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Antifungal efficacy of plant essential oil nanoemulsions against cucumber powdery mildew

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Plant essential oil nanoemulsions (PEO-NEs) offer a sustainable alternative to synthetic fungicides for managing plant diseases. This study evaluated the antifungal efficacy of PEO-NEs derived from seven medicinal plants against cucumber powdery mildew. In vitro experiments revealed that PEO-NEs from *Satureja khuzistanica* (savory), *Cymbopogon citratus* (lemongrass), and *Syzygium aromaticum* (clove) at 1, 2, and 3 g/l significantly inhibited conidia germination by 83%, 74%, and 83%, respectively, while PEO-NEs from pine, thyme, eucalyptus, and peppermint were ineffective. Comparatively, synthetic fungicides Topas and Stroby showed lower inhibition rates of 75% and 65%. Greenhouse trials demonstrated that savory, lemongrass, and clove PEO-NEs at 3 g/l reduced disease severity by 54.05%, 40.54%, and 35.13%, outperforming synthetic fungicides Topas (24.32%) and Stroby (18.91%). Preventive application 48 h before pathogen inoculation further reduced severity by 72.41%, 55.17%, and 41.3% for savory, lemongrass, and clove, respectively. Phenolic compound analysis using HPLC-DAD revealed significant differences in 3,4-dihydroxybenzoic acid, Caffeic acid, Ferulic acid, Gallic acid, Rutin, and Rosmarinic acid levels in treated plants. GC-MS identified key compounds, including carvacrol (88.6%) in savory, eugenol (56.6%) and β-caryophyllene (29.93%) in clove, and citral (42.6%) in lemongrass. These findings highlight the potential of PEO-NEs as eco-friendly alternatives for managing cucumber powdery mildew.

Keywords GC-MS, HPLC, Total phenolic compounds, Total flavonoid content, Podosphaera fusca

Cucumber (*Cucumis sativus* L.) is one of the most important and widely cultivated vegetable crops globally, grown both in greenhouses and open fields[1]. However, its productivity is significantly affected by various destructive diseases, which not only reduce yield but also increase production costs. Among these, powdery mildew, caused by *Podosphaera fusca* (Fr.) U. Braun & Shishkoff is one of the most prevalent and damaging diseases affecting cucurbits, posing a serious threat to cultivation in many countries. This pathogen infects the entire aerial parts of the plant, leading to substantial crop losses[2,3]. As a result, effective management of powdery mildew remains a critical and ongoing concern for the agricultural industry.

Currently, synthetic fungicides are the primary method for controlling this disease worldwide. However, their extensive use has led to the development of pathogen resistance, alongside adverse effects on the environment and human health[4]. Consequently, there is a growing need and trend toward non-chemical alternative control methods to mitigate crop losses[5]. In this context, several plant essential oils, known for their potent and broad-spectrum antifungal properties, have emerged as one of the most promising alternatives to synthetic fungicides.

Numerous studies have documented the antifungal activity of plant essential oils (PEOs) against various plant pathogenic fungi[6–9]. Savory (*Satureja khuzistanica* Jamzad.), for instance, is renowned for its antifungal and antioxidant properties, primarily attributed to compounds such as Rosmarinic acid[10]. Its antifungal efficacy has been demonstrated against a wide range of fungi, including *Aspergillus flavus*, *A. niger*, *Penicillium* spp., *Fusarium* spp., *Alternaria* spp., *Rhizopus* spp., *Mucor* spp., and *Fusarium solani*[10,11]. Similarly, clove (*Syzygium aromaticum* L.) oil has showed significant antifungal activity against several fungal genera[12–14]. Additionally, lemongrass (*Cymbopogon citratus* L.) oil has exhibited antifungal properties against pathogens such as *Aspergillus flavus*, *Aspergillus* spp., *Alternaria solani*, *Botrytis cinerea*, *Cladosporium herbarum*, *Colletotrichum coccodes*, *Fusarium oxysporum*, *Pythium ultimum*, *Rhizopus stolonifer*, and *Rhizoctonia solani*[15–18]. Mostafa et al.[19] evaluated the antifungal efficacy of lemongrass, lemon, thyme, and peppermint essential oils (EO) against cucumber powdery mildew both in vitro and under greenhouse conditions. Their findings revealed that the EOs significantly reduced disease incidence by up to 77.3% compared to the positive control. The study recommended applying EOs at an

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optimal dose of 2.5 ml/l to effectively protect plants against the disease. However, the authors cautioned against overdosing, as excessive application can lead to adverse effects such as leaf and flower blight.

Recent advancements in nanotechnology have demonstrated significant potential in crop production and protection, particularly as nanofertilizers and nanofungicides[20]. Among these innovations, plant essential oil based nanoemulsions (PEO-NEs) have emerged as a highly promising approach. PEO-NEs are easily formulated using a variety of ingredients and equipment, and they enhance the efficacy of phytochemicals[21]. Notably, nanoemulsions provide an effective means to encapsulate, protect, and deliver natural substances with antimicrobial properties, such as EOs[22]. The application of EOs in the form of nanoemulsions as fungal inhibitors represents a forward-looking strategy for managing plant diseases, offering a sustainable alternative to conventional methods[21].

The present study aims to evaluate the antifungal activity of PEO-NEs derived from the leaves of savory (*Satureja khuzistanica*), lemongrass (*Cymbopogon citratus*), pine (*Pinus eldarica* Medw.), garden thyme (*Thymus vulgaris* L.), eucalyptus (*Eucalyptus citriodora* Hook.), and peppermint (*Mentha piperita* L.), as well as from the flower buds of the clove (*Syzygium aromaticum*) against *P. fusca* the causal agent of cucumber powdery mildew. Following this, the most effective nanoemolsions were further assessed for their efficacy in controlling cucumber powdery mildew under greenhouse conditions. Additionally, the study analyzed cucumber phenol and flavonoid content, along with HPLC-DAD analysis, to determine the impact of nanoemulsions on plant phenolic compounds. Finally, GC-MS analysis was conducted to identify the key compounds present in the most effective nanoemulsions.

Results

Characterization of PEO-NEs

Measurements of the mean particle diameter for the nanoemulsions over a three-month period revealed a negligible increase from the 1st day to the 90th day for most PEO-NEs, with the exception of pine (Table 1). This suggests that the PEO-NEs were stable against coalescence and Ostwald ripening. Additionally, no phase separation was observed after centrifuging the nanoemulsions at 35,000 rpm, indicating that the particles were small and stable against creaming. Furthermore, the particle size distribution parameters were estimated to be less than 0.2.

Effect of PEO-NEs on conidia germination

The effects of PEO-NEs (1, 2, and 3 g/l) on *P. fisca* conidia germination are presented in Table 2. All PEO-NEs inhibited conidia germination of various time points, with savory, clove, and lemongrass demonstrating significantly higher inhibitory activity compared to the others. These three PEO-NEs were selected for further evaluation. The tested concentrations were compared with commercial fungicides (Stroby at 0.4 g/l and Topas at 0.5 g/l) as positive controls. The percentage of germinated conidia in all PEO-NEs treatments showed significant differences compared to both the positive and negative controls.

In vivo antifungal activity of selected PEO-NEs

The in vivo experiments demonstrated that all three concentrations of savory, clove, and lemongrass PEO-NEs effectively controlled powdery mildew disease five days after treatment (Figs. 1 and 2). Among the tested PEO-NEs, savory at 3 g/l exhibited the highest inhibitory rate (54.05%), followed by lemongrass at 3 g/l (40.54%), and clove at 3 g/l (35.13%).

Protective effect of selected PEO-NEs in inhibiting cucumber powdery mildew

The in vivo experiments assessing the protective effect of PEO-NEs revealed that all three concentrations of savory, clove, and lemongrass PEO-NEs effectively controlled the powdery mildew disease 10 days after pathogen inoculation (Figs. 3 and 4). Notably, all treatments demonstrated greater efficacy compared to the previous in vivo experiment. Among the tested PEO-NEs, savory at 3 g/l exhibited the highest inhibitory rate (72.41%), followed by lemongrass at 3 g/l (55.16%), and clove at 3 g/l (41.37%).

	Mean diameter of particle size (nm)		Particle size distribution (nm)	
Nano-emulsion	day 1st	day 90th	day 1st	day 90th
Savory	161.68 ± 16.24	310.87 ± 37.51	0.117	0.154
Clove	187.12 ± 18.77	199.29 ± 19.54	0.111	0.110
Lemongrass	157.22 ± 16.11	230.38 ± 27.55	0.118	0.154
Garden thyme	160.32 ± 18.06	190.78 ± 15.40	0.131	0.074
Eucalyptus	146.22 ± 14.98	170.73 ± 17.98	0.118	0.113
Peppermint	187.09 ± 18.98	201.16 ± 20.25	0.111	0.110
Pine	429.48 ± 58.32	677.21 ± 87.59	0.196	0.186

Table 1. Mean diameter and distribution of particle size of seven plant essential oil nanoemulsions obtained by DLS during 90 days' storage at 20 °C in darkness.

Treatments	Percentage of germinated conidia				
	6 h	12 h	24 h	36 h	48 h
Savory 1 g/l	12.00 ± 2.00 lm	18.33 ± 1.52 h	23.33 ± 3.05 i	33.66 ± 1.52 ji	44.33 ± 2.51 fghi
Savory 2 g/l	09.33 ± 0.57 m	14.66 ± 0.57 i	16.66 ± 1.52 j	19.00 ± 1.00 l	21.33 ± 2.08 l
Savory 3 g/l	05.66 ± 0.57 n	06.00 ± 0.001 k	07.66 ± 0.57 k	08.66 ± 1.52 m	09.33 ± 1.52 m
Clove tree 1 g/l	16.00 ± 1.00 k	18.00 ± 1.00 h	23.66 ± 1.52 hi	33.66 ± 3.50 ji	41.00 ± 2.00 i
Clove tree 2 g/l	11.00 ± 1.00 m	12.33 ± 1.52 j	14.66 ± 1.52 j	16.66 ± 1.15 l	18.00 ± 1.00 l
Clove tree 3 g/l	05.00 ± 1.00 n	07.66 ± 0.57 k	07.66 ± 1.52 k	08.66 ± 1.52 m	10.00 ± 1.00 m
Lemongrass 1 g/l	17.00 ± 2.00 jk	26.66 ± 2.50 bcd	35.00 ± 2.00 cd	41.66 ± 2.08 def	56.66 ± 4.04 b
Lemongrass 2 g/l	13.33 ± 1.15 l	20.00 ± 1.00 hg	23.66 ± 1.52 hi	25.00 ± 1.00 k	29.00 ± 1.00 k
Lemongrass 3 g/l	09.66 ± 0.57 m	14.66 ± 1.52 i	16.00 ± 1.73 j	17.33 ± 2.08 l	19.00 ± 2.00 l
Garden thyme 1 g/l	25.00 ± 1.00 b	27.66 ± 1.52 bc	34.00 ± 1.00 de	39.00 ± 1.00 fg	49.66 ± 2.08 de
Garden thyme 2 g/l	22.66 ± 1.52 cde	25.33 ± 1.52 cde	31.00 ± 1.00 f	36.00 ± 1.00 hi	46.33 ± 1.52 efgh
Garden thyme 3 g/l	20.33 ± 1.52 fgh	22.33 ± 1.15 f	26.00 ± 1.00 hg	31.00 ± 2.00 j	42.33 ± 3.50 hi
Eucalyptus 1 g/l	22.00 ± 1.00 def	25.00 ± 1.00 de	34.33 ± 0.57 de	40.66 ± 1.52 ef	47.66 ± 1.52 ef
Eucalyptus 2 g/l	18.00 ± 1.00 ijk	22.66 ± 1.52 f	32.00 ± 1.00 ef	37.00 ± 1.00 gh	43.00 ± 1.73 hgi
Eucalyptus 3 g/l	18.66 ± 0.57 hij	22.00 ± 1.00 fg	27.66 ± 1.52 g	31.66 ± 1.52 j	37.33 ± 1.52 j
Peppermint 1 g/l	24.00 ± 1.00 bcd	27.66 ± 1.52 bc	38.00 ± 1.00 b	43.00 ± 1.00 bcd	55.00 ± 3.60 bc
Peppermint 2 g/l	22.66 ± 1.52 cde	26.00 ± 1.73 cde	34.66 ± 0.57 cd	41.33 ± 1.52 def	46.66 ± 3.21 efg
Peppermint 3 g/l	24.33 ± 1.52 bc	28.66 ± 0.57 b	32.00 ± 1.00 ef	39.00 ± 1.00 fg	42.66 ± 2.08 hgi
Pine 1 g/l	21.00 ± 2.00 efg	26.33 ± 1.52 bcd	35.00 ± 1.00 cd	47.33 ± 1.15 b	56.00 ± 2.00 b
Pine 2 g/l	19.00 ± 1.00 hgij	25.66 ± 1.52 cde	37.00 ± 1.00 bc	44.00 ± 1.00 cd	54.00 ± 1.00 bc
Pine 3 g/l	19.66 ± 0.57 hgi	23.66 ± 0.57 ef	32.00 ± 2.00 ef	45.33 ± 3.78 bc	52.00 ± 3.60 cd
Stroby 0.4 g/l	16.00 ± 1.00 k	19.66 ± 0.57 h	23.00 ± 1.00 i	25.00 ± 1.00 k	27.33 ± 1.52 k
Topas 0.5 g/l	11.00 ± 1.00 m	13.66 ± 1.52 ij	15.66 ± 1.52 j	16.66 ± 1.52 l	17.66 ± 2.08 l
Control	30.00 ± 1.00 a	46.00 ± 2.00 a	59.00 ± 0.00 a	72.00 ± 3.00 a	93.00 ± 0.00 a

Table 2. Effect of plant essential oil nanoemulsions (PEO-NEs) at concentrations of 1, 2, and 3 g/l on the conidia germination of *Podosphaera fusca in vitro*. Results are mean ± SD ($n = 3$); Means with different letters in each column are significantly different according to Duncan's multiple range test ($P \leq 0.01$).

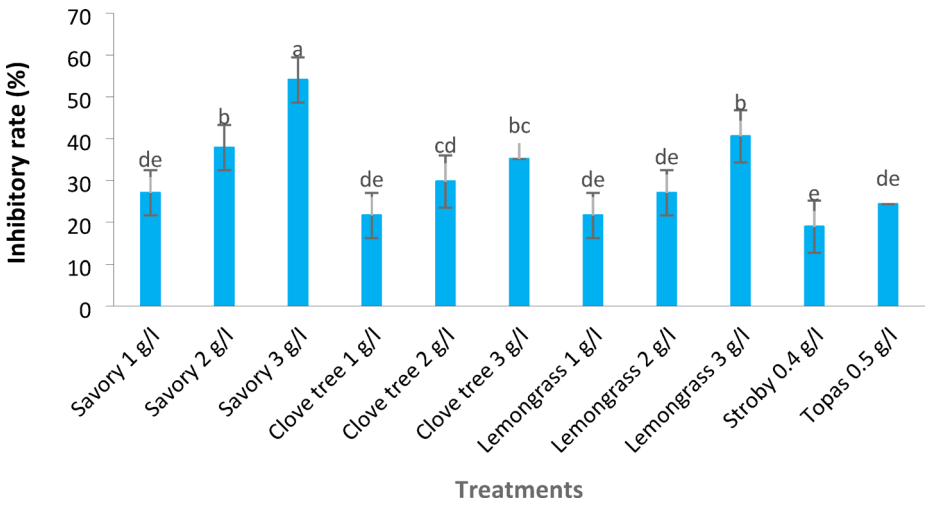

Fig. 1. Curative effect of different concentrations (1, 2 and 3 g/l) of savory, clove, and lemongrass EO-NEs, as well as Stroby (0.4 g/l) and Topas (0.5 g/l), against cucumber powdery mildew disease under greenhouse conditions. Data are mean ± SD ($n = 3$). According to Duncan's multiple range test, values with different letters indicate statistically significant differences ($P \leq 0.01$).

Effect of promising PEO-NEs on plant phenolic content

The results of the promising PEO-NEs at a concentration 3 g/l on total phenol (TPC) and flavonoid content (TFC) in cucumber leaves revealed significant differences among the treatments (Fig. 5). While savory and lemongrass PEO-NEs reduced TPC after 24 h, similar to Topas, no significant difference was observed between

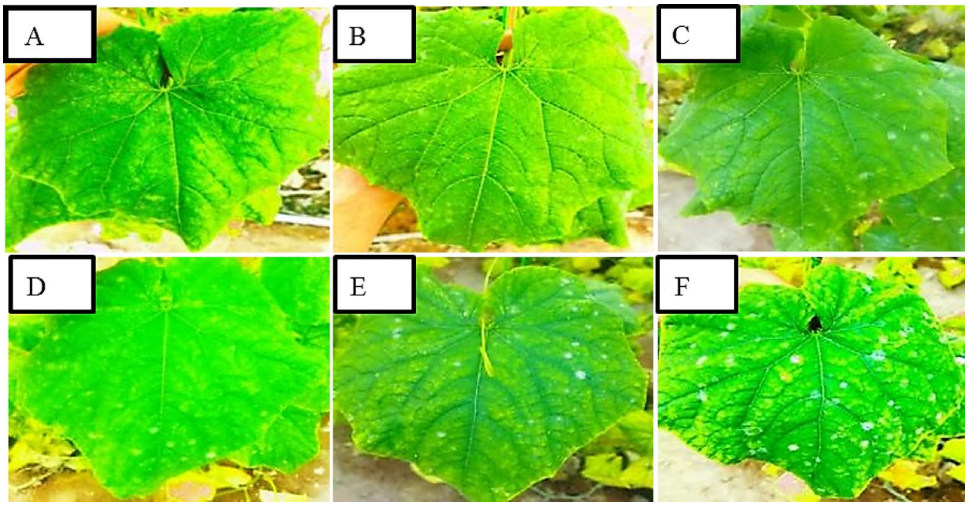

Fig. 2. Influence of PEO-NEs against cucumber powdery mildew under greenhouse conditions. (**A**) savory 3 g/l, (**B**) lemongrass 3 g/l, (**C**) clove 3 g/l PEO-NEs, (**D**) Topas 0.5 g/l, (**E**) Stroby 0.4 g/l, (**F**) control.

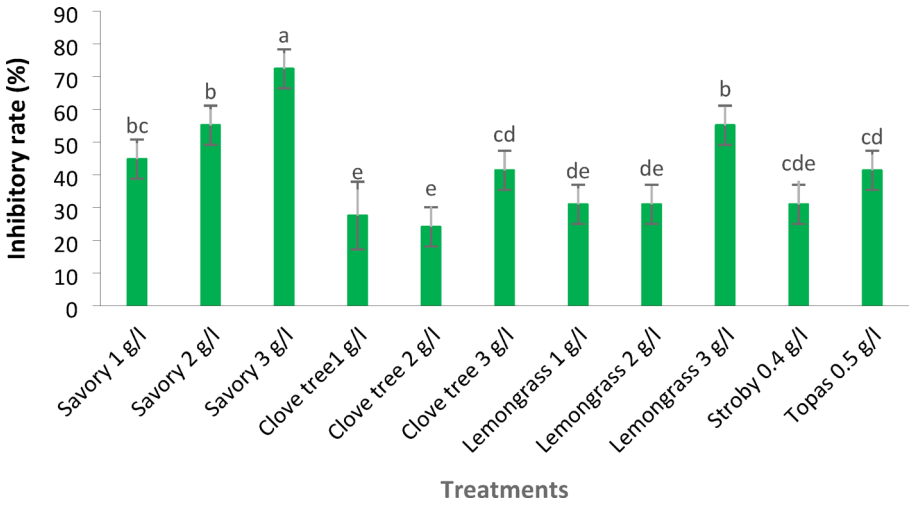

Fig. 3. Protective effect of different concentrations (1, 2 and 3 g/l) of savory, clove, and lemongrass PEO-NEs, as well as Stroby (0.4 g/l) and Topas (0.5 g/l), against cucumber powdery mildew under greenhouse conditions. Data are mean ± SD ($n = 3$). According to Duncan's multiple range test, values with different letters indicate statistically significant differences ($P \leq 0.01$).

Topas and the control at 48 h. Additionally, there was no significant difference in TPC among clove, Stroby and the control. Interestingly, savory and clove PEO-NEs significantly increased TFC at 24 h, whereas only Stroby enhanced TFC at 48 h. In contrast, lemongrass PEO-NE and Topas decreased TFC.

Furthermore, HPLC-DAD analysis of phenolic compounds indicated significant differences in cucumber leaf metabolites 48 h after treatment with nanoemulsions and fungicides. The content of 3,4-dihydroxybenzoic acid, Caffeic acid, Ferulic acid, Rutin, and Rosmarinic acid varied significantly among treatments (Fig. 6). Caffeic acid was the dominant phenolic compound in cucumber leaves, with its content increasing in response to clove PEO-NE and Topas. Conversely, lemongrass PEO-NE reduced Caffeic acid content.

GC-MS analysis of PEOs

The GC-MS analysis revealed that carvacrol (88.6%) is the dominant component of savory EO. The primary components of lemongrass EO included citral (42.6%), citronellol (20.3%), and geraniol (16.4%). Additionally, geraniol (56.6%) and β-Caryophyllene (29.93%) were identified as the major constituents of clove EO (Table 3).

Discussion

The global trend toward finding alternatives to chemical fungicides for managing plant diseases has gained momentum due to the need to reduce both pathogen-induced crop damage and the adverse effects of synthetic

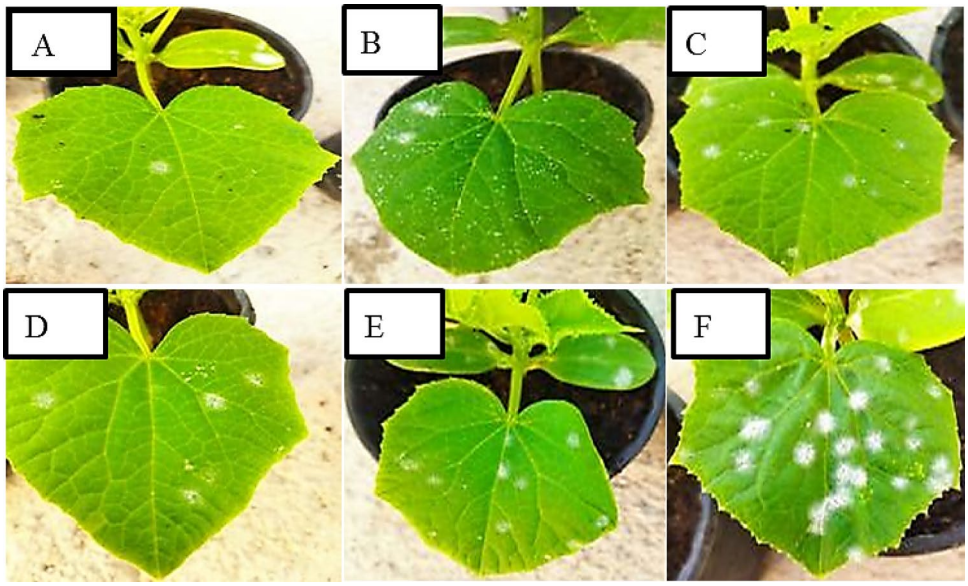

Fig. 4. Disease severity of cucumber powdery mildew after preventive application of plant essential oil nanoemulsions under greenhouse conditions. (**A**) savory 3 g/l, (**B**) lemongrass 3 g/l, (**C**) clove tree 3 g/l PEO-NEs, (**D**) Topas (0.5 g/l), (**E**) Stroby (0.4 g/l), (**F**) control.

chemicals on human health and the environment[23]. In this context, PEOs have been widely explored as antimicrobial agents against plant pathogens[24] and for food preservation[25]. Recently, nanoemulsions have emerged as a promising approach to enhance the efficacy of phytochemicals and PEOs[21]. The use of PEO-based nanoemulsions as fungal inhibitors is becoming increasingly prevalent in plant disease management[21]. This study demonstrated the potential of PEO-NEs to inhibit the fungal pathogen *P. fusca in vitro* and prevent the development of cucumber powdery mildew through direct application on cucumber plants.

In the nanoemulsion formulation, Tween 80 was used as a surfactant due to its high hydrophilic balance (HLB) value, which facilitates the formation of oil-in-water emulsions. Small-molecule surfactants like Tween 80 rapidly adsorb onto emulsion droplet surfaces, making them more effective in reducing droplet diameter compared to polymeric surfactants[26].

The PEO-NEs of savory, clove, and lemongrass exhibited significant antifungal activity by inhibiting conidia germination in vitro. Suppressing spore germination through PEO treatments can play a critical role in limiting pathogen spread by reducing the spore load on plant surfaces[9]. In contrast, PEO-NEs of pine, garden thyme, eucalyptus, and peppermint showed limited inhibitory effects against *P. fusca*, possibly due to fungal resistance to the specific compounds present in these oils. These findings align with previous studies. For instance, Ahmed[27] reported that clove oil significantly reduced conidial germination of *P. xanthii* (synonym with *P. fusca*), while Raj and Shukia[28] found that lemongrass oil completely inhibited conidial germination in opium poppy powdery mildew. Mostafa et al.[19] evaluated the efficacy of lemongrass, lemon, thyme, and peppermint essential oils (1.0–2.5 ml/l) against cucumber powdery mildew and observed that higher concentrations caused hyphal and conidial deformation leading to pathogen death. Gangavarapu and Palwai[16] also demonstrated the potent antifungal activity of lemongrass oil against *Alternaria solani*, *Botrytis cinerea*, *Fusarium oxysporum*, *Pythium ultimum*, and *Rhizoctonia solani*. Similarly, savory has shown antifungal activity against a wide range of fungi, including *Aspergillus flavus*, *A. niger*, *Penicillium* spp., *Fusarium* spp., *Alternaria* spp., *Rhizopus* spp., *Mucor* spp., and *Fusarium solani*[10,11].

The antifungal activity of PEOs is often attributed to their ability to reduce morphological changes in the fungal cell wall and disrupt enzymatic reactions involved in cell wall synthesis, thereby inhibiting fungal growth[29]. In this study, GC-MS analysis revealed that carvacrol is the dominant component of savory EO, while citral, citronellol, and geraniol are the primary constituents of lemongrass EO. Clove EO was characterized by high levels of eugenol and β-caryophyllene. Tripathi et al.[30] suggested that the fungi toxicity of PEOs may be linked to their active ingredients, such as carvacrol, which exhibit strong antifungal properties. Eugenol, a phenolic compound, is also known for its potent antifungal activity[31–33]. Similarly, citral, a key component of lemongrass oil, has been shown to possess antimicrobial properties[34]. Citronellol and geraniol, found in *Zingiber moran* EO, have also demonstrated antibacterial and antifungal activity[35].

The analysis of TPC and TFC content in cucumber leaves treated with PEO-NEs revealed significant differences among treatments. These results suggest that PEO-NEs not only exhibit direct antifungal activity but also influence the plant's biochemical defense mechanisms. Phenolic compounds and flavonoids are known to play a crucial role in plant defense against pathogens by acting as antioxidants, antimicrobial agents, and signaling molecules[36]. The observed increase in TPC and TFC in response to PEO-NE treatments indicates that these nanoemulsions may enhance the plant's innate resistance to *P. fusca* infection.

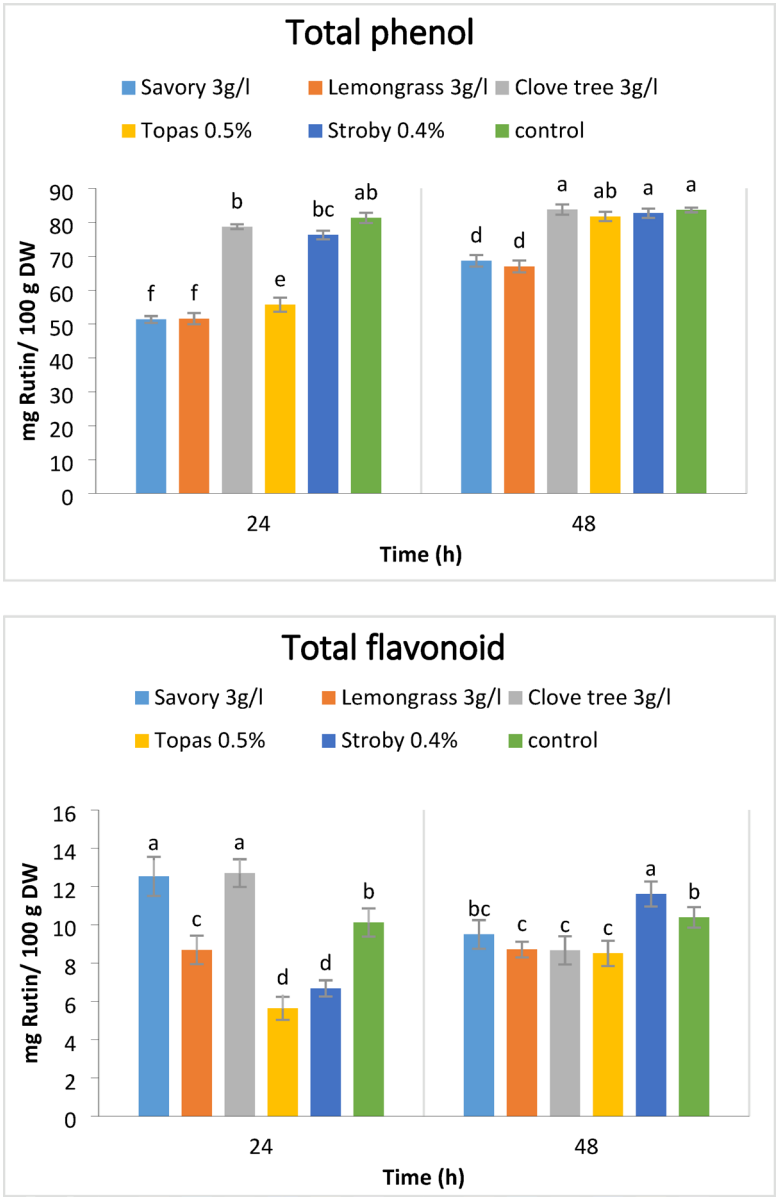

Fig. 5. Total phenol (TPC) and flavonoid (TFC) content in cucumber leaves treated with plant essential oil nanoemulsions (3 g/l) and fungicides at 24 and 48 h post-treatment.

HPLC-DAD analysis further demonstrated significant variations in the levels of specific phenolic compounds, including 3,4-dihydroxybenzoic acid, Caffeic acid, Ferulic acid, Rutin, and Rosmarinic acid, in cucumber leaves 24 and 48 h after treatment with PEO-NEs and fungicides compared to the control. Caffeic acid, the dominant phenolic compound in cucumber leaves, showed a notable increase in response to clove PEO-NE and Topas treatments. This aligns with previous studies that have highlighted the role of Caffeic acid in enhancing plant resistance to fungal pathogens through its antioxidant and antimicrobial properties[37]. In contrast, lemongrass PEO-NE reduced caffeic acid content, which may reflect differences in the mode of action of this treatment.

The increase in Rosmarinic acid and Rutin levels in response to savory and clove PEO-NEs is particularly noteworthy. Rosmarinic acid, a potent antioxidant, has been shown to inhibit fungal growth and enhance plant immunity[31]. Similarly, Rutin, a flavonoid glycoside, contributes to plant defense by scavenging reactive oxygen species (ROS) and strengthening cell walls[36]. These findings suggest that PEO-NEs may indirectly suppress *P. fusca* by stimulating the production of phenolic compounds that bolster the plant's defense system.

The differential effects of PEO-NEs on phenolic and flavonoid profiles highlight the complexity of their interaction with plant metabolism. While some treatments, such as clove PEO-NE, enhanced the accumulation of key phenolic compounds, others, like lemongrass PEO-NE, showed a contrasting effect. This variability may be attributed to differences in the chemical composition of the essential oils and their specific interactions with plant biochemical pathways. For instance, the high geraniol content in clove oil and carvacrol in savory oil

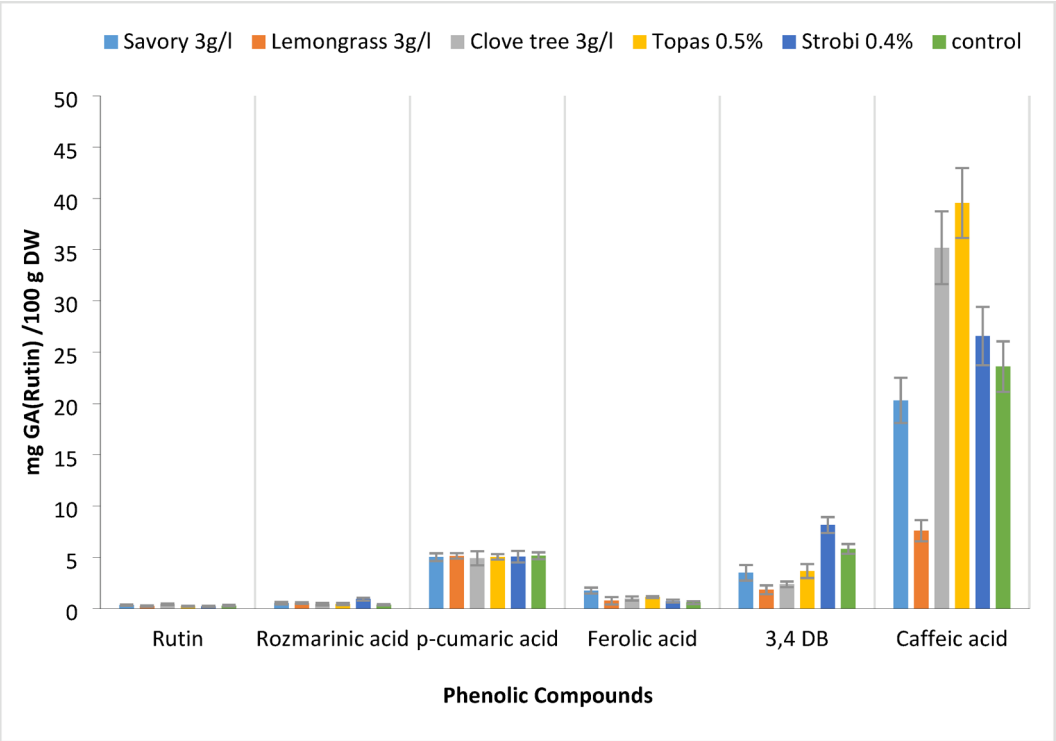

Fig. 6. HPLC-DAD analysis of phenolic compounds in cucumber leaves treated with plant essential oil nanoemulsions (3 g/l) and fungicides.

Savory			Clove			Lemongrass		
Component	**RI**	**%**	**Component**	**RI**	**%**	**Component**	**RI**	**%**
α-Pinene	933	0.6	1,8-cineole	1032	0.07	Limonene	988	1.8
Camphene	947	0.3	Menthone	1154	0.13	Linalool	1095	3.1
Myrcene	989	0.9	Neo-Menthol	1173	0.66	Citronellol	1223	20.3
delta-3-carene	1007	0.1	α–Cubebene	1348	1.40	Neral	1235	1
α-Terpinene	1013	0.5	Eugenol	1373	56.6	Citral	1242	42.6
p-Cymene	1025	1.5	α –Copaene	1377	2.88	Geraniol	1249	16.4
Limonene	1030	2.1	β-Caryophyllene	1426	29.93	Citronellyl acetate	1350	3.3
Z- β-ocimene	1036	1.3	p-Cymene	1030	0.09	Eugenol	1356	1.2
γ-Terpinene	1056	0.8	α-Humulene	1455	2.93	Geranyl acetate	1379	3.9
cis-Sabinene hydrate	1059	0.7	Alloaromadendrene	1461	0.04	d-Germacrene	1484	1.8
Thymol	1266	0.3	Trans-Cadina-1(6),4-diene	1472	0.19	Elemol	1548	1.1
Carvacrol	1282	88.6	Germacrene D	1480	0.08			
Thymyl acetate	1329	0.3	β-Guaiene	1495	0.09			
β-Bisabolene	1501	0.4	α -Farnesene	1505	0.25			
trans- β-bisabolene	1542	0.3	δ-Cadinene	1512	0.04			
			Delta-Cadinene	1522	1.23			
			Eugenol acetate	1528	2.99			
			Caryophyllene oxide	1582	0.12			
Total identified		98.7	Total identified		96.73	Total identified		96.5

Table 3. Major chemical constituents of savory, lemongrass, and clove essential oils identified by GC-MS analysis. RI: retention indices to C6-C24 n-alkanes on the DB-1 column; t, trace < 0.1%.

may act as elicitors of plant defense responses, whereas citral in lemongrass oil may have a different mode of action[33–34].

The findings of this study indicate that PEO-NEs of savory, clove, and lemongrass exhibit strong antifungal activity against *P. fusca*, the causal agent of cucumber powdery mildew, making them ideal candidates for

protective treatments in plant disease management programs. These results are consistent with previous studies. For example, Hashem et al.[38] demonstrated that clove essential oil nanoemulsion (CEO-NE) exhibits promising antifungal activity against *Neoscytalidium dimidiatum* both in vitro and in vivo. CEO-NE and clove EO also induced plant resistance by modulating proline, phenol, hydrogen peroxide, malondialdehyde, and antioxidant enzyme levels. Clove EO, rich in phenolic acids and natural antioxidants, play a vital role in scavenging free radicals and protecting plant cells from oxidative damage[36,37,39]. These properties make clove EO and CEO-NE effective stimulators of plant immunity and safe alternatives to chemical pesticides. The increase in phenolic content observed in treated plants suggests enhanced host resistance to stress[40,41].

Mostafa et al.[19] reported that lemongrass, lemon, thyme, and peppermint essential oils significantly reduced cucumber powdery mildew incidence by up to 77.3% compared to controls. The antifungal mechanism of lemongrass oil is not fully understood, but suggest that it causes morphological damage to fungal hyphae, including vesiculation and cytoplasmic disruption[42]. The high citral content in lemongrass oil, composed of geranial and neral isomers, is likely responsible for its antifungal activity. These active components may directly antagonize pathogens or activate host plant defense responses, thereby reducing disease progression[43].

Conclusion

This study highlights the potential of PEO-NEs derived from savory, clove, and lemongrass as effective antifungal agents against *P. fusca*, the causal agent of cucumber powdery mildew. The PEO-NEs demonstrated significant inhibitory effects on conidia germination in vitro and effectively reduced disease severity under greenhouse conditions. Notably, savory PEO-NE exhibited the highest efficacy, followed by lemongrass and clove. GC-MS analysis identified key bioactive compounds, such as carvacrol, citral, and geraniol, which likely contribute to the antifungal activity of these oils. Additionally, PEO-NEs influenced the phenolic and flavonoid content of cucumber leaves, suggesting their role in enhancing plant defense mechanisms. These findings underscore the potential of PEO-NEs as sustainable alternatives to synthetic fungicides for managing cucumber powdery mildew. However, further research is needed to fully elucidate the mechanisms of action and optimize their application in field conditions.

Materials and methods

Pathogen

The *Podosphaera fusca* fungus was collected from a cucumber greenhouse in Varamin, Tehran, Iran. A population of the fungus was subsequently propagated on young cucumber plants under controlled greenhouse conditions.

Plant essential oils

Seven medicinal plants—comprising leaves from six species (*Satureja khuzistanica* [savory], *Cymbopogon citratus* [lemongrass], *Pinus eldarica* [pine], *Thymus vulgaris* [garden thyme], *Eucalyptus citriodora* [eucalyptus], and *Mentha piperita* [peppermint]) and flower buds from *Syzygium aromaticum* [clove]—were selected based on their documented antifungal activity in previous studies. Savory and lemongrass were procured from the Academic Center for Education, Culture, and Research (ACECR), Lorestan Branch, Iran. The remaining plants (excluding clove) were sourced from the medicinal plant collection of the Medicinal Plants and Drugs Research Institute (MPDRI) at Shahid Beheshti University, Tehran, Iran. Clove buds were commercially obtained from local market. Botanical authentication was performed by Dr. Ali Sonboli, MDPRI, and voucher specimens for all plants were deposited in the MPDRI Herbarium (Supplementary Table 1). Plant materials were shade-dried at room temperature (25 °C) to preserve phytochemical integrity. For essential oil extraction, 50 g of dried material from each plant underwent hydrodistillation using a Clevenger-type apparatus (European Pharmacopoeia). The extracted PEOs were stored in amber glass vials at 4 °C until further analysis.

Nanoemulsion formulation

To prepare the nanoemulsions, 30 µl of PEO, 15 µl of Span® 80 (Merck Millipore, Darmstadt, Germany; CAS 1338-43-8), 35 µl of Tween® 80 (Merck Millipore, Darmstadt, Germany; CAS 9005-65-6), and lecithin (0.05% w/w; Sigma-Aldrich, Germany; CAS 8002-43-5), were mixed slowly under gentle stirring until homogenized. The volume was then adjusted to 1 ml using distilled water, following by gentle stirring for 30 min to achieve a homogeneous mixture. The mixture was emulsified using a high-energy probe sonicator (MPI, Dattwil, Switzerland) at 20.5 kHz with 400 W maximum power and controlled temperature. Subsequently, 25 µl of the PEO-NE was diluted with 1 ml of distilled water, and the droplet size was determined at 25 °C by dynamic light scattering (DLS) using a Nanophox analyzer (Sympatec GmbH, Clausthal-Zellerfeld, Germany). The nanoparticle size was expressed as the mean average of three independent batches of the nanoemulsion[44]. The particle size distribution of the PEO-NEs was evaluated after 1 day and 90 days of storage at 20 °C in darkness.

In vitro antifungal activity of PEO-NEs against *Podosphaera fusca*

Healthy and young cucumber leaves were collected from the greenhouse, washed with distilled water, and pierced with a cork borer to create 1 cm diameter disc. The discs were then soaked in a 0.5% sodium hypochlorite solution for 1 min, rinsed again with distilled water, and dried under a laminar hood for 10 min. Viable *P. fusca* conidia were obtained by gently shaking sporulating lesions with a glass rod[45]. The dried leaf discs were placed on 2.5% water agar medium, and inoculated with powdery mildew spores. Immediately after inoculation, the discs were sprayed with PEO-NEs at concentrations of 1, 2, and 3 g/l, while fungicides Topas 200 EW (0.5 g/l) and Stroby WG (0.4 g/l) were used as control treatments. The samples were placed in a desiccator at 90% relative humidity, 20 °C, and in darkness for 48 h. Conidia germination was assessed microscopically at ×40 magnification after 6, 12, 24, 36 and 48 h of inoculation. A conidium was considered germinated if the germ tube length was at least

equal to the width of the conidium. The percentage of conidia germination was calculated for 100 spores[46], with three replicates examined for each treatment. The experiment was repeated twice.

Curative effect of selected PEO-NEs in inhibiting the disease in greenhouse

Plant cultivation

The experiment was conducted in a greenhouse in Varamin, Tehran province, using a factorial design within a randomized complete block design. The design included factor A (nanoemulsions and fungicides at five levels) and factor B (concentration at three levels) with four replications. Seeds of the Nagin cucumber cultivar, a variety susceptible to powdery mildew, were planted in greenhouse experimental plots measuring 1 × 7 m, arranged in ridges and furrows. Each plot contained 20 to 30 cucumber plants, with a spacing of 90 cm between plots.

Pathogen inoculation

After six weeks, pots containing cucumber plants infected with powdery mildew were transferred to the greenhouse and placed between rows (four infected pots per row). The greenhouse condition was maintained at 20 °C and approximately 80% relative humidity.

Nanoemulsion application

Seven days after transferring the infected pots, seedlings were randomly selected and treated with different concentrations (1, 2, and 3 g/l) of selected nanoemulsions (savory, clove, and lemongrass) using a sprayer. Treatments included plants sprayed with nanoemulsions, the fungicides Stroby (0.4 g/l) and Topas (0.5 g/l) as positive controls, and plants sprayed with water as a negative control. The treated plants were continuously exposed to powdery mildew spores for an additional five days.

Disease severity

The total number of leaves per plant and the number of infected leaves per plant were counted. Disease severity was assessed using a 1–12 scale according to the Horsfall-Barratt scale[47].

Protective effect of selected PEO-NEs in inhibiting the disease in greenhouse

Plant cultivation

Seeds of the Nagin cucumber cultivar were surface-sterilized in 0.5% sodium hypochlorite (5% active chlorine) for five min and rinsed three times with sterile water[48]. The seeds were germinated in 100-cell trays filled with sterile perlite and coco peat, and the seedlings were grown in the greenhouse for two weeks. Plastic pots were filled with a pasteurized mixture of field soil, coco peat, and sand (1:1:1 ratio), and one seedling was planted in each pot. The pots were incubated at 25 °C in the greenhouse for an additional two weeks.

Nanoemulsion application

Different concentrations (1, 2, and 3 g/l) of selected nanoemulsions (savory, clove, and lemongrass) were sprayed on the entire aerial parts of the cucumber plants. The fungicides Stroby (0.4 g/l) and Topas (0.5 g/l) were used as positive controls.

Pathogen inoculation

A suspension of *P. fusca* containing 10^5 conidia/ml was sprayed on the entire aerial parts of the cucumber plants 48 h after applying the PEO-NEs. Three pots of cucumber plants were sprayed with sterile distilled water as a control. All pots were individually covered with plastic bags to maintain 100% relative humidity for 24 h. The pots were then kept in the greenhouse at > 80% humidity and 25 °C. Three pots were used for each treatment and the experiment was repeated twice.

Disease severity

Symptoms were evaluated daily, and disease severity was assessed using a 1–12 scale according to the Horsfall-Barratt scale[47].

Nanoemulsion effect on plant flavonoid and phenolic content

Preparation of extracts

Leaf samples were dried in a vacuum oven at 45 °C for 24 h, pulverized, and passed through a Number 20 sieve. A 100 mg aliquot of the sieved sample was mixed with 10 ml of methanol, vacuum-filtered through Whatman Number 1 paper, and sonicated for 45 min at 40 °C. The extract was then filtered again through Whatman Number 1 paper and dried using a rotary evaporator.

Total phenol content

The total phenolic content (TPC) was determined using the Folin-Ciocalteu method[49], with modifications. The dried extract was dissolved in 1 ml of absolute methanol, and 25 µl of the extract was mixed with 125 µl of 1 N Folin-Ciocalteu reagent (1:10). After eight min at room temperature, 100 µl of 7.5% Na_2CO_3 was added. Following a 120-min incubation at room temperature, absorbance was measured at 760 nm using Powerwave XS2 microplate reader (BioTek Instruments, Inc., USA). TPC was calculated using a gallic acid standard curve and expressed as milligrams of gallic acid equivalents per gram of extract. Analyses were performed in triplicate.

Total flavonoid content

The total flavonoid content (TFC) was determined using a modified method by Chen et al.[50]. The dried extract was dissolved in 1 ml of absolute methanol. In a two ml Eppendorf tube, 25 µl of the sample was mixed with 7.5

µl of 5% $NaNO_2$, and incubated for six min. After incubation, 7.5 µl of 10% $AlCl_3$, 100 µl of 4% NaOH, and 10 µl of distilled water were added. Absorbance was measured at 510 nm using a PowerWave XS2 microplate reader with Rutin as the standard. Results were expressed as milligrams of rutin equivalents per gram of extract.

Measurement of phenolic compounds by HPLC-DAD

The dried extract was dissolved in 1 ml of absolute methanol and analyzed using a Waters 2695 Alliance HPLC system (Waters Corporation, USA) with a diode array detector (DAD). A Gemini NX-C18 column (Phenomenex) with 3.5 µm packing, an internal diameter of 4.6 mm, and a length of 150 mm was used. The column oven was set at 25 °C, and the injection volume was 20 µl.

The elution solvents were methanol with 0.02% trifluoroacetic acid (A) and water with 0.02% trifluoroacetic acid (B), with a flow rate of 0.5 ml/min. The gradient program was as follows: 0–10 min (20% A, 80% B), 10–30 min (30% A, 70% B), 30–40 min (60% A, 40% B), 40–45 min (80% A, 20% B), 45–50 min (100% B). 50–52 min (20% A, 80% B), 52–55 min (isocratic 20% A, 80% B). The UV absorption spectra were measured between 200 and 400 nm.

Identification of phenolic compounds (Gallic acid, Caffeic acid, Ferulic acid, Rutin, Rosmarinic acid, and p-Coumaric acid) was based on retention time, UV absorption spectra, and comparison with standards.

GC-MS analysis of PEO compounds

Essential oil constituents were separated and identified using a TRACE™ GC 2000 gas chromatograph (ThermoQuest Italia S.p.A., Italy) equipped with a flame ionization detector (FID) and a DB-1 fused silica capillary column (60 m × 0.25 mm i.d.; film thickness = 0.25 µm). The injector and detector temperatures were set at 250 °C and 300 °C, respectively. Helium (99.999% purity) was used as the carrier gas at a flow rate of 1.1 ml/min. The column oven temperature was initially set at 60 °C for five min, then increased to 250 °C at a rate of 4 °C/min, and held isothermally for 10 min. GC-MS analysis was performed using a ThermoQuest Finnigan Trace GC/MS under the same conditions. The ionization voltage was 70 eV with ion source and interface temperatures of 200 °C and 250 °C, respectively. Mass spectra were recorded in the range of m/z 43–456. Compounds were identified by comparing their mass spectra with the Wiley/NBS library and authentic standards as well as by comparing retention indices with literature values. The percentage of each compound was determined based on relative peak areas from GC-FID analysis.

Statistical analysis

The experiments were conducted in a completely randomized block design. Data were analyzed using SAS v. 9.0 software. Analysis of variance (ANOVA) was performed, followed by Duncan's multiple range test to determine mean differences[51].

Data availability

All data generated or analyzed during this study are included in this published article and available from the corresponding author on reasonable request.

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Author contributions

L.E. and M.F. conceptualized and designed the study. A.T.A. prepared materials and performed experiments. L.E. and M.F. wrote the manuscript. All authors analyzed data and contributed to scientific discussions.

Declarations

Competing interests
The authors declare no competing interests.

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