated tested paint samples, yielding acceptable and favorable results. The incorporation of various additives and biocides in the paint samples exhibited effective responses (Fig. 4).

4. Conclusions

These alterations in the paint sample composition have demonstrated their potential benefits, and if further developed, they could significantly enhance the future of the paint industry. Adopting a sustainable approach has become a necessity in modern research, responding to the urgent challenge of climate change. As we progress into Industry 5.0 and 6.0, marked by a commitment to a waste-free environment and achieving net-zero carbon emissions, it is essential to shift from traditional production methods to sustainable practices. The present research represents a modest contribution, providing insights for scientists and the paint industry to embrace green synthesis in their paint formulations. The current study's findings indicate that paint, when naturally modified through the incorporation of plant extracts and their corresponding nanoparticles, enhances the insect repellent as well as antimicrobial properties of the coating materials. Further investigations are advisable, such as determining the shelf life of plant-based nanoparticles and exploring methods for extending this shelf life and to decrease the change in shade with the addition of natural plant-based materials. Given the prolonged efficacy of natural biocides, there is potential to enhance the durability of paint formulations over an extended period. A comprehensive assessment of the environmental impact of natural plant-based materials on paint weathering is strongly recommended. The study has yielded positive outcomes regarding cost-effectiveness, ease of application, and overall productivity. It highlights the transformative potential of nanotechnology within the paint industry if researchers delve into its comprehensive impact and work on the improvement and innovations in this work and contribute towards a better economy of the nation.

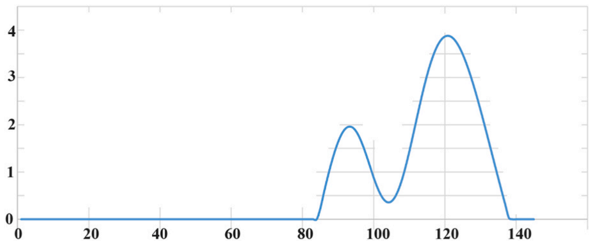

Fig. 3. Particle size analysis.

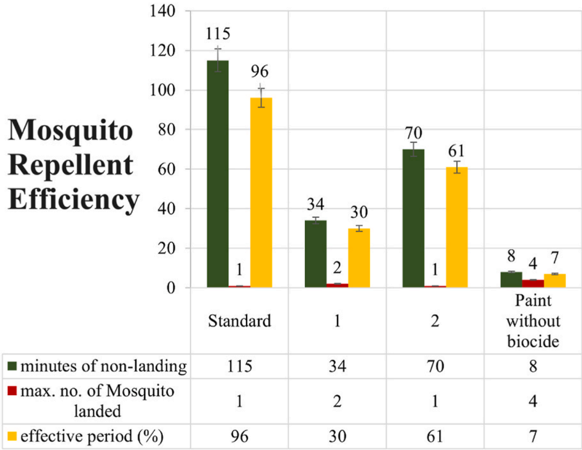

Fig. 4. Insect repellency test of all painted panels.

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Data availability

All data is included in this manuscript.

CRediT authorship contribution statement

Hammad Majeed: W. **Tehreema Iftikhar:** Visualization, Validation, Supervision, Project administration, Formal analysis, Conceptualization. **Muhammad Ashir Nadeem:** Visualization, Investigation, Formal analysis. **Muhammad Altaf Nazir:** Visualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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