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EDITED BY

Xiaoping Xin,
University of Missouri, United States

REVIEWED BY

Amit Singh Yadav,
Department of Experimental Medical
Science, Faculty of Medicine, Lund
University, Sweden

Hugo Gerardo Lazcano-Ramirez,
Autonomous University of Zacatecas,
Mexico

Yong Liu,
Zhejiang University of Science and
Technology, China

*CORRESPONDENCE

Sajad Majeed Zargar,
✉ smzargar@skuastkashmir.ac.in

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Elucidating the potential of green synthesis: formulation of nanofungicide from agricultural wastes against *Fusarium oxysporum* f. sp. *capsici*

Saika Bashir¹, Aneesa Batool¹, Mansoor Showkat¹, Suhail Ashraf²,
G. N. Yattoo³, Jebi Sudan¹, Mohsin Altaf Hajini¹,
Javed Iqbal A. Bhat⁴, Zahoor Ahmad Bhat⁵, Mudasar A. Mir¹,
Bhagyashree Dhekale⁶, Parvaze A. Sofi⁷, Antonio Masi⁸ and
Sajad Majeed Zargar^{1*}

¹Proteomics Lab, Division of Plant Biotechnology, Faculty of Horticulture, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Srinagar, Jammu and Kashmir, India, ²Department of Plant Biotechnology, Tamil Nadu Agricultural University, Coimbatore, India, ³Department of Chemistry, NIT, Srinagar, Jammu and Kashmir, India, ⁴Division of Basic Sciences and Humanity, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Srinagar, Jammu and Kashmir, India, ⁵Division of Plant Pathology, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Srinagar, Jammu and Kashmir, India, ⁶Division of Agricultural Statistics, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Srinagar, Jammu and Kashmir, India, ⁷Division of Genetics & Plant Breeding, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Srinagar, Jammu and Kashmir, India, ⁸Department of Agronomy, Food, Natural Resources, Animals and the Environment, University of Padova, Padua, Italy

Introduction: *Fusarium* wilt, caused by *Fusarium oxysporum*, is a major fungal disease leading to significant crop losses worldwide. Conventional chemical fungicides used to manage this disease pose environmental and health risks, highlighting the need for sustainable alternatives.

Methods: This study explores the antifungal efficacy of green-synthesized nanoformulations derived from Chinar (*Platanus orientalis*) and Dal weed (*Ceratophyllum demersum*) extracts for controlling *Fusarium* wilt. Nanoformulations were prepared and characterized for particle size, stability, and bioactive compound content.

Results and discussion: The Chinar-based nanoformulation exhibited superior antifungal activity due to its higher flavonoid content, smaller droplet size, and higher zeta potential, enhancing its stability and bioavailability. *In-vitro* assays revealed dose-dependent inhibition of *F. oxysporum*, with complete suppression observed at a 40% concentration. Membrane integrity assays indicated that the nanoformulations disrupted fungal cell membranes, causing leakage of cellular contents without inducing genetic damage. *In-vivo* trials on chilli plants demonstrated that treatments with 50% and 60% nanoformulation concentrations significantly reduced the incidence of *Fusarium* wilt without causing phytotoxic effects, confirming their practical applicability in agricultural conditions. Furthermore, the eco-friendly synthesis of these nanoformulations from agricultural waste materials offers environmental and economic benefits, supporting their integration into sustainable farming practices. The possible inhibitory interaction of phytoconstituents with important *Fusarium oxysporum* proteins was validated by molecular docking research. Overall, Chinar and Dal weed-based nanoformulations present an

effective, safe, and environmentally sustainable alternative to chemical fungicides, offering promising solutions for managing Fusarium wilt and improving crop health.

KEYWORDS

anti-microbials, biopesticides, crude extract, molecular docking, nanoformulation, phytochemicals

1 Introduction

Hundreds of fungal diseases impact the 168 crops identified by the Food and Agricultural Organization (FAO) of the United Nations as essential for human nutrition. Despite extensive use of fungicides and the cultivation of disease-resistant crop varieties, global farmers face losses of 10%–23% of their harvests annually due to fungal infections, with an additional 10%–20% lost after harvest (Stukenbrock and Gurr, 2023). Among such diseases, Fusarium wilt is a destructive disease impacting a wide range of plant species, leading to yield losses that can reach 100%. Due to the significant genetic diversity within the *Fusarium* pathogen, it has developed various physiological races capable of infecting different plant species across diverse geographical regions worldwide (Lal et al., 2024). Commercial agriculture largely relies on chemical fungicides to protect crops from fungal pathogens by killing or inhibiting their cells and spores. However, the convenience and affordability of these fungicides often lead to excessive or repeated use. This over-application or improper use has caused harmful effects on beneficial organisms, as well as negative impacts on human and animal health and the environment (El-Baky and Amara, 2021). Additionally, the development of resistant strains of fungal pathogens has made it increasingly difficult to manage plant fungal diseases (El-Baky and Amara, 2021). Furthermore, the rise of resistant fungal strains has made the treatment of plant fungal diseases more difficult. As a result, the development of safe, non-toxic, and environmentally friendly alternatives to chemical and synthetic fungicides is crucial for controlling plant fungal infections. Several strategies, including biological control approaches, microbial-based fungicides, botanical fungicides, agro-nanotechnology-based interventions, and fungal cell deactivation methods, have been explored to manage plant pathogenic fungi (Porteous-Álvarez et al., 2021). Plant extracts are recognized for their bioactive properties, including antioxidant, antibacterial, and antifungal activities. These biological qualities are largely attributed to the presence of phenolic compounds, which play a significant role in the plant's defense mechanisms against pathogens. For instance, in one study, extracts from *Piper aduncum* leaves have demonstrated significant antifungal activity against *Fusarium oxysporum*, potentially by disrupting the fungal cell wall or membrane. Metabolic profiling has identified three key bioactive compounds contributing to this effect: piperaduncin C, asebogenin, and (–)-methyllinderatin (Cárdenas-Laverde et al., 2021). Moreover, the use of nanotechnology offers promising alternatives to conventional pesticides by utilizing nanoscale materials to manage plant diseases more effectively and sustainably. These nano-based solutions can enhance the delivery and efficacy of

agrochemicals, reduce environmental contamination, and mitigate the adverse effects associated with traditional pesticide use (Liu et al., 2024a). For example, a study demonstrated that silver nanoparticles (AgNPs) effectively suppressed the growth of *Fusarium oxysporum* or completely eradicated the fungus. Specifically, a concentration of 25 mg/L AgNPs was shown to enhance seed germination and the growth of muskmelon seedlings while also reducing the occurrence of Fusarium wilt in muskmelon. The antifungal action of AgNPs against *F. oxysporum* is likely attributed to their ability to disrupt fungal development, damage cellular structures, and interfere with metabolic processes. These findings provide valuable insights for the widespread use of silver nanoparticles in plant disease management (Liu et al., 2024b). In recent researches, nanoemulsions have become preferred over nanoparticles due to their multiple advantages, including improved stability, which prevents issues like creaming or sedimentation. They enhance bioavailability by increasing the surface area, allowing better absorption of active compounds (Preeti et al., 2023). For instance, a study developed a stable, food-grade nanoemulsion using *Cleome viscosa* essential oils, with droplet sizes under 20 nm, which exhibited strong inhibitory effects against the foodborne pathogen *Candida albicans*. This nanoemulsion demonstrated significant fungicidal activity and caused notable morphological disruptions in the fungus, likely by interfering with cell wall biosynthesis. These essential oil nanoemulsions (EONE), which are safe and derived through a bio-based method, show promise for enhancing food safety and quality by preventing microbial spoilage, with minimal toxicity as per FDA guidelines (Krishnamoorthy et al., 2021a). Compared with metal-based nanoparticles, nanoemulsion systems offer several advantages including lower environmental persistence, reduced risk of heavy metal accumulation, and improved regulatory acceptance for agricultural applications. Plant-derived nanoemulsions are particularly attractive because they utilize biodegradable components and can serve as carriers for naturally occurring bioactive compounds. These features make nanoemulsions promising candidates for the development of environmentally compatible plant protection strategies within sustainable and circular bioeconomy frameworks (Krishnamoorthy et al., 2021a).

The primary objective of this study was to develop and evaluate eco-friendly nanoemulsion formulations derived from agricultural waste, specifically Chinar leaves and Dal weed, for the biological control of *Fusarium oxysporum*, the causative agent of Fusarium wilt. The study aimed to valorize these underutilized plant materials through green synthesis approaches, characterize their phytochemical composition, and assess their antifungal efficacy both *in vitro* and *in vivo*. In addition to evaluating disease suppression and phytotoxicity on chilli plants, the study explored the antifungal mechanism of action via membrane integrity assays, DNA laddering, and molecular docking of key fungal pathogenicity

Abbreviations: MIC, Minimum Inhibitory Concentration; EC, Electrical Conductivity; OD Optical Density; UV, Ultra Violet; PDA, Potato Dextrose Agar.

proteins. By demonstrating the effectiveness, safety, and environmental compatibility of these nanoformulations, the research contributes to sustainable waste management practices by offering biodegradable, plant-based alternatives to synthetic fungicides that align with integrated pest management (IPM) and circular bioeconomy strategies.

2 Materials and methods

2.1 Plant material

The agricultural wastes [Dal Weed (*Ceratophyllum demersum*) and Chinar (*Platanus orientalis*)] were selected because they have natural disease resistance. Chinar leaves were collected from SKUAST-K, Shalimar Campus and Dal Weed was collected from Dal Lake, Srinagar.

2.2 Extraction process

The collected samples were properly cleaned and shade dried at room temperature and then the dried samples were crushed manually. The extraction was done from the dried agricultural wastes by blending them in different solvents like ethanol, methanol, and water, based upon the criteria of increasing polarity (@10 mL per Gram). The samples were kept under dark conditions for 96 h at room temperature. After that the samples were filtered using Wattman filter paper (Grade 1), and the extra solvent in the filtrate was evaporated using a rotary evaporator at a temperature of 45 °C.

2.3 Isolation of fungal culture

For the isolation of *Fusarium oxysporum* f. sp. *capsici* wilt-infected chilli (*Capsicum annuum*) plants were gathered from experimental areas and the pathogenic fungi was isolated in the Division of Plant Pathology, SKUAST-K, Shalimar, Srinagar. Pure cultures were then obtained using the hyphal tip technique, and the isolate was kept at 25 °C ± 2 °C on Potato Dextrose Agar (PDA). For pathogenicity and antifungal bioassays, the strain was frequently employed.

2.4 Initial screening of Dal weed and Chinar extract against *Fusarium oxysporum* f. sp. *capsici*

The crude extracts were evaluated for their antifungal effect against *Fusarium oxysporum* f. sp. *capsici* by Poisoned Food Method (Ibrahim et al., 2017) using three concentrations viz., 3% (3 mg/mL), 5% (5 mg/mL) and 10% (10 mg/mL) (w/v) thereby determining the MIC (Minimum Inhibitory Concentration).

The extract was added separately to sterile potato dextrose agar medium in proper proportions and poured into petri dishes and left for solidification under aseptic conditions. One disc (3 mm) of medium was cut from 15 days old *Fusarium oxysporum* f. sp. *capsici* culture and was transferred to the centre of the petri dish under aseptic conditions. The inoculated plates were incubated at 28 °C ± 1 °C and growth of the pathogen was measured on alternate days till

7 days. Media supplemented with varying percentages of extracting solvent (10%, 50% and 100%) was taken as negative control and the petri dishes containing only media was taken as control.

2.5 Determination of total flavonoids in samples

After the initial screening of the extracts, total flavonoids were determined by the AlCl₃ method in both samples, as described by Meda (Meda et al., 2005). 1 mL of the prepared sample was blended with 0.3 mL of NaNO₃ (5%). After 2 min, 0.3 mL of 10% AlCl₃ was added to the mixture and kept for another 2 min. Then, 3.4 mL of 4 N NaOH was added to the sample solution and adjusted to 10 mL with distilled water. After 10 min at room temperature, the absorbance was measured at 510 nm using a UV-visible spectrophotometer. Total flavonoids were determined using an external standard curve of Catechin and expressed as catechin equivalent per 100 g.

2.6 Synthesis of nanoformulation

Water-in-oil nanoformulations were synthesized according to high-energy emulsification methods (Jasmina et al., 2017). Ethanol was used to extract plant materials, and a rotary evaporator was used to remove the solvent under low pressure in order to produce a dried extract. Nanoformulations were created using the dry mass that was left behind after rotary evaporation. Approximately 10 mg of crude extract was recovered from 1 kg of plant material under the extraction conditions used in this study. The obtained extract was subsequently used for the preparation of the nanoformulation and antifungal bioassays. The aqueous dispersion phase of the nanoformulation was created by dissolving 4% (w/v) of the dried extract in distilled water. In order to incorporate the dried extracts into the water-in-oil nanoemulsion system, aqueous reconstitution was used, despite the extracts' greater solubility in organic solvents.

The water-in-oil nanoformulations were synthesized using food-grade refined soybean oil (Fortune™, India) as the oil phase. Soybean oil was selected due to its biocompatibility, low toxicity, and suitability for agricultural nanoformulation development. Tween 20 was used as a stabilizing agent, and the aqueous phase consisted of plant extract prepared from agricultural waste materials. The dissolved dry mass was converted to 100 mL of nanoformulation by mixing it with vegetable oil, which constituted the oil phase, using a stabilizing agent (tween 20). The varying percentages of both phases were taken, and a particular percentage at which the formulation was stabilized, was subjected to homogenisation and then sonicated using an ultra-sonicator. A volume of 88.2 mL of oil was mixed with 1.8 mL of stabilizer and was kept at magnetic stirrer for 20 min at room temperature. After that 10 mL of the dissolved dry mass was added to it (water-in-oil nano-formulation) and again kept at magnetic stirrer for 20 min at room temperature. Initially, the emulsion was homogenized at around 13,000 rpm for 10 min to reduce the droplet size, creating a more uniform emulsion. Subsequently, sonication was performed using a probe-type ultrasonicator (KINGLAB/QUICKLAB Probe Sonicator Homogenizer, India) operated at a frequency of 20 kHz and 70% amplitude for 45 min. Sonication was carried out in pulse

mode to minimize heat generation during the synthesis of the nanoformulation. This combined method effectively improved the uniformity, stability, and performance of the nano-emulsion, offering a more efficient approach to achieving smaller, more consistent droplets (Belgheisi et al., 2021).

2.7 Characterization of nanoformulation

A Zetasizer Nano ZS (Malvern Instruments, United Kingdom) was used to measure the average droplet size, polydispersity index (PDI), and zeta potential of the produced nanoemulsions using dynamic light scattering (DLS) and electrophoretic light scattering techniques, respectively. To prevent additional scattering effects, the nanoemulsion samples were diluted 1:100 (v/v) with double-distilled water prior to testing. Every sample was examined in triplicate, and all measurements were performed at 25 °C.

Z-average diameter and accompanying PDI values, which show the homogeneity of the nanoemulsion system, are used to report the droplet size distribution. To evaluate the nanoemulsions' surface charge and colloidal stability, zeta potential values were evaluated (Inaam and Peijs, 2006).

To assess the contribution of the oil phase alone, a blank oil medium (without plant extract or surfactant) was subjected to identical dilution and measurement conditions. The blank oil medium served as a control to distinguish true nanoformulation droplet formation from background oil aggregation effects.

2.8 Studying the antifungal activity of nanoformulations against *Fusarium oxysporum* f.sp. *capsici*

Following synthesis, the nanofomulations were used directly in their liquid form and applied on a volume/volume (v/v) basis during antifungal assays. The nanoformulations were tested for their activity against the selected fungus. The poisoned food technique was again employed to the inhibitory action of the nanoformulations, as per the protocol mentioned above and thereby determining the MIC. Carbendazim (5%, v/v) was used as a commercial fungicide control to provide a reference for comparison with the antifungal activity of the developed nanoformulations.

2.9 Assessment of the optimized nanoformulation on the occurrence of *Fusarium* wilt in chilli plants

For the *in-vivo* trial, three different concentrations of the nanoformulation were chosen to investigate the antifungal effect on *Fusarium* caused wilt in chilli. Four-week-old healthy chilli seedlings were selected for the trial. The seedlings were inoculated using seedling dip method. The seedlings were soaked in a spore suspension prepared using 14 days old culture, for about 1 minute and transplanted in pots at the rate of one plant per pot. Treatment @ 20%, 30% and 40% (v/v) approximately correspond to 0.15, 0.25, and 0.50 mg/mL of crude extract, was given to the infected seedlings 21 days after inoculation. Healthy seedlings without inoculation and without treatment were taken as control and the seedlings infected with spore suspension but without treatment were taken as positive control.

2.10 Mechanism of inhibition of fungal growth

Mechanism of fungicidal action of the nanoformulation against *Fusarium oxysporum* f. sp. *capsici* was tested by measuring Electrical Conductivity (to measure the permeability changes in the fungal cell membrane), absorbance using UV visible spectrophotometer (to measure the concentration of nucleic acids released) and DNA Ladder Assay (to test for DNA damage).

Broth cultures were inoculated with 14 days old fungal culture under aseptic conditions and incubated at 28 ± 1 °C overnight. The next day, the cultures were treated with nanoformulation at MIC levels and again incubated at 28 ± 1 °C for 1 week. The medium without treatment was used as control, and the medium treated with the solvent was used as negative control.

2.10.1 Measurement of electrical conductivity

After 1 week, the cultures were diluted with distilled water at a concentration of 2% and changes in the EC of cell membrane surface was measured in triplicates by using a conductivity meter.

2.10.2 Measurement of absorbance

The cultures were diluted with distilled water after a week, at a concentration of 2% and the absorbance was measured at 290 nm using the method reported by Zhang (Zhang et al., 2016) with some modifications. All the measurements were done in triplicates using UV visible spectrophotometer.

2.10.3 DNA ladder assay

DNA was extracted from one-week-old cultures treated with 5% nanoformulation using the protocol reported by Sharma (Sharma et al., 2008) with some modifications. The culture without any treatment was used as control. Using a 1% agarose gel, extracted DNA was tested, and the Gel Documentation System was used to visualize the results.

2.11 Statistical analysis

All of the tests were conducted in triplicate, and the Completely Randomized Design method which examines the impact of a single factor on a response variable was utilized to analyse the results. Statistical significance in *in-vitro* validation was evaluated by calculating the CD value using OPSTAT software.

2.12 Assessment of the optimized nanoformulation on the occurrence of *Fusarium* wilt in chilli plants

For the *in-vivo* trial, three different concentrations of the nanoformulation were chosen to investigate the antifungal effect on *Fusarium* caused wilt on chilli. Four-week-old healthy chilli seedlings were selected for the trial. The seedlings were inoculated using the seedling dip method. The seedlings were soaked in a spore suspension prepared using 14 days old culture, for about 1 minute and transplanted in pots at the rate of one plant per pot. Treatment

@ 20%, 30% 40% and 50% (v/v) was given to the infected seedlings 21 days after inoculation.

2.13 LC-MS analysis for identification of flavonoids

Since Chinar showed better anti-fungal activity at lower concentrations, it was selected for the identification of flavonoid components in the ethanol extracts of Chinar and was profiled using LC-MS (Liquid Chromatography-Mass Spectrometry) analysis. An Agilent 1290 Infinity II LC system in conjunction with a 6545 Q-TOF mass spectrometer was used to analyze the samples using LC-MS. Mass spectra were obtained in both positive and negative ionization modes, and chromatographic separation was accomplished on a C18 reverse-phase column. Mass-to-charge ratios (m/z) and fragmentation patterns compared to the NIST and METLIN spectrum libraries were used to confirm the identification of these chemicals (Al-Rimawi et al., 2017).

2.14 In-silico molecular docking studies

In order to assess the binding potential of the main bioactive chemicals in the nanoformulation against certain survival-related proteins of *Fusarium oxysporum* f. sp. *capsici*, *in silico* molecular docking studies were conducted in light of the encouraging antifungal activity seen *in vitro*. This strategy sought to pinpoint molecular targets and clarify potential pathways that contribute to pathogen suppression. SWISS-MODEL homology modelling was used to create protein structures, which were then verified using the SAVES server, including Ramachandran plot analysis to evaluate stereochemical quality. Molecular docking investigations were conducted using the verified models.

2.14.1 Selection and identification of protein targets

The selection and identification of protein targets of *Fusarium oxysporum* f. sp. *capsici* were sorted out with the help of previous literature and the UniProt database. Protein targets selected were Fow1 (Mitochondrial Fow1 carrier protein), Fmk1, Arg1 (Arginosuccinase) and Pgx4 (Galacturonan 1,4-alpha-galacturonidase).

2.14.2 Molecular modelling of protein targets

Protein targets selected for *Fusarium oxysporum* f. sp. *capsici* were lacking experimentally and computationally solved structures. Hence, based on query coverage percentage and identity percentage obtained from BLASTp, SWISS-MODEL (Method: Rigid-body assembly) (Waterhouse et al., 2018) was used to build the structure of the identified protein targets.

2.14.3 Validation of protein models

The PROCHECK tool from the Structural Analysis and Verification Server (SAVES, Meta server) (<https://saves.mbi.ucla.edu/>) was used to ensure the model quality of modelled protein targets using Ramachandran plot. The Swiss PDB Viewer was used

for energy minimization of modelled proteins and loop construction for residues in disallowed regions (Guex and Peitsch, 1997).

2.14.4 Ligand preparation and analysis

The ligand structures were obtained in SDF format from the PubChem database (Kim et al., 2016). The commercial fungicide Carbendazim was used as a positive check. Open Babel software was used to convert the SDF file format to the PDB file format (O'Boyle et al., 2011).

2.14.5 Molecular docking and virtual screening

To accomplish molecular docking, PyRx 0.8's AutoDock vina module was used (Dallakyan and Olson, 2014). The "make macromolecule" option in PyRx programme was used to prepare the structure of proteins. To determine binding site pockets for the targets, Prankweb server was used. AutoDock4 and autogrid4 parameter files were used for molecular docking and grid configuration, respectively. BIOVIA Discovery Studio client 2021 (<https://www.3ds.com/products-services/biovia/>) was used to visualise interactions of docked conformations of protein-ligand complexes. To distinguish between the receptor, ligand, and interacting atoms, different colours were assigned.

2.15 Circos plot analysis

The binding energy of the ligands and proteins of *Fusarium oxysporum* f. sp. *capsici* was expressed as a Circos plot, where different colors represent different ligands and their binding with target proteins.

2.16 STRING analysis

The STRING database (Search Tool for the Retrieval of Interacting Genes/Proteins) was used to investigate the interactions of different proteins in *Fusarium*. *Fusarium*'s genome was queried using protein sequences from Fowl to discover the proteins interacting with each other. A degree-sorted network was constructed using genes and proteins that interacted with one another. The information regarding the query proteins, the protein domains it interacts with, and the number of other domains in the network that fall under a certain domain were all examined. For better understanding, it was investigated how the query protein and its interaction partners are related functionally. A minimum of 10 participants and a medium level of confidence of 0.40 were used to construct the network. Cytoscape 3.9.1 was used to import the STRING database result for further analysis.

3 Results

3.1 Initial bioassaying of Dal weed and Chinar extract against *Fusarium oxysporum* f. sp. *capsici*

Antifungal activities of Dal weed and Chinar extracts were prepared with various solvents. Preliminary screening against

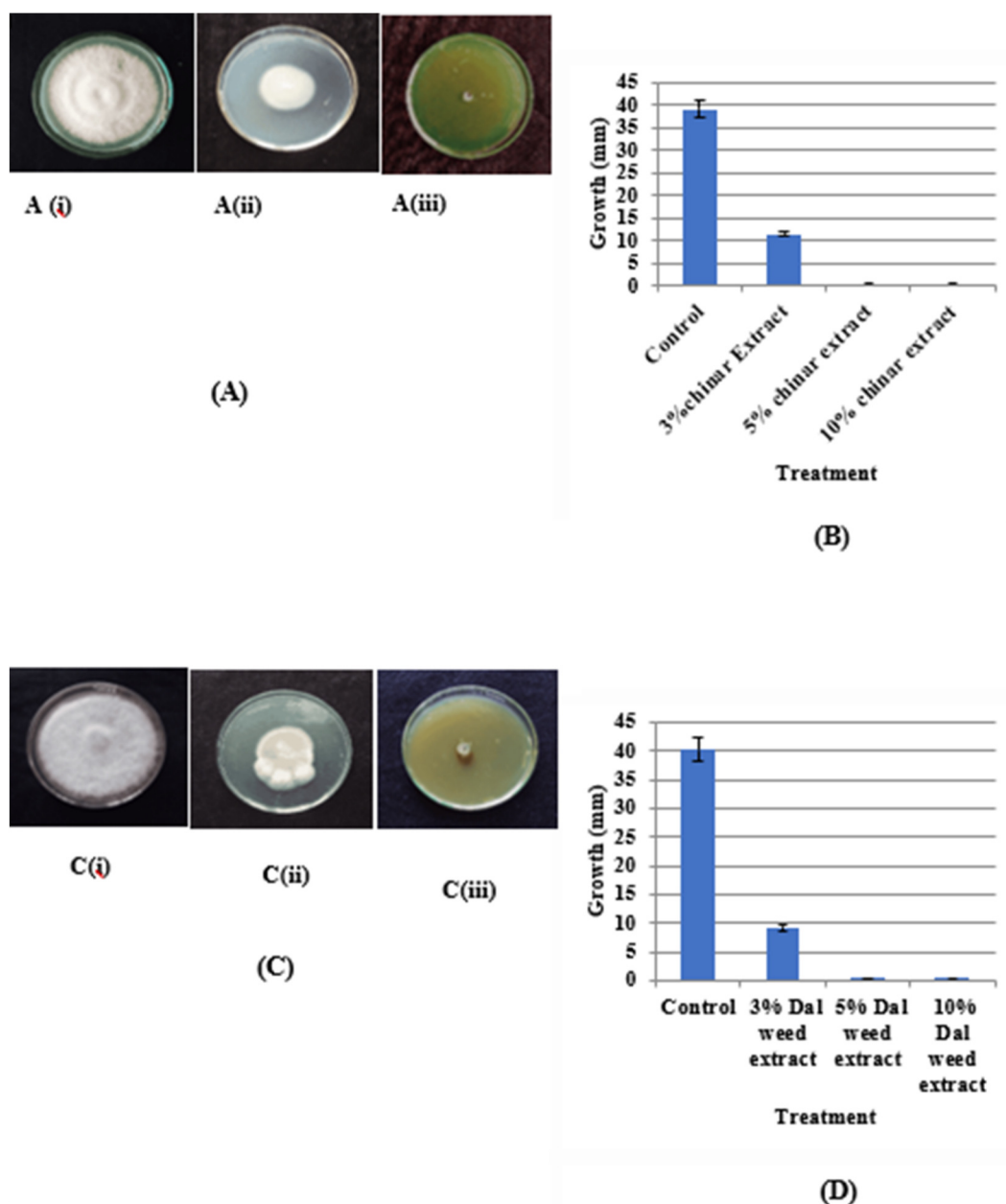


FIGURE 1

In vitro antifungal activity of Dal weed and Chinar extracts against *Fusarium oxysporum* f. sp. *capsici* assessed using the poisoned food technique. (A) Representative culture plates showing mycelial growth of *F. oxysporum* under different treatments: (i) Control (PDA only), (ii) negative control (solvent only), and (iii) treatment with Dal weed extract; (B) Quantitative analysis of mycelial growth expressed as colony diameter (mm) under different concentrations of Dal weed extract compared with the control; (C) Representative culture plates showing mycelial growth under (i) Control, (ii) negative control, and (iii) treatment with Chinar extract; (D) Quantitative comparison of colony diameter (mm) of *F. oxysporum* treated with different concentrations of Chinar extract.

Fusarium oxysporum f. sp. *capsici* was conducted using the poisoned food technique. Methanol and ethanol extracts of Dal weed exhibited distinct antifungal activities at 5% (v/v) concentration, inhibiting the mycelial growth effectively compared to the control. In case of Chinar, the ethanol extract was stronger, as effective inhibition was evident even at 3% (v/v), indicating higher potency of this extract. In contrast, the aqueous extracts of both Dal weed and Chinar did not significantly inhibit fungal growth even at the highest tested concentration of 50% (v/v), indicating poor solubility or limited extraction of the antifungal bioactives in water.

Since almost equal level of inhibition was obtained in methanolic and ethanolic extracts and aqueous extracts were ineffective, ethanol was selected as the extraction solvent for all further experiments.

Figures 1B,D compare various concentrations of ethanolic extracts from Dal weed and Chinar, respectively. Corresponding PDA plates presenting the inhibition effects of Dal weed and Chinar on the growth of *Fusarium* are depicted in Figures 1A,C. The effect of the

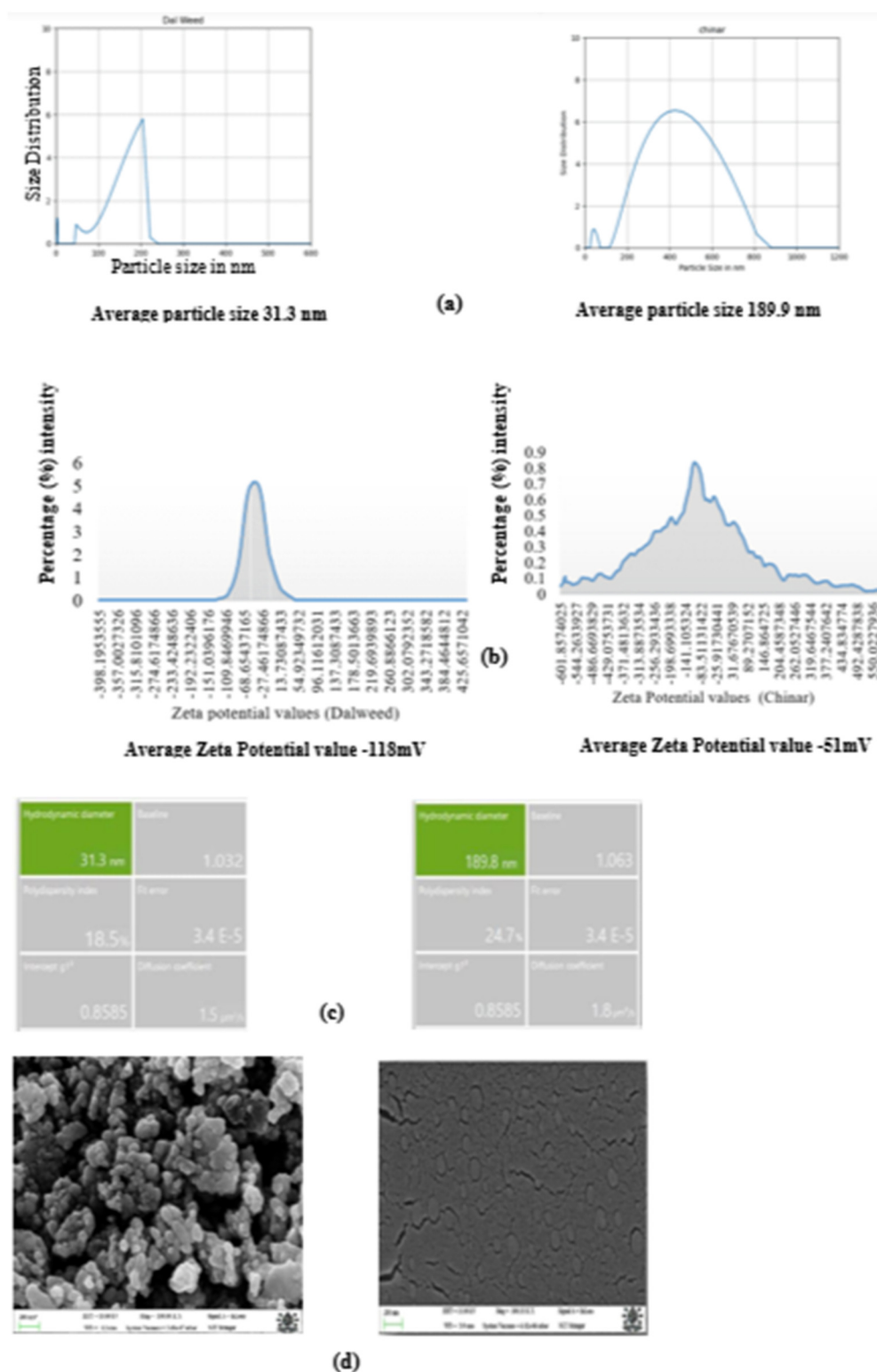


FIGURE 2

Physicochemical characterization of the synthesized Dal weed and Chinar nanoformulations. **(a)** Particle size distribution profiles of Dal weed and Chinar nanoformulations determined by dynamic light scattering (DLS), showing the hydrodynamic droplet size distribution in nanometers (nm); **(b)** Zeta potential distribution curves of Dal weed and Chinar nanoformulations, indicating surface charge and colloidal stability of the nanoemulsions. The average zeta potential values are expressed in millivolts (mV), with negative values reflecting electrostatic stabilization of the formulations; **(c)** Representative numerical outputs obtained from Malvern Zetasizer Nano ZS showing mean hydrodynamic diameter, polydispersity index (PDI), and zeta potential values of the nanoformulations; **(d)** Scanning electron microscopy (SEM) images depicting the surface morphology of the synthesized nanoformulations, illustrating differences in particle aggregation, surface texture, and structural compactness between Dal weed and Chinar nanoformulations.

various solvent extracts and concentrations on fungal growth is further highlighted in [Supplementary Figures 1a,b,c, 2a,b](#), with the respective statistical analysis along with the CD values presented in [Supplementary Table 1](#).

3.2 Total flavonoid content was higher in Chinar as compared to Dal weed

Quantitative estimation of total flavonoids showed a significant variation between the two plant extracts. The flavonoid content of the Chinar extract was estimated to be $83.31 \mu\text{g mL}^{-1}$, while the extract prepared from Dal weed contained $54.61 \mu\text{g mL}^{-1}$ of total flavonoids, which signifies that Chinar contains a much higher amount of flavonoids than Dal weed.

A statistical comparison of flavonoid content using a Student's t-test was done between the two extracts, which showed a significant difference between the samples. The comparative flavonoid content of Dal weed and Chinar extracts are graphically represented in [Supplementary Figure 3](#).

3.3 Characterization of the synthesized nanoformulations

3.3.1 Particle size distribution and zeta potential analysis

The hydrodynamic z-average diameters of the Dal weed and Chinar nanoformulations were 189.8 nm and 31.3 nm, respectively, according to dynamic light scattering studies. The mean of three successive runs taken during a single measurement is represented by the stated values. Acceptable size homogeneity was indicated by the corresponding polydispersity index (PDI) values of 0.24 for the Dal weed formulation and 0.18 for the Chinar formulation. Strong electrostatic stabilisation of the nanoformulations was suggested by the mean zeta potential values of -51 mV and -118 mV , respectively. [Figures 2A,B](#) displays the zeta potential profiles and particle size distribution. In contrast, the blank oil medium exhibited a very large hydrodynamic diameter (288–440 μm) along with extremely high PDI values (42.6–189.1) ([Supplementary Figure 4](#)). The absence of defined peaks and the high polydispersity observed for the blank oil indicate uncontrolled aggregation and poor dispersibility in the aqueous measurement medium. These results confirm that the oil phase alone does not form nanoscale droplets and lacks colloidal stability.

Over the course of a 6-month storage period, neither of the two nanoformulations showed any signs of phase separation, creaming, or sedimentation. The high absolute zeta potential values and low PDI values ([Figure 2C](#)) suggest good kinetic stability resulting from electrostatic repulsion between scattered droplets, even if visual inspection alone cannot rule out minute changes in droplet size.

3.3.2 Scanning electron microscopy analysis

The two nanoformulations showed clear morphological variations, according to SEM examination. A rough, granular, and aggregated nanostructure, typical of nanoscale particles undergoing aggregation after drying, was displayed by the Chinar nanoformulation, which had a reduced hydrodynamic diameter

(~31 nm). On the other hand, the Dal weed nanoformulation (190 nm) showed a comparatively compact and smooth surface morphology, probably as a result of film formation or droplet coalescence during sample preparation and vacuum drying. These discoveries, which show variations in solid-state organisation rather than hydrodynamic particle size, are in line with the DLS findings. [Figure 2D](#) represents the SEM images of the nanoformulations.

3.4 Study of *in-vitro* antifungal activity of Dal weed and Chinar nanoformulation

To ascertain dose-dependent effects, the antifungal effectiveness of Chinar and Dal weed nanoformulations against *Fusarium oxysporum* f. sp. *capsici* was assessed at various concentrations. Fungal growth was clearly inhibited by both nanoformulations in a concentration-dependent manner; at 40% concentration, mycelial growth was completely suppressed.

The Chinar-based nanoformulation outperformed the Dal weed nanoformulation in terms of antifungal efficacy at lower concentrations. In particular, the Chinar nanoformulation significantly inhibited fungal growth at 20% (v/v) concentration compared to the Dal weed nanoformulation, suggesting improved antifungal efficacy of the Chinar-derived formulation at lower concentrations. [Table 1](#) summarises quantitative inhibitory data for both nanoformulations across concentrations. [Figure 3](#) shows a comparison of the antifungal activity of various concentrations of the synthesised nanoformulations, together with example PDA plates that demonstrate real fungal growth inhibition. [Supplementary Figures 5a,b](#) offer more PDA plate pictures showing the impact of nanoformulations at different doses. [Supplementary Tables 4, 5](#) present the corresponding statistical analysis, which includes critical difference (CD) values. When compared with the commercial fungicide Carbendazim (5%, v/v), the nanoformulation at 20% demonstrated a nearly comparable level of inhibition of *Fusarium oxysporum*, suggesting that the formulated extract possesses antifungal activity similar to that of the standard fungicide under the experimental conditions ([Supplementary Figure 5a](#)).

3.5 Antifungal assessment of optimized nanoformulation against Fusarium wilt in chilli plants

The outcomes of our *in vitro* research are supported by the data from *in-vivo* results conducted in polyhouses. 10% and 20% (v/v) of the nanoformulation did not show any significant reduction in the incidence of wilt disease in chilli plants. At 40% (v/v) the symptoms were reduced to some extent, however, at 50% and 60% (v/v) of the concentration, the nanoformulation showed promising results, demonstrating the antifungal activity of the nanoformulation on *Fusarium* wilt as depicted in [Figure 4A](#).

3.6 Determination of the mechanism of inhibition of fungal growth

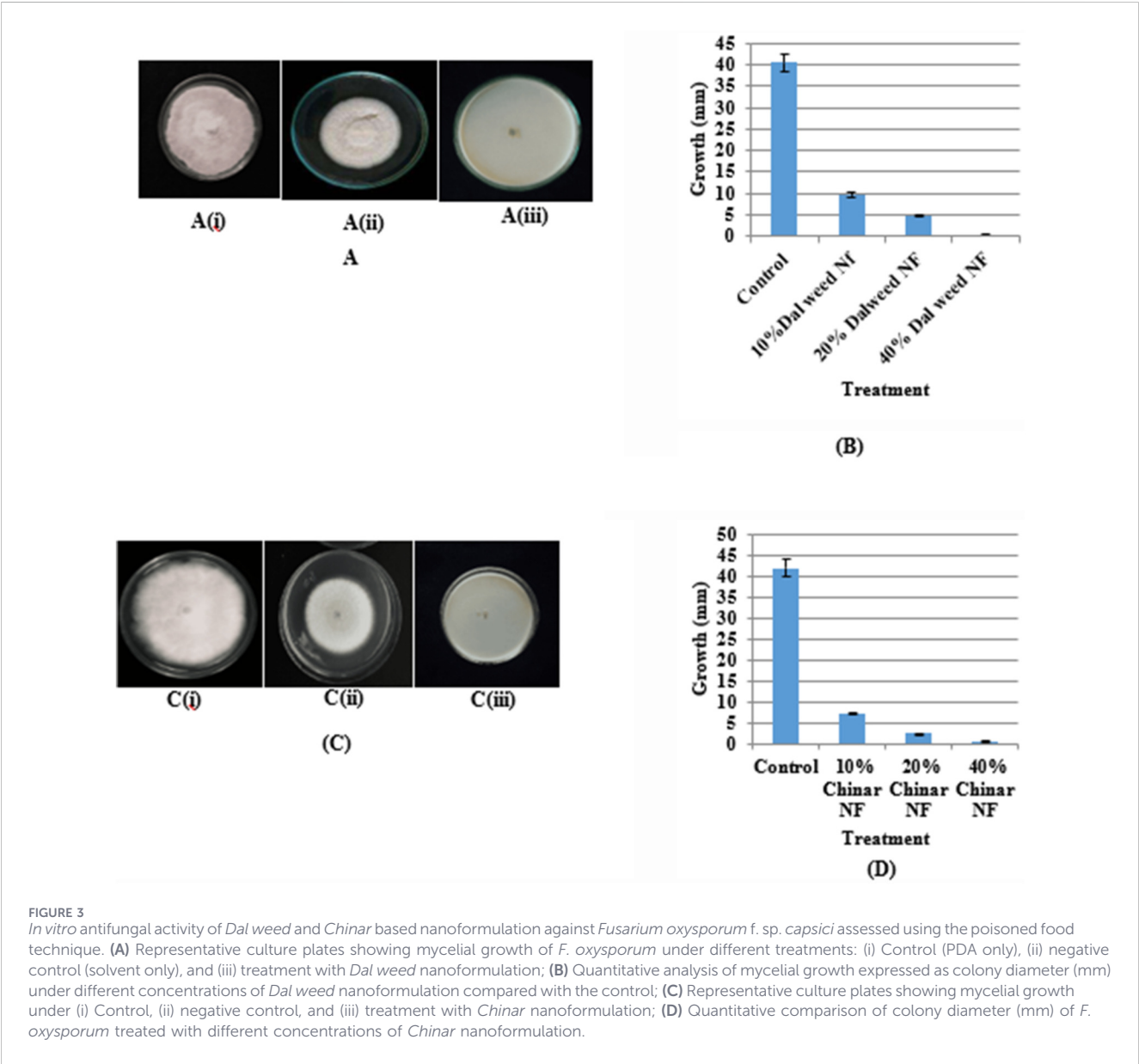
3.6.1 Determination of electrical conductivity (EC)

After being treated with the nanoformulation, membrane permeability of *Fusarium oxysporum* f. sp. *capsici* was

TABLE 1 Effect of different concentrations of Dal weed and Chinar nanoformulations on the mycelial growth of *Fusarium oxysporum* f. sp. *capsici*.

Samples	10% concentration	20% concentration	40% concentration	50% concentration	CD values
Chinar	7.16	3.10	0.50	0.50	0.38
Dal weed	9.70	4.50	0.50	0.50	0.37

Values represent mean colony diameter (mm) of *F. oxysporum* measured after incubation. Nanoformulations were tested at 10%, 20%, 40%, and 50% (v/v). CD, indicates critical difference at $p \leq 0.05$. Each value represents the mean of three independent replicates.



evaluated using electrical conductivity (EC) measurements. The control, negative control, and treatment cultures had EC values of 6.36 μ S, 6.42 μ S, and 11.58 μ S, respectively. In comparison to both control conditions, the treated culture's much higher EC value suggests more intracellular electrolyte loss. Figure 4Bi shows the comparing EC values.

3.6.2 Determination of absorbance

To assess the release of intracellular components into the extracellular media, absorbance analysis was performed using a UV-visible spectrophotometer. The control, negative control, and treatment cultures had optical density values of 1.067, 1.069, and

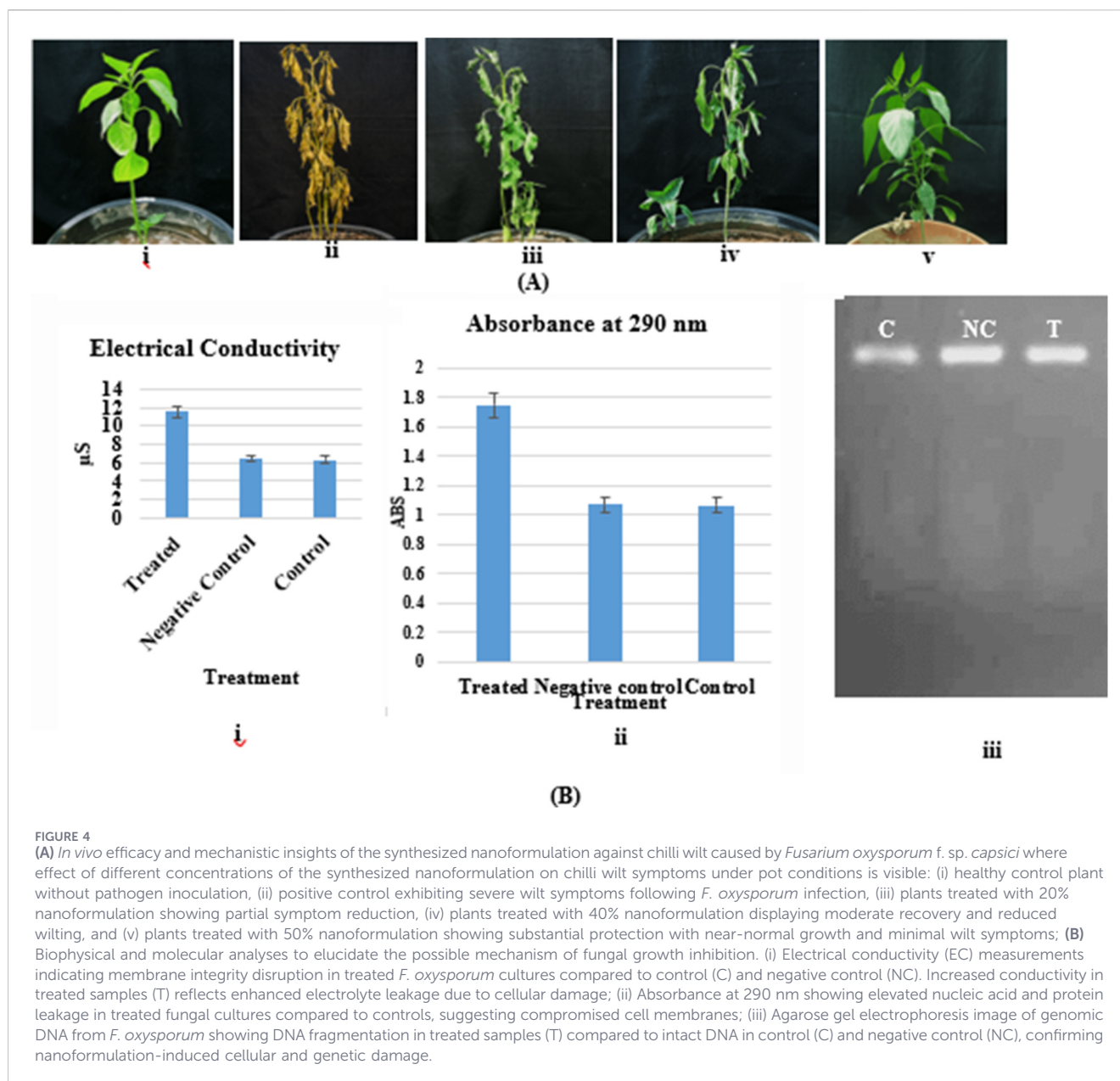


FIGURE 4

(A) *In vivo* efficacy and mechanistic insights of the synthesized nanoformulation against chilli wilt caused by *Fusarium oxysporum* f. sp. *capsici* where effect of different concentrations of the synthesized nanoformulation on chilli wilt symptoms under pot conditions is visible: (i) healthy control plant without pathogen inoculation, (ii) positive control exhibiting severe wilt symptoms following *F. oxysporum* infection, (iii) plants treated with 20% nanoformulation showing partial symptom reduction, (iv) plants treated with 40% nanoformulation displaying moderate recovery and reduced wilting, and (v) plants treated with 50% nanoformulation showing substantial protection with near-normal growth and minimal wilt symptoms; (B) Biophysical and molecular analyses to elucidate the possible mechanism of fungal growth inhibition. (i) Electrical conductivity (EC) measurements indicating membrane integrity disruption in treated *F. oxysporum* cultures compared to control (C) and negative control (NC). Increased conductivity in treated samples (T) reflects enhanced electrolyte leakage due to cellular damage; (ii) Absorbance at 290 nm showing elevated nucleic acid and protein leakage in treated fungal cultures compared to controls, suggesting compromised cell membranes; (iii) Agarose gel electrophoresis image of genomic DNA from *F. oxysporum* showing DNA fragmentation in treated samples (T) compared to intact DNA in control (C) and negative control (NC), confirming nanoformulation-induced cellular and genetic damage.

1.747, respectively. Increased leakage of UV-absorbing cellular components is suggested by the treated samples' noticeably higher absorbance when compared to both controls. Figure 4Bii displays these findings.

3.6.3 DNA ladder assay

A DNA ladder experiment was used to determine if nanoformulation treatment produced observable DNA fragmentation. As seen in Figure 4Biii, all three samples, the control, negative control, and treated cultures had intact genomic DNA bands. Under the experimental conditions, no evidence of DNA fragmentation or degradation was found.

3.7 Identification of flavonoids by LC-MS

Several flavonoid chemicals were found in the ethanolic Chinar extract, according to liquid chromatography-mass spectrometry (LC-MS) research. According to Table 2, the main flavonoids found were naringenin, quercetin, rutin, myricetin, kaempferol, epigallocatechin, epicatechin gallate, and catechin.

Rutin, quercetin, myricetin, kaempferol, and naringenin were chosen for further molecular docking research based on their relative quantity in the extract and previously documented antifungal activity. Comparison of MIC of our extracts and nanoformulations with other studies has been shown in Supplementary Tables 6, 7 respectively where the fungicidal effect

TABLE 2 LC–MS–based identification and relative abundance of major flavonoids detected in dried Chinar leaves. Values represent peak area/intensity obtained from LC–MS analysis for individual compounds across three technical replicates. Compound identification was performed based on retention time and mass spectral data.

Samples	Naringenin	Quercetin	Rutin	Myricetin	Kampeferol	Epigallocatechin	Epicatechin gallate	Catechin
Dried chinar	4.241	4.370	194.006	0.756	2.980	19.518	0.105	24.60
Dried chinar	4.247	4.467	171.482	0.785	2.680	15.254	−0.097	25.70
Dried chinar	4.426	0.921	176.893	0.856	2.973	18.099	−0.049	25.40

has been reported due to flavonoids. The identification of these flavonoids supports quantitative flavonoid quantification and provides a logical foundation for assessing their possible interactions with important *Fusarium oxysporum* f. sp. *capsici* proteins using *in silico* methods.

3.8 In-silico molecular docking studies

According to the docking results, significant interactions between the chosen phytochemicals and the modelled *Fusarium oxysporum* f. sp. *capsici* proteins were shown. Naturally occurring binding was shown by all ligands' negative binding affinities; the greatest affinities across several targets were shown by quercetin and rutin.

3.8.1 Molecular modelling of target proteins of *Fusarium oxysporum* f. sp. *capsici*

The Fow1, Fmk1, Arg1 and Pgx4 target proteins used SWISS-MODEL software. The 3D structure and modeling details of the target proteins are mentioned in [Supplementary Figure 6](#) and [Supplementary Table 8](#). While the structure of ligands used is provided in [Supplementary Figure 7](#).

3.8.2 Validation of target proteins of *Fusarium oxysporum* f. sp. *capsici* using the SAVES server

SAVES server validated Fow1, Fmk1, Arg1 and Pgx4 target proteins via Ramachandran plot. It revealed that Fow1 had 95% of the residues that were found in the most favoured region and 5% in the additionally allowed region, Fmk1 had 92.4% of the residues in the most favoured region, 6.9% in the additionally allowed region, Arg1 had 94.5% of the residues in the most favoured region, 5.3% in the additionally allowed region and Pgx4 had 86.5% of the residues in the most favoured region, 13.2% in the additionally allowed region ([Figure 5](#)).

3.8.3 Molecular docking of the selected ligands with the protein target of *Fusarium oxysporum* f. sp. *capsici*

Molecular docking tools were employed to evaluate the binding energy of the modelled target proteins Fow1, Fmk1, Arg1 and Pgx4 with different ligand molecules. The binding affinity of ligands Naringenin, Quercetin, Rutin, Myricetin, Kampferol and Carbendazim (Positive control) with the target protein Fow1 was −7.5, −7.4, −8.9, −7.2, −7.4 and −5.7 kcal/mol,

respectively. Likewise, binding affinity of ligands with the target protein Fmk1 was −7.8, −8.4, −9.0, −8.4, −8.3 and −6.9 kcal/mol, respectively. Similarly, the binding energy of ligands with the target protein Arg1 was −7.0, −7.4, −8.4, −7.1, −7.2 and −6.1 kcal/mol respectively. The binding affinity of ligands with the target protein Pgx4 was −7.8, −8.3, −10.1, −8.6, −7.8 and −6.5 kcal/mol respectively ([Figure 6](#)) and [Table 3](#).

3.9 Circos plot analysis

The binding interactions of the ligands Naringenin, Quercetin, Rutin, Myricetin, Kampferol and Carbendazim with the target proteins Fow1, Fmk1, Arg1 and Pgx4 is represented in the form of a circos plot ([Figure 7](#)). The Circos plot illustrates the interaction landscape between selected bioactive ligands and key fungal target proteins (Fow1, Fmk1, Arg1, and Pgx4) identified through molecular docking analysis. In the circular layout, proteins and ligands are represented as distinct sectors along the outer ring, while the colored connecting ribbons denote docking interactions between individual ligands and target proteins.

The width and continuity of the ribbons reflect the relative strength and frequency of interactions, where broader ribbons indicate stronger binding affinities or multiple favourable contacts between a ligand and a given protein. The multicoloured ribbons highlight that several ligands interact with more than one target protein, suggesting a multi-target mode of action.

3.10 Analysis of protein-protein interaction of Fow1 using STRING software

Interactions of Fow1 protein with other proteins are suggested, including a large family of proteins with a wide range of functions. It was found that the Fow1 protein was making a network with 10 other proteins ([Supplementary Figure 8](#)).

4 Discussion

In contrast to traditional emulsions, nanoemulsions are kinetically stable dispersions that are usually distinguished by droplet sizes less than 200 nm and limited size distributions ([McClements, 2012](#)). From a regulatory perspective, plant-based nanoformulations are increasingly considered safer alternatives to conventional agrochemicals due to their biodegradable nature and lower ecotoxicological footprint. Nevertheless, systematic evaluation of environmental safety and regulatory compliance remains

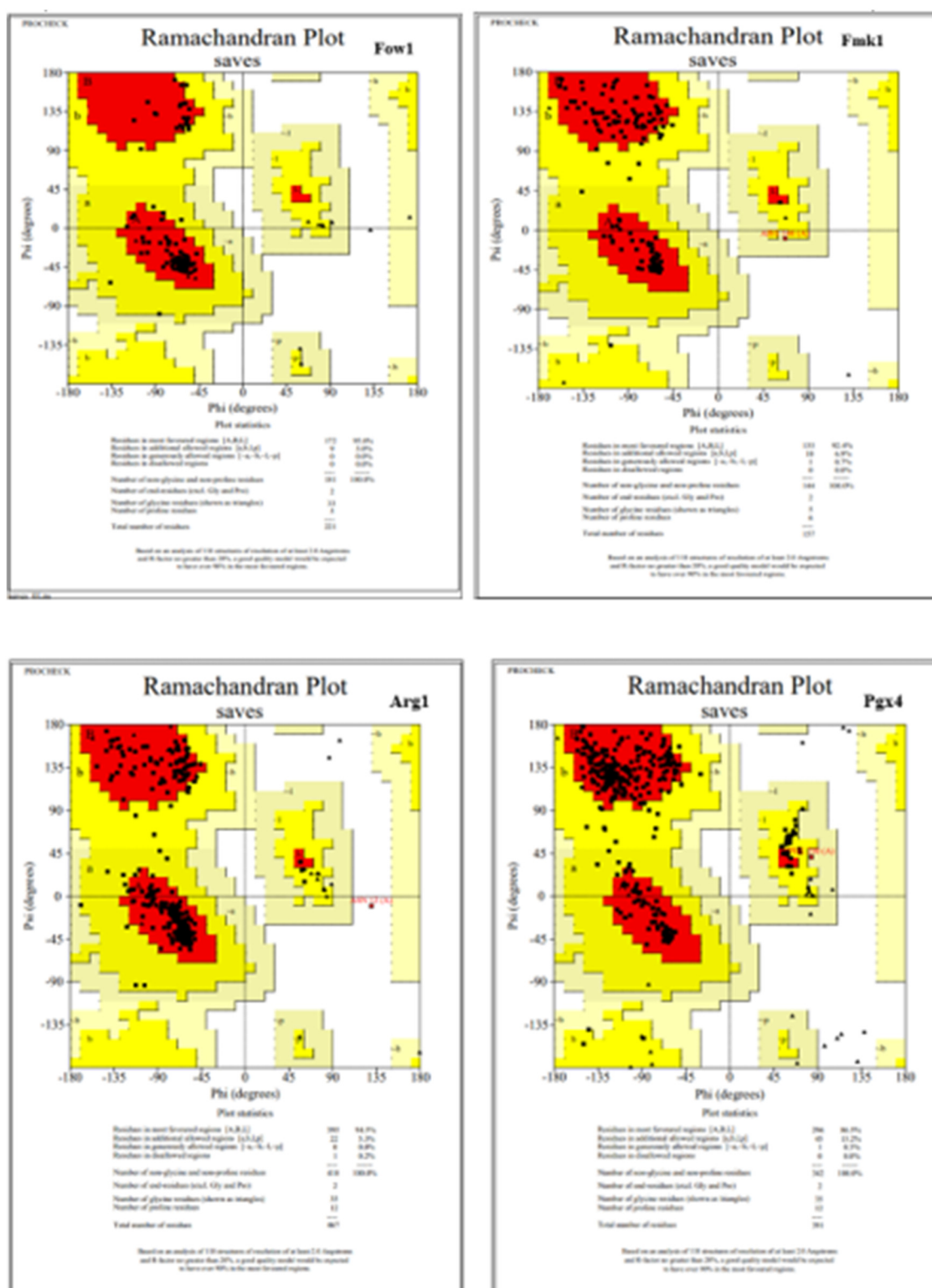


FIGURE 5
Ramachandran plot for the target proteins Fow1, Fmk1 and Arg1, Pgx4 respectively.

essential before large-scale agricultural deployment (Krishnamoorthy et al., 2021a). *Fusarium oxysporum* f. sp. *capsici*, a prominent soil-borne pathogen that causes Fusarium wilt in chillies, was significantly inhibited by green-

synthesised nanoemulsions made from Chinara and Dal weed extracts in the current study. Bioactive phytochemicals, such as flavonoids, phenolic acids, tannins, saponins, and terpenoids, which are well known for their antifungal, antioxidant, and antibacterial

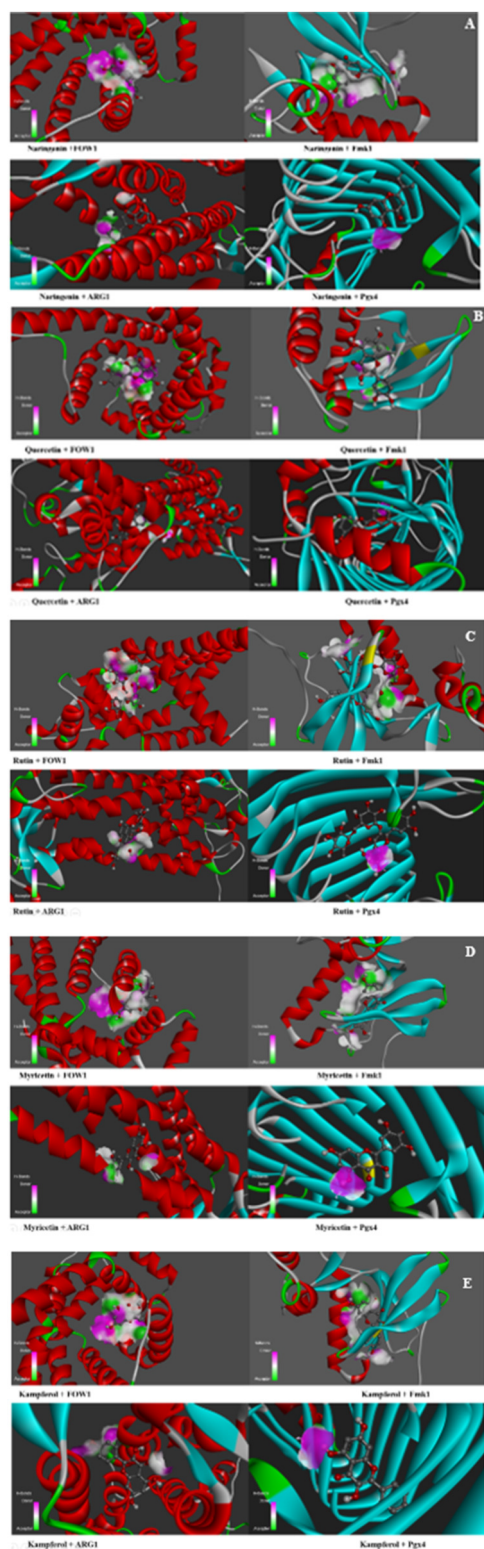


FIGURE 6
3D interaction of the ligands (A) Naringenin (B) Quercetin (C) Rutin (D) Myricetin and (E) Kampferol with the target proteins.

phytochemical studies have revealed substances including kaempferol, caffeic acid, quercetin, and rutin in Chinar (Devi et al., 2019; Baibakov et al., 2021). Because of their superior bioavailability, better solubilisation of hydrophobic chemicals, and increased kinetic stability, which together improve biological performance, nanoemulsions have become a viable alternative to traditional formulations (Feng et al., 2018; Sambhakar et al., 2023).

The increased flavonoid content and advantageous physicochemical characteristics of the Chinar-based nanoemulsion may be responsible for its improved antifungal action, especially at lower concentrations. It is well known that flavonoids interfere with enzyme function, damage fungal cell membranes, and block vital metabolic processes (Ngu et al., 2021). The improved antifungal effectiveness seen in this investigation is supported by prior research showing that kaempferol and caffeic acid hinder fungal cell wall production and inhibit mycelial development (Benoutman et al., 2022). Because of their high surface area-to-volume ratios and tiny droplet sizes, nanoemulsions improve cellular absorption and enable effective interactions between bioactive substances and fungal cells (Sharma et al., 2018; Iqbal et al., 2020). Metal-based nanoparticles, including silver nanoparticles, have been shown to exhibit comparable membrane-targeted antifungal activities against *F. oxysporum* (Liu et al., 2024b). Both Dal weed and Chinar nanoemulsions demonstrated concentration-dependent antifungal action in the current investigation, with total inhibition seen at 40% concentration. Because of its higher zeta potential and smaller droplet size, which encourage improved membrane contact and prolonged release of active ingredients, the Chinar nanoemulsion may be more effective at lower concentrations (Iqbal et al., 2020). Essential oil-based nanoemulsions have demonstrated similar antifungal effects against *Penicillium verrucosum* and *Candida albicans* (Krishnamoorthy et al., 2021b). The relatively high negative zeta potential observed particularly for the Chinar-based formulation may be attributed to the presence of ionizable phytochemical constituents such as flavonoids and phenolic compounds. These molecules can contribute to surface charge through deprotonation of hydroxyl groups, thereby enhancing electrostatic stabilization of the nanoemulsion droplets (Liu et al., 2024b).

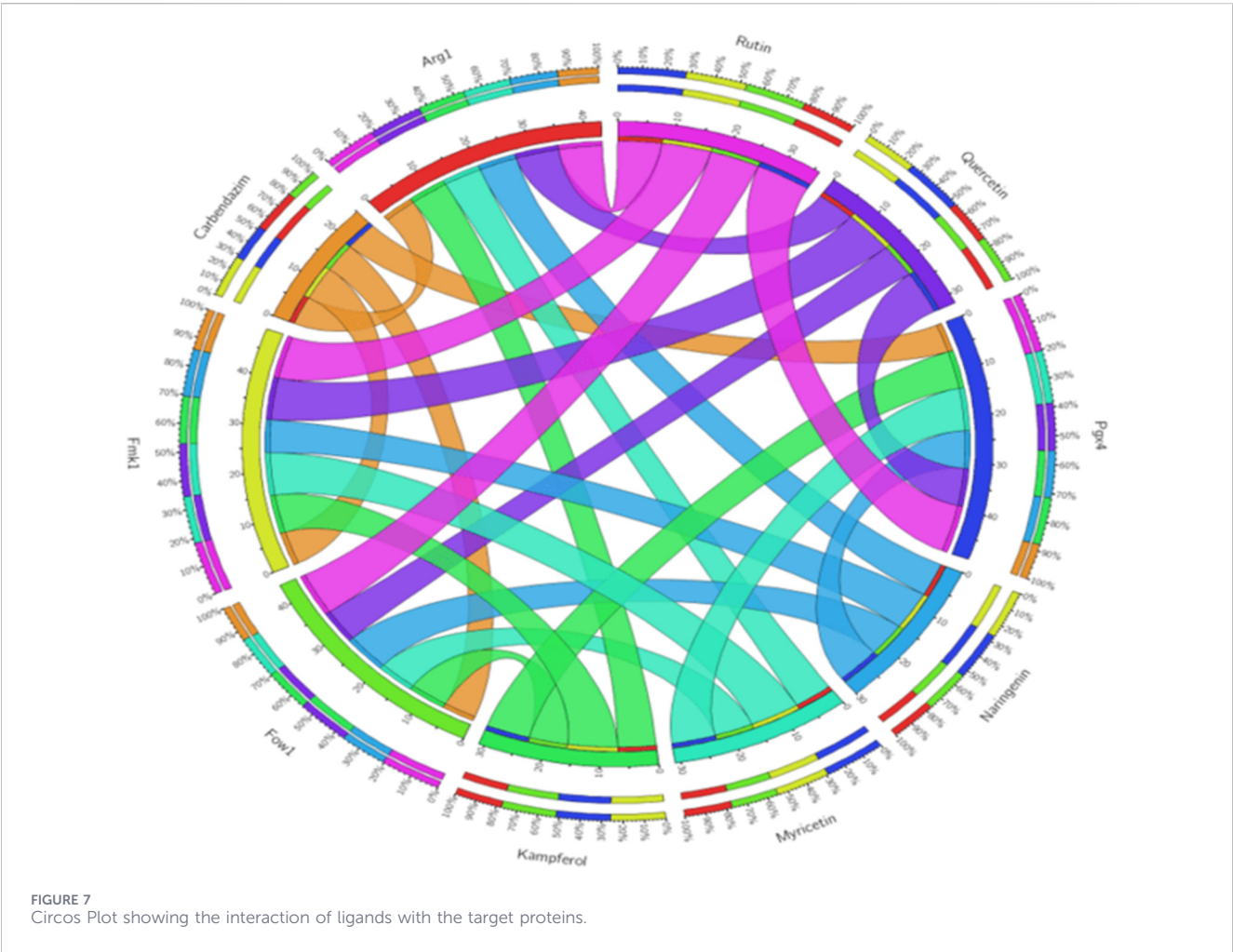
In terms of characterising nanoemulsions, it is crucial to remember that they are categorised according to kinetic stability rather than thermodynamic stability, and that physicochemical parameters like droplet size, polydispersity index (PDI), and zeta potential are used to identify them (McClements, 2012; Feng et al., 2018). The production of stable nanoemulsion systems rather than traditional emulsions was confirmed by the nanoemulsions in this work, which showed nanoscale droplet sizes, low PDI values indicating a limited size distribution, and sufficient zeta potential. As frequently reported in nanoemulsion-based plant protection and pharmaceutical studies, the maintenance of favourable droplet size distribution, PDI, and zeta potential values provides dependable evidence of kinetic stability, even though visual homogeneity alone is insufficient to confirm long-term stability (Iqbal et al., 2020; Krishnamoorthy et al., 2021b).

Mechanistic studies revealed that the primary mechanism of the nanoemulsions' antifungal action is the destruction of the integrity of the fungal cell membrane. Increased electrical conductivity (EC)

qualities, are abundant in both Chinar and Dal weed (Devi et al., 2019; Baibakov et al., 2021). While Dal weed is known to possess saponins and terpenoids that contribute to antifungal activity, earlier

TABLE 3 Binding energy (kcal/mol) of ligands with the targeted proteins.

Proteins	Ligands					
	Naringenin	Quercetin	Rutin	Myricetin	Kampferol	Carbendazim
Fow 1	−7.5	−7.4	−8.9	−7.2	−7.4	−5.7
Fmk 1	−7.8	−8.4	−9.0	−8.4	−8.3	−6.9
Arg 1	−7.0	−7.4	−8.4	−7.1	−7.2	−6.1
pgx4	−7.8	−8.3	−10.1	−8.6	−7.8	−6.5



and optical density (OD) readings after treatment, which indicate intracellular electrolyte and cytoplasmic content leakage, were used to infer this. EC and OD measures are commonly used as preliminary and suggestive assays for evaluating membrane permeability and cellular leakage in fungal systems, even if they are not direct visual confirmation methods (Lan et al., 2019; Hashem et al., 2023). Even in the absence of electron microscopy, a number of earlier investigations have effectively used similar assays to infer membrane damage induced by plant extracts, essential oil nanoemulsions, and green-synthesized nanomaterials (Lan et al., 2019; Hashem et al., 2023). Significantly, the lack of DNA

fragmentation in the DNA ladder test indicates that the nanoemulsions target specific membranes instead of causing genotoxic damage, which is in line with previous findings on plant-based nanoemulsions (Lan et al., 2019). Although no clear DNA fragmentation pattern was observed in the ladder assay, this result should be interpreted with caution. The absence of detectable laddering does not necessarily exclude the possibility of other forms of genomic or cellular stress, such as localized DNA damage, oxidative stress, or sublethal effects that may not produce a characteristic fragmentation pattern detectable by agarose gel electrophoresis.

The antifungal effectiveness of the nanoemulsions under biologically relevant circumstances was further confirmed by the *in vivo* assessment. Without causing any phytotoxic effects, application of the formulations at 50%–60% doses dramatically decreased the incidence of Fusarium wilt in chilli plants. These results demonstrate the nanoemulsions' usefulness for managing diseases in greenhouse or field settings, where crop safety and efficacy are crucial. Under typical agricultural spraying conditions, approximately 400–600 L of spray solution per hectare is applied for foliar treatments in vegetable crops. Based on this estimation, the tested concentrations of 50%–60% (v/v) would correspond to approximately 250–300 L of nanoformulation per hectare when using a spray volume of 500 L ha⁻¹. At an average planting density of about 25,000 plants per hectare, this would represent roughly 10–12 mL of nanoformulation per plant, providing a preliminary indication of the practical application requirements under field conditions. Further understanding of the antifungal mechanism at the molecular level was obtained using molecular docking research. This study's molecular docking analysis offers initial insights into possible interactions between the target proteins linked to *Fusarium oxysporum* and the flavonoids discovered by LC-MS. It is crucial to remember that these docking results are predictive in nature and were produced utilizing protein structures that were computationally predicted instead of crystal structures that were resolved through experimentation. As a result, rather than being conclusive proof of particular protein targeting, the reported binding affinities should be regarded cautiously as suggestive of potential molecular interactions. However, the docking analysis provides a helpful foundation for comprehending the possible molecular foundations of the antifungal activity seen *in vitro*. Interestingly, a number of flavonoids with comparatively high binding affinities were also among the more prevalent components found in the extract's LC-MS analysis. This association between docking affinity and compound quantity suggests that these phytochemicals may work together to enhance the extract's antifungal effectiveness. The main flavonoids in the nanoformulation, namely rutin and quercetin, showed substantial binding affinities toward important pathogenicity-related proteins of *Fusarium oxysporum* (Fow1, Fmk1, Arg1, and Pgx4), according to molecular docking study. It is well recognized that these proteins are essential for signal transduction, amino acid synthesis, cell wall breakdown, and mitochondrial function. The high binding affinities found, which were on par with or more potent than the commercial fungicide carbendazim, provide light on the mechanisms underlying the antifungal action seen in experiments, including membrane disruption and fungal growth suppression (Moin et al., 2024).

The results of the *in silico* docking process offer computational insights into the possible interactions between important phytoconstituents and crucial *Fusarium oxysporum* proteins. These results add to the experimental antifungal evidence and point to potential molecular targets for the bioactivity that has been discovered. Nevertheless, this work did not directly validate the connections between nanoformulation and proteins through experimentation, which calls for more research (Rathore et al., 2024). Similar insights into protein-ligand interactions have been reported for other green-synthesized nanoparticles: (Aseel et al., 2024); showed antiviral activity of biosynthesized copper oxide

nanoparticles through docking studies, and (Nashath et al., 2025) described effective binding of iron oxide nanoparticles to bacterial virulence proteins. (Azhar et al., 2022). also demonstrated strong binding of ZnO nanoparticles to fungal proteins affecting carrot pathogens, adding further support to our mechanistic conclusions.

The nanoemulsions have significant economic and environmental benefits in addition to their biological performance. In line with the concepts of sustainable agriculture and environmental protection, the use of agricultural biomass, such as Chinar and Dal weed, promotes waste reduction, resource valorisation, and a reduced dependency on synthetic fungicides (Sharma et al., 2018). Their eco-friendliness and biodegradability reduce hazards to non-target organisms and facilitate incorporation into integrated pest management (IPM) plans (Sambhakar et al., 2023).

Overall, this work shows that green-synthesized nanoemulsions made from biomass from agricultural waste successfully reduce *Fusarium oxysporum* and provide a sustainable substitute for traditional chemical fungicides. The current work emphasises the potential of agricultural waste valorisation in sustainable plant disease management and promotes the development of ecologically safe, nano-enabled biofungicides by integrating physicochemical characterisation, biological validation, and computational analysis.

While the present study demonstrates promising antifungal activity of the developed nanoformulation against *Fusarium oxysporum*, certain limitations should be acknowledged. The current study did not evaluate potential impacts on non-target soil microbiota or conduct comprehensive ecotoxicological assessments. Furthermore, although the *in vivo* experiments demonstrated effective disease suppression under controlled conditions, large-scale field validation was beyond the scope of this work. Future studies should therefore focus on detailed physicochemical characterization, environmental safety assessments, and field-level evaluations to further establish the practical applicability of this nanoformulation for sustainable plant disease management.

5 Conclusion

The present study aimed to create and characterize a stable, non-phytotoxic plant-based nanoformulation with potent antifungal properties against *Fusarium*. The results showed that the Chinar nanoformulation exhibited significantly higher antifungal activity compared to the Dal weed nanoformulation. Molecular and microscopic analyses revealed a substantial disruption of the fungal cell membrane, which contributed to the enhanced efficacy of the Chinar-based formulation. As a result, a notable reduction in wilt was observed in chili plants during the *in-vivo* tests. Rutin and quercetin, two important phytochemicals in the nanoemulsion, bind significantly to essential virulence proteins of *Fusarium oxysporum* (Fow1, Fmk1, Pgx4, Arg1), according to molecular docking study. This supports the nanoemulsion's potential as a long-lasting antifungal drug by indicating that it not only has physical antifungal effects but also molecularly alters pathogen metabolism. These findings suggest that the Chinar nanoformulation could serve as an effective alternative to conventional synthetic pesticides, addressing the challenges of

high costs and environmental pollution associated with their use. This nanoformulation offers a promising biocontrol solution with potential for large-scale agricultural application. Further studies are needed to optimize its formulation, better understand the underlying mechanisms of action, and evaluate its real-world performance in various agricultural contexts.

Data availability statement

The original contributions presented in the study are included in the article/[Supplementary Material](#), further inquiries can be directed to the corresponding author.

Author contributions

SB: Formal Analysis, Investigation, Methodology, Validation, Writing – original draft, Writing – review and editing. AB: Conceptualization, Investigation, Writing – review and editing. MS: Methodology, Writing – review and editing. SA: Methodology, Writing – review and editing. GY: Validation, Writing – review and editing. JS: Writing – review and editing. MH: Methodology, Writing – review and editing. JB: Visualization, Writing – review and editing. ZB: Data curation, Writing – review and editing. MM: Visualization, Writing – review and editing. BD: Data curation, Writing – review and editing. PS: Visualization, Writing – review and editing. AM: Visualization, Writing – review and editing. SZ: Conceptualization, Project administration, Resources, Supervision, Writing – review and editing.

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Conflict of interest

The author(s) declared that this work was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Generative AI statement

The author(s) declared that generative AI was not used in the creation of this manuscript.

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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fnano.2026.1727211/full#supplementary-material>

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