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Research Article

Optimization of lemongrass essential oil nanoemulsion formulation and antifungal activity against *Colletotrichum musae* QB6 causing anthracnose in bananas

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ABSTRACT

Response Surface Methodology (RSM) was used in this study to optimize the formulation of lemongrass essential oil nanoemulsion (LEO-NE) for improved stability and antifungal activity. The formulation of LEO-NE was optimized using a Box-Behnken Design (BBD), which examined the effects of Tween-80 (T-80) concentration, stirring speed, and ultrasonic time on droplet diameter (DD) and polydispersity index (PDI). The optimized nanoemulsion showed a DD of 153.2 nm and a PDI of 0.22 after being prepared with a T-80 concentration of 17.22 g/L, a stirring speed of 10,180 rpm, and an ultrasonic treatment time of 20 min. For more than 120 days, this formulation showed exceptional stability at room temperature. Furthermore, *Colletotrichum musae* (*C. musae*) QB6, the causative agent of banana anthracnose, was effectively inhibited by the optimized LEO-NE. *Musa* AAA bananas were used for both *in vitro* and *in vivo* testing. The antifungal activity of the LEO-NE was significantly higher than that of pure lemongrass essential oil (LEO) and benzimidazole. These results demonstrate the promising potential of optimized LEO-NEs as safe, environmentally friendly substitutes for synthetic fungicides in fruit preservation, providing improved stability and bioefficacy for use in farming.

1. Introduction

Lemongrass essential oil (LEO) is widely recognized for its valuable biological activities, including antibacterial, antifungal, anti-inflammatory, and antioxidant properties (Falcão *et al.*, 2012; Tran *et al.*, 2019; Antonioli *et al.*, 2020; Gao *et al.*, 2020; Mukarram *et al.*, 2022; Schweitzer *et al.*, 2022; Tang *et al.*, 2024; Van Do and Vu 2024). The chemical components of LEO, such as geraniol and citral, exhibit potent antagonistic effects against bacteria and pathogenic fungi, and these effects are highly influenced by climate, temperature, humidity, and soil composition (Gao *et al.*, 2020; Mahmoud *et al.*, 2022; Schweitzer *et al.*, 2022; Das and Prakash 2024; Salimi *et al.*, 2024). In particular, citral is acknowledged as an environmentally safe potential for employing compounds derived from plants in sustainable applications (Shukla *et al.*, 2025). Notwithstanding its benefits, LEO's hydrophobicity and high volatility are significant drawbacks that limit its usefulness in real-world applications (Turek and Stintzing 2012). To overcome these limitations and increase its applicability, a nanoemulsion system containing LEO has been developed using nanotechnology (Salvia-Trujillo *et al.*, 2013; Gao *et al.*, 2020; Faheem *et al.*, 2022; Liu *et al.*, 2023; Torres Neto *et al.*, 2024).

In addition to the promising potential of LEO, the use of nanoemulsions made from various essential oils (EOs) has been extensively investigated for the control of postharvest diseases on a variety of fruits. With stable oil droplets ranging from 20 to 200 nm and a large surface area in the water phase, nanoemulsion is an ideal bioavailability system (Gupta *et al.*, 2016; McClements and Jafari 2018; Blancas-Benitez *et al.*, 2022; Dhanasekaran *et al.*, 2024). Research has demonstrated that converting LEO into a nanoemulsion can prolong storage duration and shield active ingredients from deterioration due to temperature, light, and oxygen (Barradas and de Holanda e Silva 2021; Preeti *et al.*, 2023; Alsakhawy *et al.*, 2024). Although Lemongrass essential oil-nanoemulsions (LEO-NEs) have potential in antimicrobial applications, there is currently no optimized preparation method for achieving the desired physical properties (like low polydispersity index-PDI and minimal droplet diameter-DD) for maximum and consistent biological efficacy.

Ultrasonic homogenization has emerged as a promising technique for effectively producing nanoemulsions with desired characteristics. This method can effectively produce an optimized nanoemulsion system (Kentish and Ashokkumar 2011; Peshkovsky *et al.*, 2013). This procedure utilizes ultrasound to generate micro-bubbles in the solution,

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which in turn help break down oil droplets to the nanoscale. However, to achieve optimal efficiency, technical parameters such as emulsifier concentration, homogenization speed, and ultrasonic time need to be adjusted. Response surface methodology (RSM) is a powerful tool that could be employed to optimize these technological processes (Leyva-Jiménez *et al.*, 2022). BBD, a popular RSM approach, facilitates the precise formulation of nanoemulsions from plant EOs with targeted characteristics by enabling the simultaneous optimization of multiple variables. Many studies on creating nanoemulsions from different natural EOs, such as cinnamon EO, peppermint EO, and cajeput EO, have been successfully optimized using RSM to optimize the process (Pongsumpun *et al.*, 2020). However, the optimization of the preparation process of nanoemulsion from LEO and evaluation of its antifungal ability are still limited, especially for application in agricultural product preservation (Barradas and de Holanda e Silva 2021; Hunde *et al.*, 2023).

While chemical treatments for anthracnose, a serious postharvest hazard in banana brought on by *C. musae*, are effective, they raise deep concerned regarding food safety and environmental impact (Carvalho 2017; Heneberg *et al.*, 2018; de Oliveira Filho *et al.*, 2021; Santos *et al.*, 2025). Therefore, developing an optimized LEO-NE system is essential as a natural, safe, and effective alternative to control *C. musae*. This study hypothesized that optimizing LEO-NE properties (small DD and a low PDI) through RSM would lead to significantly higher antifungal activity against *C. musae* compared to bulk LEO. Furthermore, the enhanced nanoemulsion system's antifungal efficacy against *C. Musae* was assessed, with the goal of creating a natural solution that was both safe and efficient for preserving agricultural products, particularly bananas.

2. Materials and Methods

2.1 Materials

Lemongrass (*Cymbopogon citratus*) was collected, and LEO extraction was performed following the procedure detailed in Fig. S8. Bananas (*Musa* AAA) were harvested, and the fungus *C. musae* QB6 was cultured, as described in Fig. S9.

Tween® 80, a polysorbate non-ionic surfactant with purity >58.0% (GC), and benzimidazole, with purity >97.5% (HPLC), were employed in the experiments and were purchased from Sigma-Aldrich company. All other chemicals used were of analytical grade and obtained from reputable suppliers.

2.2 Preparation and characterization of LEO Nanoemulsion

Nanoemulsions were prepared by adapting the method of Hunde *et al.* (Hunde *et al.*, 2023) and Gupta *et al.* (Gupta *et al.*, 2016) with modifications. The emulsions were formed by combining the oil phase and the water phase, as described in Fig. S10.

The DD and PDI of the LEO-NE were evaluated by dynamic light scattering with non-invasive backscattering using a particle size analyzer (Zetasizer Nano ZS90, Malvern Instruments Ltd., UK).

2.3 Optimization of LEO-NE for DD and PDI

RSM was employed to optimize the preparation conditions of the LEO nanoemulsion, as shown in Table S4. BBD was employed for the optimization study (Table 1) with a total of 17 trials. The collected experimental data were analyzed using Minitab 21.2 software to construct a generalized quadratic regression equation, representing the relationship between the independent variables and the response variables, as follows:

$$Y_n = a_o + \sum_{i=1}^{3} a_i X_i + \sum_{i=1}^{3} a_{ii} X_i^2 + \sum_{i=1}^{2}\sum_{j=1+1}^{3} a_{ij} X_i X_j$$

Where:

- Y_n: The response variables,
- a_0: constant,
- a_i, a_{ii}, a_{ij}: linear, quadratic, and interaction coefficients, respectively,
- X_i and X_j: levels of the independent variables.

Table 1.
BBD and the corresponding experimental results.

Run	T-80 concentration (X_1) (g/L)	Homogenization speed (X_2) (rpm)	Ultrasonication time (X_3) (min)	DD (Y_1) (nm).	PDI (Y_2)
1	17.5 (0)	8000 (-1)	10 (-1)	193.21	0.31
2	20 (+1)	11000 (0)	20 (+1)	212.55	0.40
3	15 (-1)	11000 (0)	10 (-1)	187.64	0.27
4	20 (+1)	11000 (0)	10 (-1)	264.68	0.55
5	15 (-1)	11000 (0)	20 (+1)	204.58	0.32
6	20 (+1)	14000 (+1)	15 (0)	235.17	0.48
7	17.5 (0)	14000 (+1)	10 (-1)	171.93	0.25
8	20 (+1)	8000 (-1)	15 (0)	281.62	0.51
9	17.5 (0)	8000 (-1)	20 (+1)	178.84	0.26
10	15 (-1)	14000 (+1)	15 (0)	225.63	0.29
11	17.5 (0)	14000 (+1)	20 (+1)	209.18	0.34
12	15 (-1)	8000 (-1)	15 (0)	207.51	0.32
13	17.5 (0)	11000 (0)	15 (0)	156.24	0.22
14	17.5 (0)	11000 (0)	15 (0)	149.73	0.19
15	17.5 (0)	11000 (0)	15 (0)	146.88	0.22
16	17.5 (0)	11000 (0)	15 (0)	150.56	0.26
17	17.5 (0)	11000 (0)	15 (0)	157.18	0.24

(-1, 0, +1): represents the coded coefficient levels of the input elements corresponding to low, medium, and high levels. The significance of the parameters was tested based on the statistical significance level p <0.05.

2.4 Determination of nanoemulsion stability

The LEO nanoemulsions were stored in transparent glass bottles, wrapped in aluminum foil to prevent direct light exposure to the samples. Systems exhibiting phase separation or creaming were considered unstable. DD and PDI values were measured after storage periods at 0, 30, 60, 90, and 120 days of storage. Electrophoretic mobility was measured using a Zetasizer Nano ZS (Malvern, UK) via laser Doppler velocimetry at 25°C.

2.5 Antifungal activity to anthracnose on banana fruit

Antifungal activity of the optimized LEO-NE on *C. musae* was evaluated following the method, as designated in Fig. S11. The inhibitory effect was calculated as the percentage (%) of inhibition of the growth rate of the colony diameter, Percentage inhibition of radial growth (PIRG%).

$$PIRG\% = \frac{(R_1 - R_2)}{R_1} \times 100\%$$

Where R_1: radial growth of fungi on control plates, R_2: the radial growth of fungi on treated plates.

Effect of the optimized LEO-NE on *C. musae* biomass formation *in vitro,* as described in Fig. S12. The percentage inhibition of fungal biomass growth was calculated based on the dry weight of the biomass (Long *et al.*, 2020).

In antifungal assays of optimized LEO-NE against *C. musae in vivo,* as detailed in Fig. S13. The diameter of the lesions was measured daily using a digital calliper over a 6-day period, with three replicates for each treatment (five bananas per replicate).

2.6 Statistical analysis

All data were processed and optimized using Minitab software with the experimental design based on the Box-Behnken Design (BBD) method. Statistical significance was determined at a confidence level of $p < 0.05$.

Table 2.
ANOVA of the regression coefficients for the responses.

Variable	DD (Y_1)				PDI (Y_2),			
	Coefficient	Adjusted Mean square	F-value	P-value	Coefficient	Adjusted Mean Square	F-value	P-value
Constant	2595				6.167			
Model		3103.2	26.25	0.000		0.024535	25.83	0.000
Linear		3448.2	29.16	0.000		0.028625	30.14	0.000
X1	-270.0	8733.0	73.86	0.000	-0.6634	0.056373	59.36	0.000
X2	-0.0459	692.3	5.85	0.042	-0.000136	0.011076	11.66	0.008
X3	14.40	329.6	2.79	0.134	0.0428	0.002915	3.07	0.114
Square		9151.6	77.40	0.000		0.043873	46.20	0.000
X1*X1	9.223	14028.2	118.64	0.000	0.02173	0.077853	81.98	0.000
X2*X2	0.000003	3446.0	29.14	0.001				
X3*X3								
2-Way Interaction		967.0	8.18	0.008		0.007450	7.84	0.011
X1*X2	-0.002152	1042.3	8.82	0.018				
X1*X3	-1.381	1192.7	10.09	0.013	-0.00400	0.010000	10.53	0.010
X2*X3	0.000860	666.2	5.63	0.045	0.000002	0.004900	5.16	0.049
Lack-of-Fit			11.10	0.019			1.71	0.311
R^2	0.9633				0.9526			
Adjusted R^2	0.9266				0.9157			

3. Results and Discussion

3.1 Optimization of LEO nanoemulsions by RSM

The experimental data for LEO-NE optimization have been presented in Table 1. The DD (Y_1) ranged from 146.88 to 281.62 nm, which significantly influences the stability, optical properties, and viscosity of nanoemulsions (Sharma *et al.*, 2010; Uluata *et al.*, 2016). The PDI (Y_2), reflecting DD uniformity, varied from 0.19 to 0.55, indicating a stable nanoemulsion system. Regression analysis and ANOVA were employed to evaluate the significance of the model terms, with the R^2 and adjusted R^2 values demonstrating strong predictive capability (Table 2). Specifically, the R^2 values of 0.9633 for DD (Y_1) and 0.9526 for PDI (Y_2), along with their adjusted counterparts of 0.9266 and 0.9157, confirm that the quadratic polynomial model can explain more than 90% of the variability in the response variables. ANOVA results indicated that linear, quadratic, and interaction terms of independent variables significantly influenced both DD and PDI ($p < 0.05$), justifying the non-linear relationships and the use of a quadratic polynomial model, as visualized in Fig. 1 and Fig. 2. The final equations according to the coded factors for the responses Y_1 and Y_2 produced the following polynomial formulas:

$$Y_1 = 2595 - 270.0X_1 - 0.0459X_2 + 14.40X_3 + 9.223X_1^2 + 0.03\times10^4X_2^2 - 21.52\times10^4X_1X_2 - 1.381X_1X_3 + 8.6\times10^4X_2X_3$$

$$Y_2 = 6.167 - 0.6634X_1 - 1.36.104X_2 + 0.0428X_3 + 217.3\times10^4X_1^2 - 0.004X_1X_3 + 0.02\times10^4X_2X_3$$

The DD (Y_1) was primarily influenced by T-80 concentration (X_1) and the homogenization speed (X_2) (Fig. 1a). The smallest DD (<100 nm) was achieved at high T-80 concentrations (~20 g/L) and homogenization speeds (12,000-14,000 rpm), attributed to enhanced interfacial tension reduction and efficient droplet dispersion. However, exceeding the optimal T-80 level led to system destabilization due to excess micelle formation, while excessive homogenization speeds generated localized heating, promoting droplet re-coalescence. Conversely, lower levels of both factors resulted in larger DD (>300 nm), indicating reduced nanoemulsion stability. These findings align with previous studies on emulsifier concentration and mechanical energy for optimal DD reduction and stability (Sharma *et al.*, 2010; McClements and Jafari 2018). In addition, the interaction between X_1 and X_3 (Fig. 1b) and that between X_2 and X_3 (Fig. 1c) significantly impacted DD, with optimal conditions leading to smaller sizes and excess leading to aggregation or re-agglomeration. This confirms the synergistic effect of shear forces and ultrasonic cavitation facilitated efficient droplet breakdown and uniform dispersion.

The PDI (Y_2) was significantly influenced by the T-80 concentration (X_1) and the homogenization speed (X_2), with the ultrasonication time ($X_3 = 0$) held constant (Fig. 2a). Increasing the concentration of T-80 from 15 g/L to 20 g/L led to a decrease in PDI due to enhanced surface stabilization and improved droplet dispersion. However, excessively high homogenization speeds (~14,000 rpm) resulted in an increase in PDI due to localized heating effects, which destabilized the emulsion and caused droplet re-coalescence. The optimal conditions were identified at a T-80 concentration of 20 g/L and a homogenization speed of 12,000 rpm, yielding the lowest PDI (~0.2), indicating a highly uniform nanoemulsion system. The PDI was significantly affected by X_1 and X_3 (Fig. 2b), which underscores the necessity of optimizing both factors simultaneously to maintain nanoemulsion stability. X_2 and X_3 exhibited a synergistic effect on PDI (Fig. 2c), emphasizing the critical importance of simultaneous optimization of homogenization speed and ultrasonication time to achieve stable nanoemulsions with uniform DD distribution.

The optimization results using the BBD revealed that the ideal conditions for producing LEO nanoemulsions were a T-80 concentration of 17.22 g/L, a homogenization speed of 10,180 rpm, and an ultrasonication time of 20 min (Table 3). Under these parameters, the nanoemulsion achieved a DD of 153.02 nm and a PDI of 0.22, signifying a highly stable and uniform system. The measured zeta potential of -12.0 mV for the optimized nanoemulsion indicates the presence of negative charges on the droplet surface, contributing to electrostatic repulsion among droplets (Fig. S5). These optimized conditions underscore the importance of harmonizing emulsifier concentration, homogenization speed, and ultrasonication time to attain the desired nanoemulsion properties.

3.2 Stability of LEO nanoemulsions

The stability of LEO nanoemulsions is essential for their efficacy in various applications (Gago *et al.*, 2019; Barradas and de Holanda e Silva

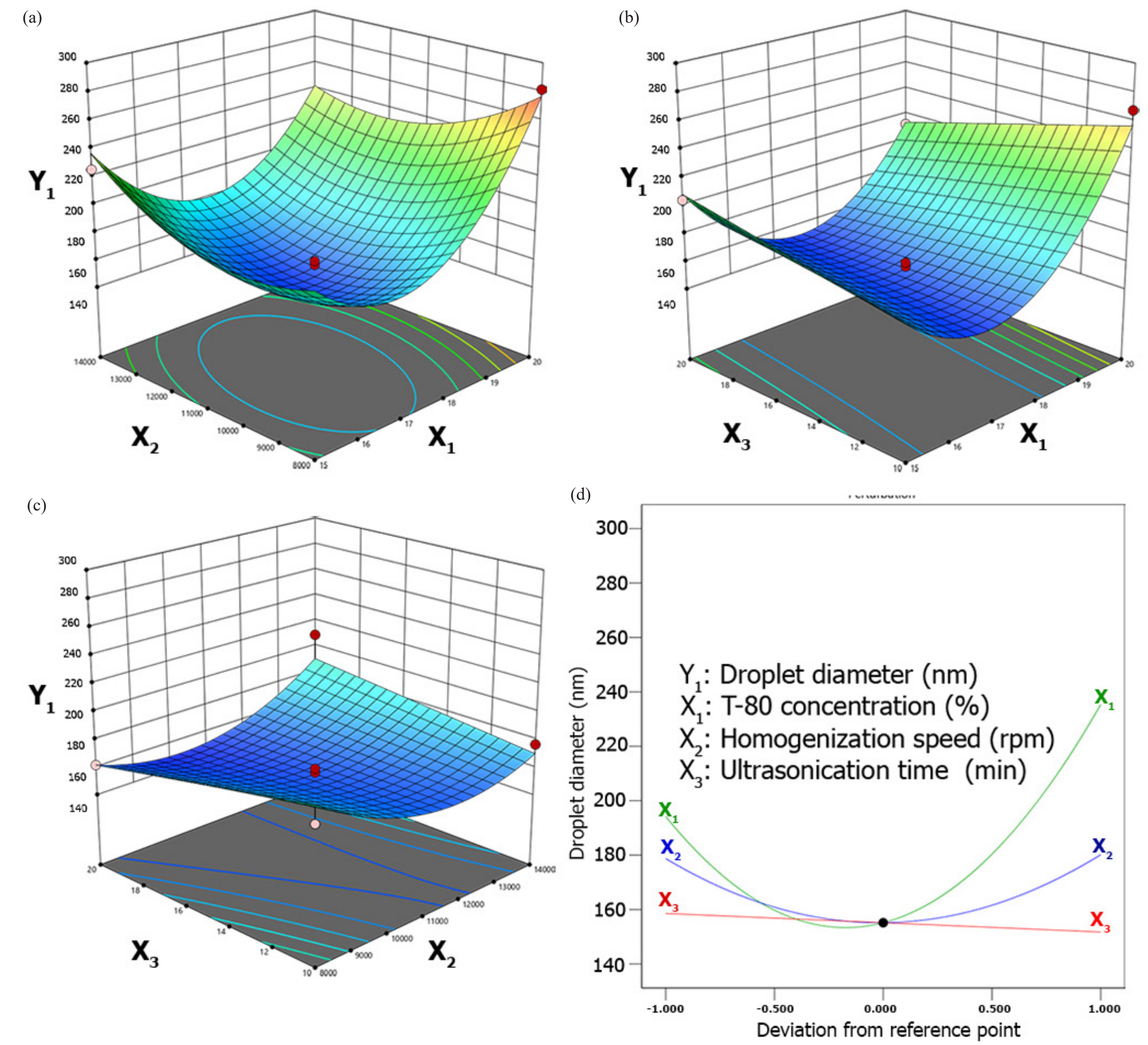

Fig. 1. Response surface plots of interaction between (a) T-80 concentration and homogenization speed; (b) T-80 concentration and ultrasonication time; (c) ultrasonication time and homogenization speed on DD of the LEO nanoemulsions, and (d) The influence of factors on DD at (X1:X2:X3) of (17.5:11000:15).

2021). Key stability indicators, such as DD and PDI, were evaluated in this study. Nanoemulsions were formulated under optimized conditions of T-80 concentration, homogenization speed, and ultrasonication time. The results, as depicted in Fig. 3, confirm the exceptional stability of LEO nanoemulsions over 120 days, with DD consistently remaining below 200 nm, and PDI stayed under 0.3. The absence of creaming, phase separation, or notable size variation reflects an optimal balance between surface tension reduction and coalescence prevention, achieved through precise control of the emulsifier concentration and processing parameters. These findings are consistent with contemporary research highlighting the critical role of optimized emulsifier levels and energy input in maintaining nanoemulsion stability, making prepared LEO-NE highly suitable for applications in pharmaceuticals, food preservation, and cosmetics (Gupta *et al.*, 2016; McClements and Jafari 2018; Gago *et al.*, 2019; Barradas and de Holanda e Silva 2021).

A batch of LEO-NE was made especially with these ideal conditions after RSM determined the optimized parameters as a validation experiment. This optimized nanoemulsion batch's experimentally measured properties at time zero included a DD of 153.2 nm and a PDI of 0.22 (Tab. S5 and Fig. S4), both of which are very close to the values that the RSM model predicted.

3.3 Antifungal activity against anthracnose on banana fruit

Effect of nanoemulsion on the colony diameter growth of *C. musae* after 10 days of monitoring. The results presented in Table 3 and Fig. 4 demonstrate exceptional antifungal activity against *C. musae* QB6. The optimized LEO-NE showed complete inhibition of fungal growth with a PIRG of 100% comparable to the synthetic fungicide Benzimidazole. In contrast, non-nanoemulsified LEO partially inhibited fungal growth, with a PIRG of 85.94%. This finding reinforces the growing evidence of nanoemulsions as sustainable and efficient antifungal agents.

The effect of LEO-NE on biomass formation of C. musae QB6 after 10 days of incubation at 28°C was measured. Figs. 5 and 6 indicate that both the optimized LEO-NE and Benzimidazole exhibited superior antifungal activity compared to pure LEO and the negative control (distilled water). In the absence of antifungal agents, *C. musae* QB6 biomass reached 259.3 mg, indicating unrestricted growth. The

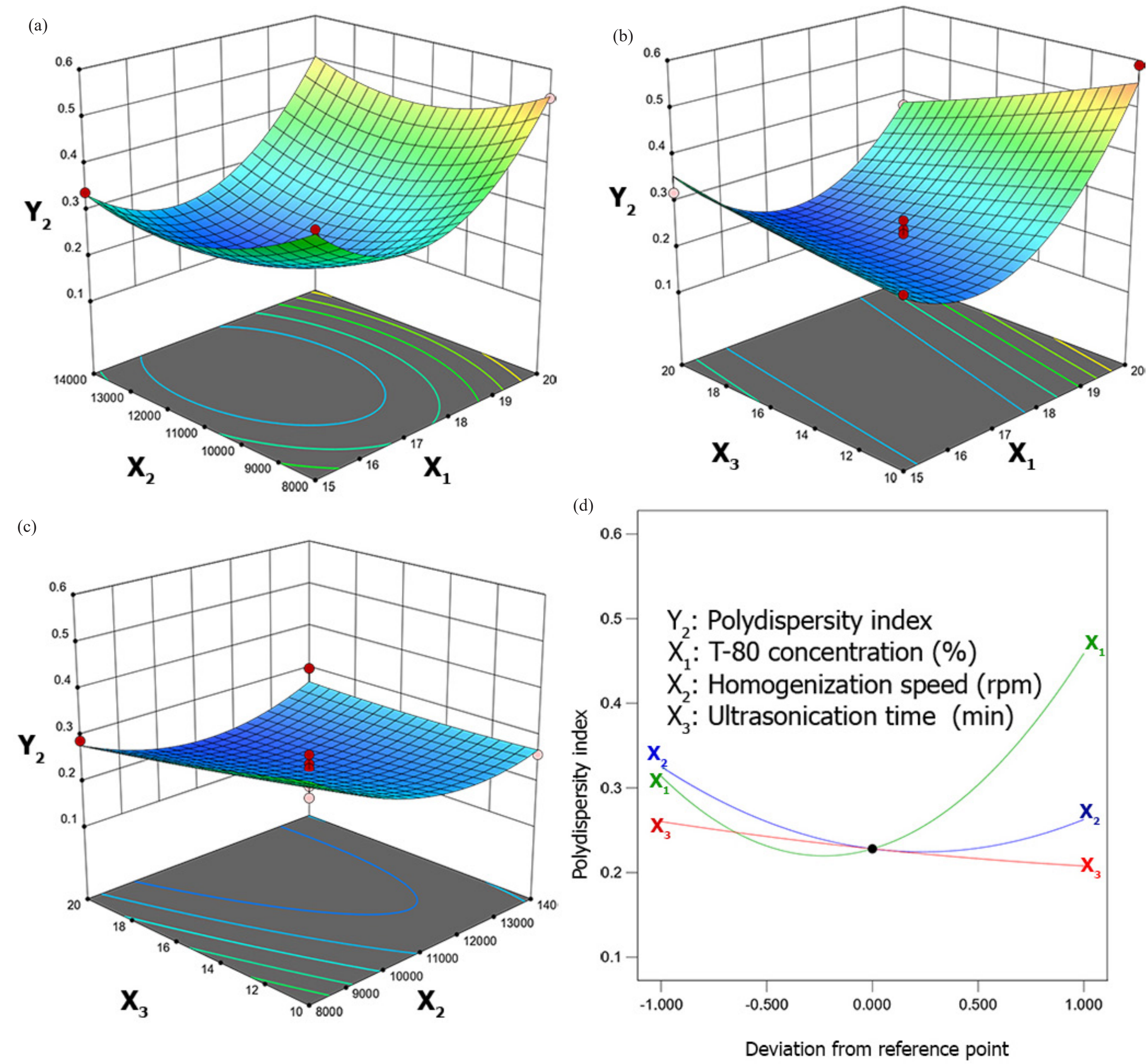

Fig. 2. Response surface plots of interaction between (a) T-80 concentration and homogenization speed; (b) T-80 concentration and ultrasonication time; (c) ultrasonication time and homogenization speed on PDI of the LEO nanoemulsions; (d) The influence of factors on PDI at (X1:X2:X3) of (17.5:11000:15)

Table 3.
Antifungal effect of LEO-NE on the inhibition zone diameter of *C. musae* QB6 after 10 days of observation.

Sample	Diameter of fungal colony (mm)					PIRG (%) after 10 days
	2 days	4 days	6 days	8 days	10 days	
Distilled water (control -)	1,910^{bA} (±0,105)	3,923^{bB} (±0,112	5,387^{bC} (±0,517)	6,480^{bD} (±0,431)	7,677^{bE} (±0,442)	0,000
Benzimidazole (control +)	0,000^{a} (±0,000)	0,000^{a} (±0,000)	0,000^{a} (±0,000)	0,000^{a} (±0,000)	0,000^{a} (±0,000)	100,00
LEO	0,000^{aA} (±0,000)	0,000^{aA} (±0,000)	0,000^{aA} (±0,000)	0,237^{aB} (±0,015)	0,986^{aC} (±0,083)	85,94
LEO-NE	0,000^{a} (±0,000)	0,000^{a} (±0,000)	0,000^{a} (±0,000)	0,000^{a} (±0,000)	0,000^{a} (±0,000)	100,00

Mean fungal colony diameters within the same column sharing the same lowercase letter and those within the same row sharing the same uppercase letter indicate significant differences at a confidence level of $p < 0.05$.

optimized LEO-NE completely inhibited biomass formation, achieving a 100% inhibition rate, comparable to Benzimidazole. In contrast, pure LEO exhibited significant but lower antifungal efficacy, reducing biomass to 30.1 mg with an inhibition rate of 88.39%. These findings align with various studies, which highlight the role of nanoemulsions in enhancing the bioactivity of EOs by improving cellular uptake and reducing required dosages for efficacy (Maurya *et al.*, 2021; Guidotti-Takeuchi *et al.*, 2022).

Banana fruit antifungal activity against anthracnose was evaluated *in vivo* (Fig. 7). Benzimidazole and LEO nanoemulsion-treated bananas showed no outward signs of damage after 6 days, demonstrating total inhibition of fungal growth. In the meantime, bananas treated with pure LEO developed minor lesions by the end of the observation period, indicating delayed but partial inhibition, and significant lesion development was seen in the untreated control (sterile distilled water). By providing a sustainable method for managing fungal diseases in fruits after harvest, this study highlights the potential benefits of

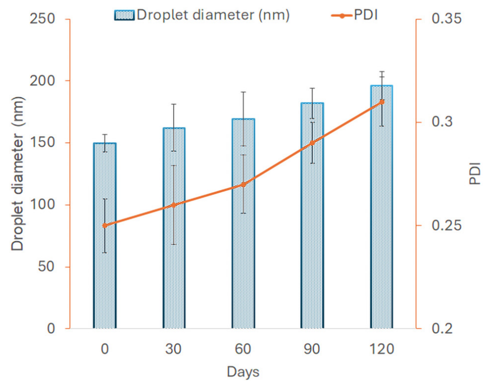

Fig. 3. DD and PDI of optimized LEO nanoemulsion samples over storage time.

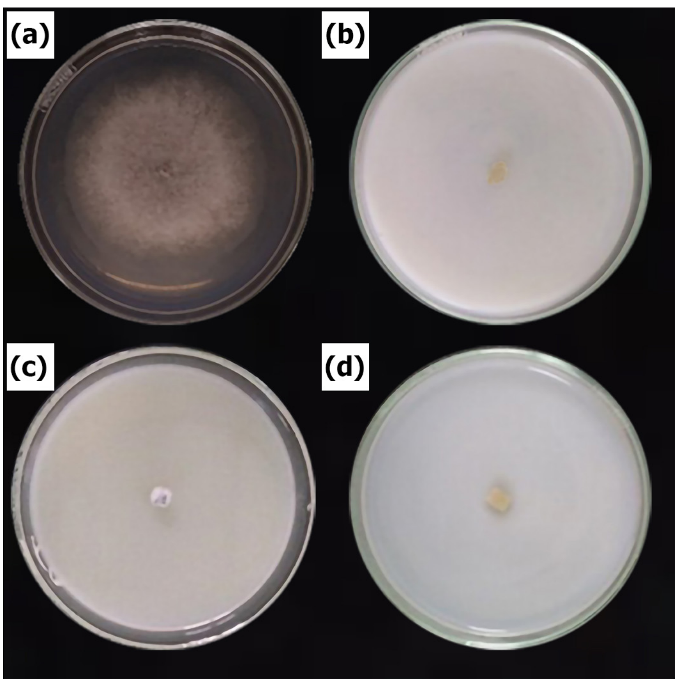

Fig. 4. Growth of *C. musae* QB6 colonies after 10 days of incubation under different treatments: (a) Distilled water (negative control), (b) Benzimidazole (positive control), (c) Pure LEO, and (d) Optimized LEO nanoemulsion.

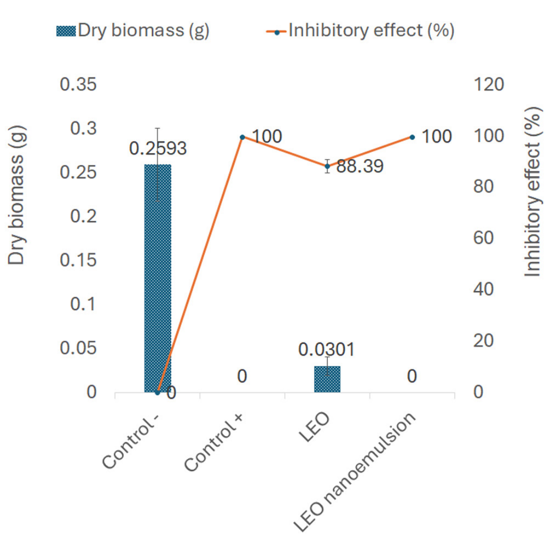

Fig. 5. Inhibitory effect of optimized LEO nanoemulsion on *C. musae* QB6 biomass after 10 days of incubation at 28°C.

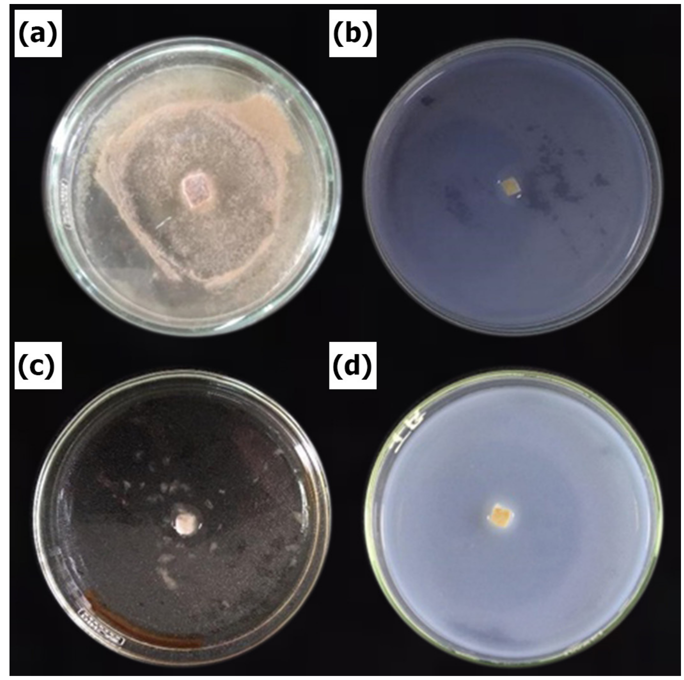

Fig. 6. Biomass formation of *C. musae* QB6 after 10 days of incubation in PDB medium under different treatments: (a) Distilled water (negative control), (b) Benzimidazole (positive control), (c) Pure LEO, and (d) Optimized LEO nanoemulsion

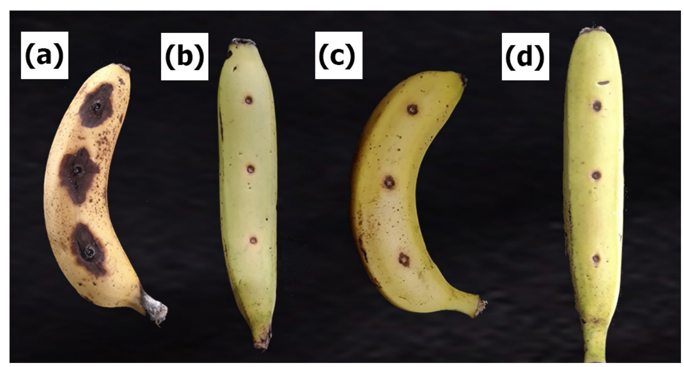

Fig. 7. Effect of different treatments on anthracnose lesion development in bananas inoculated with *C. musae* QB6 *in vivo*. (a) Distilled water (negative control), (b) Benzimidazole (positive control), (c) LEO, (d) Optimized LEO nanoemulsion

using nanoemulsified EOs as efficient and environmentally friendly substitutes for synthetic fungicides.

The enhanced antifungal efficacy of LEO-NE over bulk LEO is likely attributable to its nanoscale properties, including increased surface contact, improved membrane permeability, and stabilization of volatile actives. These physicochemical advantages support sustained inhibition in both *in vitro* and *in vivo* settings, as reported in similar studies, reinforcing the potential of nanoemulsions in postharvest disease control.

Nonetheless, several limitations should be acknowledged. The *in vivo* assays were conducted under controlled laboratory conditions, which may not fully reflect commercial postharvest environments. Additionally, the scope of biological validation and storage stability assessments remains restricted. Future research should address these aspects to better inform real-world applicability.

4. Conclusions

This study successfully applied RSM to optimize the formulation of LEO nanoemulsion. The nanoemulsion formulation was optimized using the BBD to develop a second-order polynomial model for optimizing

DD and PDI. The results demonstrated that T-80 concentration, stirring speed, and ultrasonic vibration time significantly influenced the characteristics of the LEO nanoemulsion. The optimized conditions, 17.22 g/L T-80, stirring speed of 10,180 rpm, and ultrasonic vibration for 20 min, produced a stable nanoemulsion system with a DD of 153.2 nm and a PDI of 0.22, which exhibited remarkable antifungal activity against *C. musae* QB6 and achieved complete inhibition of fungal growth both *in vitro* and *in vivo* on *Musa* AAA bananas. The enhanced properties of the optimized LEO-NE highlight its great potential in fruit preservation technology and the effective prevention of anthracnose-related fungal diseases. This study demonstrated the potential advantages of EO nanoemulsions in improving stability and biological efficacy, suggesting their suitability for further investigation towards practical applications in agricultural product preservation and protection.

CRediT authorship contribution statement

Anh-Quang Dao: Concepts, Design, Experimental studies, Data acquisition, Data analysis, Statistical analysis, Manuscript preparation, Manuscript editing and review. **Le Thi Kim Anh:** Experimental studies, Data acquisition. **Tuan Le Minh Dao:** Data acquisition. **Bao-Khanh Tran:** Data analysis, Manuscript preparation. **Nguyen Quoc Sinh:** Literature search, Manuscript preparation. **Diem-Huong Thi Nguyen:** Concepts, Literature search, Experimental studies, Data acquisition, Statistical analysis. **Hanh Nhu Thi Hoang:** Experimental studies, Data analysis, Manuscript preparation. **Hoang Duc An:** Experimental studies, Data acquisition. **Van-Phuong Nguyen:** Concepts, Experimental studies, Data analysis, Statistical analysis, Manuscript preparation. **Tinh Thi Thanh Nguyen:** Concepts, Literature search. **Le Lam Son:** Data acquisition, Data analysis. **Quoc-Bao Vo-Van:** Concepts, Design, Experimental studies, Data acquisition, Data analysis, Statistical analysis, Manuscript preparation, Manuscript editing and review.

Declaration of competing interest

The authors declare that they have no competing financial interests or personal relationships that could have influenced the work presented in this paper.

Declaration of Generative AI and AI-assisted technologies in the writing process

The authors confirm that there was no use of Artificial Intelligence (AI)-Assisted Technology for assisting in the writing or editing of the manuscript and no images were manipulated using AI.

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Supplementary data

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