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Control of potato leaf spot caused by *Alternaria alternata* using lemongrass oil microemulsions

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Abstract

Alternaria alternata, the causal agent of potato leaf spot, is a destructive foliar pathogen responsible for major yield losses worldwide, including in Egypt. This study evaluated the efficacy of lemongrass (*Cymbopogon citratus*) oil microemulsions compared with Index fungicide 77% WP (copper hydroxide) under laboratory, greenhouse, and field conditions. *In vitro* assays, conducted using the poisoned food technique, showed a clear dose-dependent inhibition of mycelial growth, ranging from 6.3% at 60 µl/ml to complete suppression (100%) at 450 and 800 µl/ml. Greenhouse trials confirmed a concentration-dependent reduction in disease severity, with 300 µl/ml achieving the highest suppression (74.8%), while Index fungicide (250 g/100 L water) consistently provided superior control (87.4%). Biochemical analyses revealed that lemongrass oil significantly increased chlorophyll a and carotenoid contents but had no notable effect on chlorophyll b, suggesting selective stimulation of photosynthetic pigments. Enhanced peroxidase, polyphenol oxidase, and phenolic compound levels indicated activation of host defense pathways, while sugar and flavonoid contents also increased following treatment. Field trials across Beheira, Sharkia, and Qalyubeia governorates confirmed the protective effects of lemongrass oil, with 300 µl/ml consistently reducing disease severity, though less effectively than Index fungicide. Collectively, these findings demonstrate that lemongrass oil microemulsions represent a promising eco-friendly alternative for integrated management of potato leaf spot, with potential to reduce reliance on synthetic fungicides.

Keywords: Potato, *Alternaria alternata*, leaf spot, lemongrass oil microemulsion, oxidative enzymes, biochemical defense.

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1. Introduction

Potato (*Solanum tuberosum* L.) is a globally important food crop, with annual production exceeding 383 million tons (FAOSTAT, 2023). In Egypt, potato cultivation contributes significantly to the national economy, producing approximately 6.87 million tons annually. However, potato crops are increasingly affected by fungal diseases, including early blight, late blight, and brown leaf spot (Park *et al*., 2024; Van der Waals *et al*., 2004). Potato leaf spot, caused by *Alternaria alternata*, is a destructive foliar disease leading to up to 60% infection under humid and warm conditions (Choi *et al*., 2023). The pathogen causes characteristic brown necrotic lesions, progressing to leaf yellowing, curling, and premature senescence (Park *et al*., 2024; Schmey *et al*., 2024; Smith, 2010). Severe epidemics substantially reduce photosynthetic area and yield. Leaf spot is traditionally managed through repeated applications of synthetic fungicides. Although effective, these chemicals pose risks to human and environmental health, including acute toxicity, chronic exposure, and ecological imbalance (Ahmad *et al*., 2024; Gikas *et al*., 2022). Continuous reliance on synthetic fungicides also increases the likelihood of pathogen resistance, underscoring the urgent need for safer alternatives. Growing interest in sustainable crop protection has led to investigations into essential oils (EOs) as eco-friendly plant disease management tools. EOs are valued for their biodegradability and reduced hazards to human health and the environment, aligning with the principles of integrated pest management (Regnault-Roger *et al*., 2012). Their antimicrobial bioactivity, derived from compounds such as terpenes, phenolics, and aldehydes, is well documented against pathogens including *Botrytis cinerea*, *Fusarium* spp., and *Phytophthora infestans* (Pavela and Benelli, 2016). Among EOs, lemongrass (*Cymbopogon citratus*) oil is notable for its richness in citral, geraniol, and myrcene, compounds that contribute to strong antifungal properties against diverse pathogens such as *Aspergillus flavus*, *Fusarium oxysporum*, and *Penicillium expansum* (Boukhatem *et al*., 2014; Mohamed *et al*., 2019; Shreaz *et al*., 2016). However, practical use is constrained by volatility, rapid degradation, and potential phytotoxic effects (Pavela and Benelli, 2016). To overcome these limitations, microemulsion and nanoemulsion technologies have been developed to improve solubility, stability, and bioavailability, thereby enhancing cellular penetration and antifungal efficacy (Ali *et al*., 2017; Clausse *et al*., 2018; Noveriza and Manohara, 2023; Youssef *et al*., 2021). The incorporation of emulsifiers improves solubility and bioavailability, and enhances penetration of active compounds into fungal cells. Although essential oils have shown promise in pathogen control, few studies have evaluated their efficacy across multiple scales, from laboratory assays to field trials, or explored their effects on host physiological and biochemical defenses. Moreover, while nanoemulsions have been widely studied, microemulsions remain less investigated despite their formulation stability and ease of preparation. To address these gaps, this study aimed to evaluate the antifungal efficacy of lemongrass oil microemulsions compared

with Index fungicide 77% WP (copper hydroxide) against potato leaf spot caused by *A. alternata* under laboratory, greenhouse, and field conditions in Egypt.

2. Materials and methods

2.1 Isolation and identification of the causal organism

Potato leaves exhibiting typical symptoms of leaf spot disease were collected from the Egyptian Governorates of Qalyubeia (Moshthour), Ismailia, Beheira (Nubaria), and Al Sharkia. Potato leaves were collected, thoroughly rinsed to eliminate surface contamination, cut into small pieces, sterilized with 1%sodium hypochlorite, and transferred into potato dextrose agar (PDA) enriched with antibiotics, followed by incubation at 25°C. Pure isolates that were morphologically similar to Alternaria fungus were transferred to PDA slant tubes and kept in a refrigerator at 5°C for further studies. Pure isolates were identified based on their morphology, as outlined by Singh (1982) and Barnett and Hunter (1987).

2.2 Pathogenicity tests

A controlled greenhouse experiment was conducted at the Department of Vegetable Diseases, Plant Pathology Research Institute, Egypt to investigate the effects of a fungal pathogen on potato plants (*Solanum tuberosum* L. Spunta cv.). Healthy imported potato tubers (30–50 g) were individually planted in 30 cm plastic pots. The growth medium was a randomized mixture of peat moss and sandy soil (2:1), sterilized with formalin (5%), and used in three replicates for each of the treatments. Each pot contained a single tuber of the respective cultivar. At 40 days after planting, the foliage was inoculated with a spore suspension of the fungal pathogen. The suspension was adjusted to a final concentration of 1×10^6 conidia/ml. The potato plants were then sprayed with 30 ml of spore suspension per plant. Following inoculation, the plants were placed covered with polyethylene bags for 48 hours to maintain high humidity levels and promote infection. Control plants were treated with sterile water and covered with polyethylene bags. Disease severity was recorded every 10 days, with three measurements taken beginning 10 days after inoculation, utilizing a 0–9 scale (0 = healthy; 1 less than 10%; 2= 10-20%; 3= 20-30%; 4= 30-40%; 5=40-50%; 6= 50-60%; 7 = 60-70%; and 8 = 70-80 %; 9= more than 80%) of the infected leaf area infected (Ghosh *et al*., 2009).

2.3 Preparation of oil microemulsion

A microemulsion of lemongrass oil-in-water (O/W) was prepared using lemongrass oil, a surfactant (Tween 80), a co-surfactant, and distilled water. Microemulsions were created by diluting the oil-surfactant combination with water using a magnetic stirrer for continuous agitation until a homogeneous solution was achieved. Subsequently, distilled water was added to the mixture to bring

the final volume to 100 mL. The surfactant-to-cosurfactant ratio was 3:1, and the solution changed from turbid to light yellow, indicating the formation of a microemulsion (Al-Shahrani *et al*., 2017).

2.4 Characterization of microemulsion

The UV-Visible light absorption of the microemulsions was evaluated via spectrophotometry (Spectronic 601, Milton Roy, USA) at 600 nm to determine the apparent transparency of the samples.

2.4.1 Particle size analysis

The particle size of the microemulsions was assessed using a dynamic light scattering device (Zetasizer, Particle Size Analyzer, PSS NICOMP Nano, N 3000). All samples were filtered using a syringe filter to remove impurities and were diluted to a concentration of 0.05% prior to testing. The observations were derived from the Brownian motion of the hydrated particles, yielding information on the hydrodynamic diameter (nm) of the microemulsion particles. The sizes provided are the z-average mean of the hydrodynamic diameter of the microemulsion (nm).

2.5 In vitro antifungal assay

The antifungal effects of the lemongrass microemulsion were evaluated *in vitro* against a highly virulent *A. alternata* isolate. Different concentrations of the lemongrass microemulsion were evaluated using the poisoned food technique, with three replicates. All Petri dishes were incubated at 25 ± 1°C until the fungal growth in the control dishes was almost complete (Sirirat *et al*., 2009):

$$\text{Reduction \%} = \left(\frac{\text{de} - \text{di}}{\text{de}}\right) \times 100$$

Where de = mean diameter of radial growth in control and di = mean diameter of radial growth in treatment.

2.6 Greenhouse experiments

The greenhouse experiment evaluated the effect of different concentrations of lemongrass microemulsion on the prevalence of leaf spot disease in potato induced by *A. alternata*. The cultivation was carried out as previously described in Pathogenicity assay, with three replicates for each concentration (100, 200, and 300 µl/ml) in a Completely Randomized Design. When the plants reached 40 days post-planting, they were treated with lemongrass oil microemulsion at different concentrations compared to Index fungicide (copper hydroxide) 77% WP at the recommended rate of 250 g/100 L of water. An additional control group was sprayed with sterile water. After 4 days of spraying, the plants were inoculated with a conidial suspension at a rate of 1 × 10^6 conidia/ml (30 ml per plant). The plants were covered with polyethylene bags for 48 hours to maintain high humidity. The plants received a total of three foliar sprays of their designated treatments, applied at 10-day intervals (e.g., 0, 10, and 20 days post-inoculation). Randomized plants were collected for the assessment

of disease severity and for biochemical analysis at 10, 20, and 30 days post-inoculation. Greenhouse environmental conditions were maintained at 25 ± 2°C, 70-80% relative humidity, and a 16/8 h light/dark photoperiod. The assessment of disease severity started 10 days post-inoculation (dpi). Subsequent disease severity assessments were performed at 10-day intervals, with a total of three readings per treatment group collected across the study period (i.e., at 10, 20, and 30 dpi), using the 0-9 scale (Ghosh *et al*., 2009).

2.7 Biochemical analyses

2.7.1 Determination of free and total phenols

Total and free phenolic compounds were measured using the Folin-Ciocalteu reagent method, as explained by Gomaa *et al*. (2016). The density of the colored product was measured at 520 nm using catechol as a reference. Measurements were taken using a UV-VIS spectrophotometer (Spectronic 601, Milton Roy, USA).

2.7.2 Total flavonoids

Total flavonoid content was determined as described by Zhishen *et al*. (1999). The reaction was measured at 510 nm using a spectrophotometer (Spectronic 106). The results were reported as µg quercetin/g dry weight (DW).

2.7.3 Sugar content

Total soluble sugars and reducing sugars were colorimetrically determined at 540 nm using the picric acid technique, as described by Thomas and Dutcher (1924). The density of the generated color was assessed at 540 nm using a blank and glucose as reference standards.

2.7.4 Oxidative enzyme assay

Crude enzyme preparation for the assay was described by Aluko and Ogbadu (1986). Enzyme activity was assayed using spectrophotometry. Peroxidase enzyme assay (PO) activity was assessed using pyrogallol as a substrate, following the modified method of Chance and Maehly (1955), as described by Falade *et al*. (2019). The absorbance change of the reaction mixture was measured at 420 nm. The activity of the polyphenol oxidase enzyme (PPO) was assessed using the methodology outlined by Arnnok *et al*. (2010). The absorbance change of the reaction mixture was measured at 495 nm.

2.7.5 Determination of chlorophyll

The Arnon methodology, as delineated by Gu *et al*. (2016), was used to quantify the chlorophyll (total, a, and b) and carotenoid contents in plant leaves.

2.8 Field experiments

All field experiments were carried out during the summer growing season (June to September) in Qalyubeia (Moshthour), Beheira (Nubaria), and Al Sharkia Governorates, Egypt. Potato tubers were planted in plots consisting of 4 lines, each 9 m long and 0.8 m wide, with tubers planted 25 cm deep and 30 cm apart

within lines. The experiment was arranged in a randomized block design with three replications (plots) per treatment. Treatments identical to those in greenhouse conditions were applied. Natural disease pressure was monitored, and no additional inoculation was performed. Standard agricultural practices for weed and pest control (excluding fungicides targeting *Alternaria*) were followed.

2.9 Statistical analysis

Data collected were analyzed using MSTAT-C software (version 1991). Analysis of variance (ANOVA) was performed, and the differences between the mean values of various treatments were compared using the least significant difference (LSD) test at $p \leq 0.05$.

3. Results

3.1 Microemulsion characterization

Physicochemical characterization of the lemongrass oil microemulsion was performed (Table 1, Figure 1). The microemulsion exhibited a UV/Vis absorbance of 0.004 at 600 nm, indicating high transparency. Dynamic Light Scattering (DLS) analysis revealed a mean hydrodynamic diameter of 16.4 nm with a polydispersity index (PDI) of 0.353, confirming the formation of a stable microemulsion. The visual appearance of the stable microemulsion after 30 days of storage at room temperature is shown in Figure (1a).

Table (1): Physicochemical characteristics of lemongrass oil microemulsion.

Parameter	Value
Mean diameter (nm)	16.4
Polydispersity Index (PDI)	0.353
Normalized Standard Deviation (Coefficient of Variation)	0.594
Z-Average Diffusion Coefficient (cm^2/s)	2.84E-007

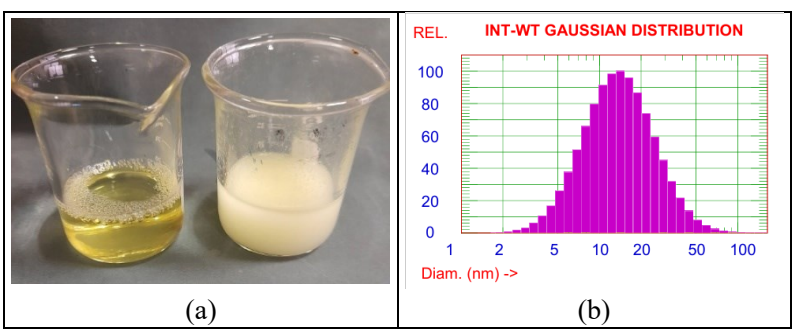

Figure (1): (a) Visual appearance of the lemongrass microemulsion after 30 days of storage. (b) Hydrodynamic size distribution of the lemongrass microemulsion as determined by Dynamic Light Scattering (DLS).

3.2 Pathogenicity tests

Table (2) presents the results of the pathogenicity test conducted on potato plants (Spunta cv.) using various *A. alternata* isolates collected from different geographical locations. While all isolates induced similar leaf symptoms, considerable variation in virulence was observed across the three post-inoculation intervals (10, 20, and 30 days). The isolate Beheira 3 (AL-11) exhibited significantly higher disease severity (DS%) compared to all other isolates, with values of 27.5%, 35.15%, and 52.46% at 10, 20, and 30 days post-inoculation (dpi), respectively. In contrast, Qalyubeia 2 (AL-2) was the least virulent, demonstrating significantly lower DS% values of 3.09%, 7.32%, and 12.98% at the corresponding time points. Several isolates displayed intermediate levels of virulence, including Sharkia 1 (AL-5) with DS% values of 22.01%, 31.63%, and 42.24%, and Ismailia 2 (AL-8) with DS% values of 16.04%, 22.3%, and 43.64%. Beheira 2 (AL-10) also exhibited intermediate virulence.

Table (2): Pathogenicity test of different isolates of *A. alternata* on potato (Spunta cv.) under greenhouse conditions.

Locations	Isolate No.	After 10 days (DS% ± SD)	After 20 days (DS% ± SD)	After 30 days (DS% ± SD)
Qalyubeia	AL-1	20.80 ± 1.57	26.94 ± 1.61	37.63 ± 1.99
	AL-2	3.09 ± 0.77	7.32 ± 0.76	12.98 ± 1.17
	AL-3	13.53 ± 0.60	20.32 ± 0.91	26.74 ± 0.73
	AL-4	8.01 ± 0.64	12.02 ± 1.27	17.84 ± 0.36
Al Sharkia	AL-5	22.01 ± 0.69	31.63 ± 1.37	42.24 ± 1.18
	AL-6	15.67 ± 1.55	19.83 ± 1.72	26.58 ± 1.29
Ismailia	AL-7	8.01 ± 0.64	12.02 ± 1.27	29.64 ± 0.97
	AL-8	16.04 ± 1.01	22.30 ± 0.79	43.64 ± 1.30
El Beheira	AL-9	11.26 ± 0.75	16.29 ± 1.07	24.51 ± 1.28
	AL-10	19.04 ± 1.02	26.63 ± 1.35	33.50 ± 1.18
	AL-11	27.50 ± 0.64	35.15 ± 1.11	52.46 ± 1.14
L.S.D. ($p \leq 0.05$)		1.77	2.20	2.11

3.3 In vitro antifungal assay

Table (3) shows that lemongrass oil microemulsion exhibited a strong antifungal effect against *A. alternata* linear growth under laboratory conditions. All tested concentrations significantly inhibited fungal growth compared to the untreated control. The lowest concentration (60 µL/ml) resulted in a 6.30% reduction in growth (84.33 mm). As the concentration increased, a progressive decline in fungal growth was observed. At 240 µL/ml, linear growth decreased to 47.00 mm, corresponding to a 47.78% inhibition. A pronounced antifungal effect was recorded at 360 µL/ml, where growth was limited to 24.33 mm with a 72.96% reduction. The highest levels of inhibition were achieved at 450 and 800 µL/ml, both completely suppressing fungal development (0.00 mm), indicating a full 100% inhibition rate.

Table (3): Effect of lemongrass oil microemulsion on *A. alternata* under *in vitro* conditions.

Concentrations (µL/ml)	Linear Growth (mm ± SD)	% of Reduction
60	84.33 ± 1.16	6.30
120	75.00 ± 2.00	16.67
180	66.67 ± 0.58	25.93
240	47.00 ± 1.00	47.78
360	24.33 ± 1.16	72.96
450	0.00 ± 0.00	100.00
800	0.00 ± 0.00	100.00
Control	90.00 ± 0.00	0.00
L.S.D. (p≤0.05)	1.857	--

3.4 Greenhouse experiments

Table (4) shows the efficacy of lemongrass oil microemulsion at varying concentrations (100, 200, and 300 µl/ml) in mitigating leaf spot disease caused by A. alternata under greenhouse conditions. The highest concentration (300 µl/ml) of microemulsion exhibited the most pronounced disease-suppressive effect, evidenced by a reduction in disease severity (DS%) of 65.79% at 10 days post-inoculation (dpi), 72.04% at 20 dpi, and 74.79% at 30 dpi. The 200 µl/ml concentration showed a moderate level of disease control, with DS% reductions of 48.49% at 10 dpi, 57.93% at 20 dpi, and 63.53% at 30 dpi. The lowest concentration (100 µl/ml) was the least effective in reducing disease severity, with reductions ranging from 40.96% (15.44 ± 1.69 DS%) at 10 dpi to 49.95% (24.59 ± 0.83 DS%) at 30 dpi. For comparative purposes, a positive control using Index fungicide exhibited the highest reduction in DS% at all time points: 86.36% at 10 dpi, 87.52% at 20 dpi, and 87.44% at 30 dpi.

Table (4): Effect of lemongrass oil microemulsion on leaf spot disease progression caused by *A. alternata* under greenhouse conditions.

Concentrations	DS% (10 dpi ± SD)	Reduction% (10 dpi)	DS% (20 dpi ± SD)	Reduction% (20 dpi)	DS% (30 dpi ± SD)	Reduction% (30 dpi)
100 µl/ml	15.44 ± 1.69	40.96	20.77 ± 0.45	49.46	24.59 ± 0.83	49.95
200 µl/ml	13.47 ± 0.50	48.49	17.29 ± 1.148	57.93	17.91 ± 1.52	63.53
300 µl/ml	8.95 ± 1.60	65.79	11.49 ± 1.17	72.04	12.38 ± 1.38	74.79
Fungicide Index 77%	3.57 ± 0.58	86.36	5.13 ± 0.98	87.52	6.17 ± 0.90	87.44
Control	26.15 ± 2.57	0.00	41.10 ± 1.98	0.00	49.18 ± 1.01	0.00
L.S.D. (p≤0.05)	2.71	--	2.69	--	2.35	--

3.5 Biochemical analyses

Table (5) indicates significant differences in chlorophyll a and carotenoid content between treatments and control. Conversely, no significant differences were observed in chlorophyll b content between the 200 µl/ml lemongrass treatment and the control after 30 days. The activity of PPO and PO enzymes, along with the accumulation of phenolic compounds, were higher at the 300 µl/ml concentration after 30 days (Table 6). However, PO enzyme activity at 100

µl/ml and 200 µl/ml concentrations showed no significant differences compared to the control after 20 and 30 days, respectively.

Table (5): Effect of oil microemulsion on plant content of chlorophyll and carotenoids.

Concentrations	Chlorophyll A (mg/g)			Chlorophyll B (mg/g)			Chlorophyll Total (mg/g)			Carotenoids (mg/g)		
	10 days	20 days	30 days	10 days	20 days	30 days	10 days	20 days	30 days	10 days	20 days	30 days
100 µl/ml	8.31	6.50	2.31	5.69	5.11	3.52	13.99	11.61	5.83	1.48	0.93	1.563
200 µl/ml	6.35	6.86	4.53	6.34	6.11	2.29	12.69	12.97	6.82	0.77	1.82	0.81
300 µl/ml	6.73	10.36	3.63	4.51	6.49	4.18	11.24	16.85	7.81	1.26	1.38	0.56
Fungicide Index 77%	3.96	8.79	3.01	5.34	6.60	4.29	9.30	15.39	7.31	0.10	0.20	1.06
Control	5.95	4.02	1.52	4.23	3.85	2.73	10.17	7.87	4.25	0.62	1.19	0.46
LSD 0.05 (T)	0.26			0.55			0.52			0.05		
LSD 0.05 (I)	0.20			0.42			0.41			0.04		
LSD 0.05 (T × I)	0.45			0.95			0.91			0.09		

T= Treatments, I= Time.

Table (6): Effect of oil microemulsion on plant content of oxidative enzymes and phenolic compounds.

Concentrations	PPO (Units mg^{-1} solid)			PO (Units mg^{-1} solid)			Free Phenolic Compounds (mg/g)			Total Phenolic Compounds (mg/g)		
	10 days	20 days	30 days	10 days	20 days	30 days	10 days	20 days	30 days	10 days	20 days	30 days
100 µl/ml	1.01	0.44	1.01	89.83	20.32	8.98	80.60	102.04	91.68	19.93	15.59	56.50
200 µl/ml	1.54	0.32	0.97	72.98	55.37	18.67	79.64	55.70	103.21	43.50	30.72	64.25
300 µl/ml	1.91	0.89	1.37	51.51	47.81	62.96	52.16	79.37	130.86	29.83	42.47	72.25
Fungicide Index 77%	0.85	0.87	3.10	28.81	52.14	31.78	54.22	39.79	92.04	28.32	45.28	89.18
Control	0.41	2.68	0.95	29.64	51.44	5.49	59.98	74.29	87.94	50.02	66.74	65.68
LSD 0.05 (T)	0.08			4.30			2.12			1.36		
LSD 0.05 (I)	0.06			3.33			1.64			1.05		
LSD 0.05 (T × I)	0.13			7.44			3.68			2.35		

T= Treatments, I= Time.

Table (7) shows that total flavonoids, total sugar, and reducing sugar in potato leaves were affected by the treatments. All treatments exhibited significant differences compared with the control, except for the total sugar compounds at lower concentrations after 20 and 30 days. After 30 days, the recorded levels of total flavonoids, total sugar, and reducing sugar were higher across all treatments.

Table (7): Effect of oil microemulsion on plant content of total flavonoids and sugars.

Concentrations	Total Flavonoids (µg quercetin/g)			Total Sugar (mg/g)			Reducing Sugar (mg/g)		
	10 days	20 days	30 days	10 days	20 days	30 days	10 days	20 days	30 days
100 µl/ml	144.33	119.33	152.00	23.53	17.90	27.98	10.28	15.41	22.50
200 µl/ml	141.67	100.67	185.00	11.98	17.99	31.03	10.33	12.48	22.66
300 µl/ml	125.33	199.67	191.33	8.60	29.02	36.32	3.52	20.58	32.70
Fungicide Index 77%	106.33	112.00	137.33	18.36	31.23	30.45	6.60	6.93	22.65
Control	144.33	155.00	123.33	20.68	17.12	27.36	11.55	8.73	21.07
LSD 0.05 (T)	12.58			0.87			1.36		
LSD 0.05 (I)	9.75			0.68			1.05		
LSD 0.05 (T × I)	21.80			1.51			2.35		

T= Treatments, I= Time.

3.6 Field experiments

3.6.1 Beheira Governorate

Table (8) shows that foliar application of lemongrass oil microemulsion at varying concentrations effectively mitigated the progression of leaf spot disease caused by *A. alternata* in field trials conducted within Beheira Governorate. Disease severity (DS%) was expressed as a percentage and significantly reduced with increasing microemulsion concentrations. The concentration 300 µl/ml achieved reductions of 45.58%, 47.70%, and 61.15% at 10, 20, and 30 days post-treatment (dpt), respectively, compared to the untreated control. Index fungicide exhibited the highest efficacy, with DS% reductions of 54.04%, 62.52%, and 71.93% over the same time points. The reduction in disease severity was statistically significant (LSD < 0.05) across all treatments and time points.

Table (8): Effect of lemongrass oil microemulsion on leaf spot disease progression caused by *A. alternata* under Beheira Governorate field conditions.

Concentrations	DS% (10 dpt ± SD)	Reduction% (10 dpt)	DS% (20 dpt ± SD)	Reduction% (20 dpt)	DS% (30 dpt ± SD)	Reduction% (30 dpt)
100 µl/ml	18.15 ± 2.64	20.80	21.48 ± 1.40	28.11	27.78 ± 2.66	39.56
200 µl/ml	15.52 ± 1.70	32.27	19.33 ± 1.81	35.32	23.90 ± 4.29	48.00
300 µl/ml	12.47 ± 1.08	45.58	15.63 ± 1.67	47.70	17.85 ± 1.86	61.15
Fungicide Index 77%	10.53 ± 1.97	54.04	11.20 ± 3.00	62.52	12.90 ± 2.00	71.93
Control	22.92 ± 2.40	0.00	29.88 ± 2.39	0.00	45.96 ± 3.65	0.00
L.S.D. (p≤0.05)	4.26	--	5.14	--	4.79	--

3.6.2 Sharkia Governorate

Table (9) shows that the field trials conducted in Sharkia Governorate demonstrated that lemongrass oil microemulsion effectively suppressed leaf spot disease caused by *A. alternata*, with its efficacy exhibiting a concentration-dependent relationship. In the untreated control group, disease severity (DS%) progressed naturally over time, with values of 15.81%, 26.41%, and 33.49% recorded at 10, 20, and 30 days post-treatment (dpt), respectively. Application of lemongrass oil emulsion at 100 µl/ml resulted in a moderate level of disease suppression, with DS% reductions of 22.1%, 32.45%, and 25.66% across the same time periods. Increasing the concentration to 200 µl/ml enhanced disease reduction to 25.08%, 40.38%, and 43.18%, while the highest concentration tested, 300 µl/ml, achieved the most significant reductions among the emulsion treatments at 31.25%, 53.6%, and 60.50%. Index fungicide consistently exhibited the highest efficacy, with reductions in DS% of 57.37%, 68.93%, and 72.79% throughout the observation period. Statistical analysis (LSD < 0.05) confirmed the significant differences observed between treatments and the control group.

Table (9): Effect of lemongrass oil microemulsion on leaf spot disease progression caused by *A. alternata* under Sharkia Governorate field conditions.

Concentrations	DS% (10 dpt ± SD)	Reduction% (10 dpt)	DS% (20 dpt ± SD)	Reduction% (20 dpt)	DS% (30 dpt ± SD)	Reduction% (30 dpt)
100 µl/ml	12.32 ± 1.75	22.10	17.84 ± 2.19	32.45	24.90 ± 2.37	25.66
200 µl/ml	11.84 ± 1.57	25.08	15.75 ± 1.53	40.38	19.03 ± 1.81	43.18
300 µl/ml	10.87 ± 1.80	31.25	12.25 ± 1.89	53.60	17.78 ± 1.69	60.50
Fungicide Index 77%	6.74 ± 1.89	57.37	8.21 ± 2.16	68.93	9.11 ± 2.36	72.79
Control	15.81 ± 1.51	0.00	26.41 ± 1.71	0.00	33.49 ± 3.37	0.00
L.S.D. (p≤0.05)	3.27	--	3.52	--	3.89	--

3.6.3 Qalyubeia Governorate

Table (10) shows that the field trials conducted in the Qalyubeia Governorate demonstrated that lemongrass oil emulsion significantly reduced the progression of leaf spot disease caused by *A. alternata*, with efficacy positively correlating with increasing concentration. The untreated control group exhibited the highest disease severity (DS%), with values of 18.83%, 24.85%, and 37.32% at 10, 20, and 30 days post-treatment (dpt), respectively. Foliar application of lemongrass oil emulsion at 100 µl/ml resulted in a modest level of disease suppression, with DS% reductions of 23.03%, 24.34%, and 38.49% across the same time periods. Increasing the concentration to 200 µl/ml enhanced these reductions to 32.67%, 33.49%, and 46.59%, while the highest concentration tested, 300 µl/ml, achieved the most substantial reductions among the emulsion treatments, with values of 39.05%, 44.16%, and 59.66%. Index fungicide treatment consistently outperformed all other treatments, achieving reductions of 57.58%, 60.34%, and 73.97% at 10, 20, and 30 dpt, respectively, confirming its superior efficacy in controlling the disease. Statistical analysis (LSD < 0.05) indicated that all treatments significantly reduced disease severity compared to the control, with significant differences observed among the treatment groups.

Table (10): Effect of lemongrass oil microemulsion on leaf spot disease progression caused by *A. alternata* under Qalyubeia Governorate field conditions.

Concentrations	DS% (10 dpt ± SD)	Reduction% (10 dpt)	DS% (20 dpt ± SD)	Reduction% (20 dpt)	DS% (30 dpt ± SD)	Reduction% (30 dpt)
100 µl/ml	14.49 ± 1.55	23.03	18.54 ± 1.89	24.34	22.96 ± 1.98	38.49
200 µl/ml	12.68 ± 1.48	32.67	16.30 ± 1.84	33.49	19.93 ± 2.22	46.59
300 µl/ml	11.48 ± 1.63	39.05	13.69 ± 1.65	44.16	15.06 ± 1.69	59.66
Fungicide Index 77%	7.99 ± 1.57	57.58	9.72 ± 2.24	60.34	9.71 ± 2.36	73.97
Control	18.83 ± 1.91	0.00	24.85 ± 1.39	0.00	37.32 ± 2.18	0.00
L.S.D. (p≤0.05)	2.74	--	3.28	--	3.89	--

4. Discussion

Species of the genus *Alternaria* are recognized as major plant pathogens responsible for significant yield losses, which can reach up to 80% in susceptible crops (Choi *et al*., 2023). Among these, *A. solani* and *A. alternata* are the most prevalent species affecting potato production worldwide. Specifically, *A. alternata* has been identified as a causal agent of leaf spots in potato. In the current greenhouse trials on *Solanum tuberosum* L. Spunta cv., the *A. alternata* isolates collected from various geographic regions in Egypt exhibited differential virulence. Although all isolates induced similar leaf spot symptoms, the observed variation in disease severity (DS%) across isolates suggests underlying genetic and physiological diversity. This variability may also reflect environmental adaptations that influence the pathogenic potential of each isolate. This finding is consistent with numerous studies (Böhme *et al*., 2013; Leiminger and Hausladen, 2014; Park *et al*., 2024; Shunping *et al*., 2019), which have observed that *A. alternata* is the causative agent of leaf spot disease in potato and exhibits wide-ranging virulence patterns among fungal isolates. This variability may be attributed to the mycotoxins secreted by the fungus, which are toxic to plants and play a vital role in disease development (Garganese *et al*., 2016; Habib *et al*., 2021; Meena *et al*., 2019; Youssef *et al*., 2021). For instance, Meena *et al*. (2017) similarly demonstrated that *A. alternata* isolates from different Indian regions varied significantly in their virulence on tomato and chili plants, with some strains consistently causing more severe symptoms. Similarly, Esfahani (2018) found that *A. alternata* isolates from distinct climatic zones in Iran exhibited differential pathogenic effects on potato cultivars, reflecting both environmental adaptation and host-pathogen interaction dynamics. Ismail *et al*. (2023) also illustrated significant diversity in virulence among *A. alternata* isolates on tomato, noting that specific isolates exhibited varying degrees of disease severity. Lemongrass oil microemulsions exhibit enhanced antifungal activity due to improved solubility, stability, and cellular penetration, which facilitates the delivery of active compounds like citral and geraniol (Cofelice *et al*., 2021; Deepika *et al*., 2022; Liu *et al*., 2023). The primary mechanism involves disruption of fungal membrane integrity and interference with metabolic processes, leading to cell death (Liu *et al*., 2023; Shaikh *et al*., 2020). Furthermore, it diminishes the expression of genes related to quorum sensing, peptidoglycan, and fatty acid biosynthesis (Gao *et al*., 2020). The antifungal mechanism of lemongrass oil involves disrupting fungal cell membrane integrity, increasing permeability, and leading to cell lysis and death (Alsakhawy, 2024; Mukarram *et al*., 2021). Additionally, lemongrass oil has been reported to inhibit the biosynthesis of fungal toxins, such as aflatoxins and fumonisins, which pose serious threats to food safety and public health (Hua et al.,

2014; Rasooli, 2007). These microemulsions thus provide an effective and natural alternative for antifungal applications. The present results are consistent with those of Mohammad *et al*. (2019), who reported that lemongrass emulsion inhibited the mycelial growth of various phytopathogens, including *Pestalotia longisetula*, *Fusarium oxysporum*, *Macrophomina phaseolina*, and *Alternaria raphani*, at concentrations ranging from 31.25 to 500 µl/ml. Liu *et al*. (2023) also found that lemongrass essential oil-loaded microemulsion had high antifungal activity against Aspergillus flavus. Furthermore, Tripathi *et al*. (2024) found that peppermint oil microemulsion at 0.5% concentration reduced *A. alternata* biomass by 96%, with treated mycelia exhibiting structural damage and osmotic imbalance, underscoring the efficacy of essential oil microemulsions as antifungal agents. Greenhouse and field trials conducted in three different regions (Beheira, Sharkia, and Qalyubeia Governorates) consistently demonstrated the efficacy of lemongrass microemulsion in suppressing the development of leaf spot disease, particularly at a concentration of 300 µl/ml (Tables 4, 8, 9, 10). Nguyen *et al*. (2021) reported similar findings, where a ternary combination of chili oil-encapsulated lipid nanoparticles (LNs), garlic oil-encapsulated LNs, and cinnamon oil-encapsulated LNs at 200 µl/ml exhibited over 80% effectiveness in preventing and treating leaf spot disease. They also noted no negative effect on tomato plant growth under ex vitro conditions. Likewise, El-Shennawy and Abozid (2017) found that suppressing the seeds of peas pre-planting in lemongrass oil (10%) significantly reduced damping-off disease incidence with a 72% reduction. Lemongrass essential oil was also identified as one of the top three essential oils exhibiting the highest antifungal activity among a group of eleven tested oils (Xiang *et al*., 2020). Lemongrass oil enhanced the levels of chlorophyll a, total chlorophyll, and carotenoids in potato leaves compared with the control (Table 5), while chlorophyll b showed no significant changes at certain concentrations/time points. These findings confirm previous research indicating that the application of essential oils as alternative pesticides can positively affect plant physiology. Massoud *et al*. (2015) and Thakur *et al*. (2023) have shown that these oils can increase chlorophyll and carotenoid content in plants. This improvement is often an indirect effect of a healthier plant. Carotenoids are essential for preserving membrane integrity and supporting antioxidant defenses. Furthermore, applying lemongrass oil led to an increase in the total soluble sugar content within the plant (Table 7), consistent with findings by Massoud *et al*. (2015) and Ahmed *et al*. (2024). These sugars are crucial for the plant's defense response for several reasons. Sugars and other osmolytes help maintain cell turgor, enabling the plant to tolerate various stresses, including those caused by pathogen invasion (Jeandet *et al*., 2022). Lemongrass oil also enhanced

the activity of peroxidase (PO) and polyphenol oxidase (PPO) (Table 6), which are associated with heightened resistance to fungal infections in many plant systems. PPO facilitates the oxidation of phenols to quinones, which are toxic to pathogens, while PO is involved in lignin synthesis and phenol oxidation (Ahmed *et al*., 2024; Kgang *et al*., 2023). Prasannath (2017) noted that PO transforms phenols into quinones, which inhibit fungal proliferation and spore germination, and also contribute to lignin synthesis. Zyton and Ahmed (2016) found that an active phenol oxidase system is essential for utilizing endogenous phenolic compounds in plant disease resistance. These studies support earlier research, indicating that the accumulation of phenolic compounds, defense-related enzymes, pathogenesis-related proteins, and systemic acquired resistance suggests that applying chemicals and biotic inducers may effectively assist in managing plant diseases (Ahmed *et al*., 2024; Omara *et al*., 2022). Furthermore, total flavonoids, a class of polyphenolic compounds, increased after lemongrass oil microemulsion treatment (Table 7). Patil *et al*. (2024) highlighted the multifunctional roles of flavonoid compounds in plant physiology, defense, symbiosis, and stress mitigation. While lemongrass oil microemulsion consistently demonstrated significant efficacy against potato leaf spot, the Index fungicide (copper hydroxide) generally exhibited superior disease control across all experiments (Tables 4, 8, 9, 10). This difference in efficacy highlights a common trade-off when considering eco-friendly alternatives to synthetic pesticides. Although the synthetic fungicide provides a higher level of immediate disease suppression, its extensive use poses serious hazards to humans and the environment, including acute toxicity, chronic health effects, and ecological imbalance (Ahmad *et al*., 2024; Gikas *et al*., 2022). In contrast, lemongrass oil microemulsions offer a safer, sustainable, and biodegradable alternative, aligning with principles of integrated pest management and sustainable agriculture (Regnault-Roger *et al*., 2012). The slightly lower efficacy of the lemongrass microemulsion may be acceptable in contexts where environmental impact and consumer safety are prioritized, or as part of a rotational strategy to manage fungicide resistance. This study provides valuable insights into the potential of lemongrass oil microemulsions for potato leaf spot management. However, certain limitations should be acknowledged. The long-term stability of the microemulsion under diverse field conditions, particularly regarding varying temperatures and UV exposure, warrants further investigation. While the study demonstrated efficacy, a detailed cost-benefit analysis comparing the economic viability of lemongrass microemulsions versus synthetic fungicides for large-scale agricultural application was not conducted. Furthermore, the impact of repeated applications of lemongrass oil microemulsion on soil microbial communities and non-target

organisms was not assessed. Future research should focus on optimizing the microemulsion formulation to further enhance its stability and efficacy, potentially through the use of different co-surfactants or encapsulation techniques. Exploring synergistic combinations of lemongrass oil with other natural compounds or biocontrol agents could lead to improved disease control. Additionally, studies on the residual effects of lemongrass microemulsion on potato tubers and the overall impact on yield and quality under various environmental conditions would be beneficial. Finally, conducting comprehensive economic analyses and assessing the environmental footprint over the entire product lifecycle would provide a more complete picture of its sustainability and practical utility in integrated disease management programs.

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