SCIENTIFIC NOTE

Antifungal effect of limonene against different pathogenic *Fusarium* species

Efecto antifúngico del limoneno sobre diferentes especies patógenas de *Fusarium*

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ABSTRACT

Alternative control methods of fungal diseases have been studied with an emphasis on finding new compounds derived from plants, such as essential oils and extracts, which are considered safer for consumers and the environment. Limonene, a cyclic monoterpene widely found in nature, is the main component of essential oils obtained from the peels of citrus fruits such as grapefruit, lemon, lime, and particularly orange. Despite its prevalence and its use as an antifungal agent, especially against fungi that cause diseases in major crops worldwide, studies on its application in greenhouse assays have been limited. The aim of this research was to evaluate the antifungal activity of limonene against cereal-pathogenic *Fusarium* species and to assess its effectiveness in controlling Fusarium head blight through plant bioassays. Limonene inhibited mycelial growth *in vitro* for all tested species, showing effective fungistatic action on pathogens. Regarding plant bioassays, the most significant effect was observed when limonene was applied simultaneously with and after the pathogen, indicating that limonene is not acting as a defense-inducing agent in the plant but directly on the pathogen. When limonene was applied before the pathogen, no significant inhibition of incidence was detected. Further studies are necessary to explore the use of limonene in controlling Fusarium head blight in major crops such as *Triticum aestivum* L. This study presents promising results for controlling this disease using limonene.

Keywords: pathogen control, essential oil, Fusarium head blight, wheat.

RESUMEN

Se han estudiado métodos alternativos para el control de enfermedades, con énfasis en la búsqueda de nuevos compuestos derivados de plantas, como aceites esenciales y extractos, que se consideran más seguros para los consumidores y el medio ambiente. El limoneno, un monoterpeno cíclico ampliamente encontrado en la naturaleza, es el principal componente de los aceites esenciales obtenidos de las cáscaras de frutas cítricas como pomelo, limón, lima y, en particular, naranja. A pesar de su prevalencia, su uso como antifúngico, especialmente contra hongos que causan enfermedades en cultivos extensivos a nivel mundial, y los estudios sobre su aplicación en ensayos bajo invernadero han sido poco explorados. El objetivo de este trabajo fue evaluar la actividad antifúngica del limoneno contra especies de *Fusarium* patógenas de cereales y evaluar su eficacia mediante bioensayos en plantas. El limoneno inhibió el crecimiento micelial *in vitro* en todas las especies analizadas, mostrando su eficaz acción fungistática sobre los patógenos. El efecto más significativo se observó cuando el terpeno se aplicó simultáneamente con y después del patógeno. Esto indicaría que el limoneno no estaría ejerciendo una acción como agente inductor de defensa en la planta, sino que su acción es directa sobre el patógeno, dado que en el tratamiento en el que se aplicó limoneno antes del patógeno, no se detectó una inhibición significativa en su incidencia. Se requieren más estudios para explorar el uso del limoneno en el control de la fusariosis de la espiga en cultivos extensivos como *Triticum aestivum* L. Este trabajo presenta resultados prometedores para el control de esta enfermedad mediante el uso de limoneno.

Palabras clave: control de patógenos, aceite esencial, fusariosis de la espiga, trigo.

Introduction

The control of fungal diseases has been based for years on the use of phytosanitary products. However, alternative methods for disease control have been studied with an emphasis on finding new compounds derived from plants, such as essential oils and extracts, which are considered safer for consumers and the environment (Zaker, 2016). Most plant essential oils are chemically complex in their composition, which improves their efficacy due to synergy among their compounds (Dhifi *et al*., 2016). Terpenes and their derivatives are the main components of essential oils and are of great importance due to their application in the agricultural, food, cosmetic, and pharmaceutical

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industries. Agronomic applications include their potential use as antifungals (Marei *et al*., 2012; Pawar & Thaker, 2007), insecticides (Huang *et al*., 2002), and nematicides (Duschatzky *et al*., 2004). Their action as fungicides involves reducing mycelial growth (by modifying the structure of cell membranes), inhibiting spore germination, and affecting the enzymatic activity of fungi (Marei *et al*., 2012; Zhou *et al*., 2014). There are also many other compounds applied as elicitors in plants, which act as inducers and trigger an immune response, promoting long-term inhibition of the pathogen's action. The use of fungicides and elicitors can be carried out together at different times of the vegetative cycle of the crops, triggering a favorable additive response (Jones & Dangi, 2006; Martinez *et al*., 2024; Thakur & Sohal, 2012). Limonene is the most widely distributed cyclic monoterpene in nature and is the major component of essential oils obtained from the peel of citrus fruits such as grapefruit, lemon, lime, and mainly orange (Pérez Mosquera *et al*., 2015). It is extensively used to produce fragrances and beverages, as a flavor additive in food, and as a biotransformation substrate. Nevertheless, limonene has been little explored as an antifungal agent in plants, mainly against those species that cause diseases in major crops worldwide (Gupta *et al*., 2021; Ravichandran *et al*., 2018; Ünal *et al*., 2012). *Fusarium* is a genus of fungi with a broad distribution that includes the causal agent of Fusarium head blight (FHB), one of the most relevant diseases of cereals, in humid and semi-humid wheat-growing areas (Bai & Shaner, 1994; Wiese, 1987). The importance of this disease lies in its impact on yield and grain quality as well as the production of mycotoxins that remain stable in barley malt, wheat flour, and even final products, thus adversely affecting human and animal health (Champeil *et al*., 2004; Xu, 2003). Considering that the use of phytosanitary products poses risks to human health and contributes to environmental contamination, as well as the well-documented negative effects caused by *Fusarium* spp. through crop losses and production of mycotoxins in grains. The aim of this research was to evaluate *in vitro* the antifungal activity of limonene against cereal-pathogenic *Fusarium* species as well as its effectiveness in controlling FHB in plant bioassays.

Materials and methods

In vitro antifungal activity

A mycelial growth inhibition test was performed for seven pathogenic species of *Fusarium*.: *F. avenaceum* (A3; MH362768), *F. cerealis* (Herrero 5; Castañares *et al*., 2013), *F. culmorum* (FC115; Toth *et al*., 2004), *F. poae* (25/5; Nogueira *et al*., 2018), *F. graminearum sensu stricto* (Ass5; Castañares *et al*., 2016), *F. pseudograminearum* (LPSC 1154; Castañares *et al*., 2012), and *F. subglutinans* (1.1; MG857113).

The stock solution of limonene, (R) - (+)- limonene 97%, Sigma-Aldrich (#183164) was prepared with absolute EtOH and Tween-20 (5% v/v). Different volumes of this solution were incorporated into 25 ml of potato dextrose agar (PDA) culture medium at 40-45°C, in 9 cm diameter Petri dishes, and the following treatments were defined: T1: 350 µl, T2: 400 µl, and T3: 450 µl of limonene. The controls were: Control 1, the pathogen without limonene and with 1 ml EtOH (solvent used to solubilize limonene) and Control 2, without limonene and without EtOH (Tsao & Zhou, 2000). For each *Fusarium* species, a disc of mycelium from an actively growing colony (7 d in a growth chamber under 20+2°C with alternating light/darkness for 12 h 3,500 lx) was inoculated in the center of the plate, which was then sealed with Parafilm to prevent the evaporation of limonene. Plates were incubated in a culture oven at 20±2°C with alternating light/darkness for 12 h (3,500 lx). Each treatment was conducted in triplicate. Colony diameter was measured every 24 h until the control treatment reached the edge of the plate (4-7 d). The percentage of mycelial inhibition was calculated for each concentration using the following formula (Marei *et al*., 2012):

$$\% \, Inhibition = \left(\frac{DC - DT}{DC} \right) \times 100\% \quad (1)$$

where:

DC=diameter of the control, DT=diameter of the treatment. This assay determined the minimum inhibitory concentration (MIC) to be used in the next assay.

Greenhouse assay and experimental design

The assay was carried out in the greenhouse of the Faculty of Agronomy (36°41'00" S, 59°48'00" W) at the National University of Central Buenos Aires Province (Argentina). The average temperature of the greenhouse was recorded every 24 h.

The experimental design was completely randomized, with three replicates per treatment. The test with *F. poae* was carried out using the minimum concentration of limonene that had demonstrated the greatest inhibitory effect *in vitro* (Li *et al*., 2015). The treatments were: T1, the limonene solution and the pathogens applied at the same time; T2, the limonene solution applied 24 h before the inoculation of the pathogens; T3, the limonene solution applied 24 h after inoculation of the pathogens. The controls were: C0,

the pathogen inoculated with a solution without limonene and with ethanol; C1, pathogen inoculated with a solution without limonene and without Ethanol (only water); C2, only the pathogen inoculated and C3, only the limonene solution applied.

For treatments, in each replicate, 10 seeds of *Triticum aestivum* L. (hexaploid wheat) Apogee variety, reported as susceptible to FHB (Li *et al*., 2017), were sown in 20 L pots, containing clay loam soil without prior sterilization obtained from the farm of the Faculty of Agronomy of the National University of Central Buenos Aires Province. An aliquot of 350 µl of stock solution (MIC) of limonene (50% v/v), prepared with absolute ethanol and 5% Tween-20 (v/v) in 25 ml of sterile distilled water, was used. Inoculation of the pathogen and application of the monoterpene solution were done in Zadoks 6.0 (Zadoks *et al*., 1974) until dripping using sprinklers. They were left for 48 h with polyethylene bags to achieve a humid chamber effect. Humidity was maintained by irrigating twice a day and the fertilizer conditions were set according to Dinolfo *et al*. (2022). Symptom assessment was conducted by evaluating the severity percentage (number of symptomatic spikelets per spike) 21 d after inoculation (Martinez *et al*., 2020).

Inoculum production

Fusarium poae was grown in Petri dishes with PDA 2%. Cultures were incubated for 2 weeks in a culture oven at 20±2°C with alternating light/darkness for 12 h (3,500 lx). To obtain the spore suspension, 15 ml of sterile Tween 20® solution (0.85% NaCl, 0.1% Tween 20 in water) was added to each Petri dish, the mycelium was scraped with a previously flamed slide or scalpel, and filtered through sterile gauze. The resulting suspension was adjusted to $1x10^5$ conidia/ml using a Neubauer hemocytometer under a binocular optical microscope (Brennan *et al*., 2007; Dinolfo *et al*., 2022).

Statistical analysis

Mycelial inhibition (%) was evaluated using software R v.4.2.1 (R Core Team, 2022). A statistical analysis was carried out using a mixed model with repeated measures (species). Results are reported as the mean ± standard error of the mean (SEM) at a significance level of $\alpha = 0.05$.

To evaluate the antifungal efficacy of the treatment across different *Fusarium* species, a partitioned linear regression analysis was performed using Infostat software (Di Rienzo *et al*., 2015). Mycelial inhibition (%) was used as the dependent variable, while treatment concentration (in µl) served as the continuous explanatory variable. The model was stratified by species to account for interspecific variation in sensitivity. This approach allowed for the empirical estimation of species-specific dose–response trends and the identification of the minimum inhibitory concentration (MIC) associated with a 50% reduction in mycelial growth (MIC_{50}). The analysis provided insight into the differential effectiveness of the treatment across the *Fusarium* complex and facilitated quantitative comparisons among species (Li, 2015; Marei *et al*., 2012).

Disease severity (%) was evaluated using software R v.4.2.1 (R Core Team, 2022). The main factor was limonene treatment (L) nested within *F. poae* (F) treatments with two levels (presence or absence), with three blocks for each combination of treatments. Due to non-normal errors, comparisons were performed using a generalized linear mixed model (GLMM). The function glmer from the lme4 package was used for variance analysis (Bates *et al*., 2015). Data assumptions were verified graphically using plots of fitted values versus residuals for homogeneity of variances. Normal Q-Q plots and the Shapiro-Wilk test were used to check the normality of the residuals. Furthermore, fitted values versus residuals were graphically verified using plots to check variance homogeneity. The lsmeans function was used to test significant effects (emmeans package) (Lenth, 2018). The results are reported as the mean ± standard error of the mean (SEM), using a significance level of $\alpha = 0.05$.

Results

Inhibition of mycelial growth

The treatments showed a significant effect on the fungal growth of all pathogenic species of crops tested. Furthermore, significant growth was observed across the measurement days, with no interaction between the two variables (treatments and measurement days) (Tab. 1). All treatments significantly inhibited the mycelial growth of *Fusarium* species compared to the controls; however, no significant differences were observed among the treatments themselves (Tab. 2). Linear regression analyses were performed separately for each *Fusarium* species to assess the relationship between treatment concentration and the percentage of mycelial inhibition. The species that achieved at least 50% inhibition (using 350 µl of limonene in the Petri dishes) were *F. avenaceum, F. culmorum, F. cerealis, F. pseudograminearum, F. subglutinans, F. poae and F. graminearum*. However, only *F. avenaceum*, and *F. culmorum* showed significant difference at higher concentrations of limonene (Tab. 3). The minimum inhibitory concentration (MIC_{50}) was determined to be 350 µl of limonene solution.

TABLE 1. Analysis of the effect of limonene application on fungal mycelial growth of *Fusarium* spp. through time.

Source of variation	Df.	*P-value*	
Treatments	8	$<2^{-16}$	***
Day-measurement	1	$<2^{-16}$	***
Treatment x day-measurement	8	0.4986	n.s.

Treatments: T1: 350 µl limonene, T2: 400 µl limonene, T3: 450 µl limonene, C1: the pathogen without organic compound and with 1 ml EtOH (solvent used to solubilize limonene), C2: without organic compound and without EtOH. Day of measurement: every 24 h until the control treatment reached the margin of the Petri dish (4-7 d). Statistical analyses were performed using a mixed model with repeated measures (species). df: degrees of freedom ***significant differences, ns: not significant at α-value$\leq$ 0.05.

TABLE 2. Effect of treatments with limonene on the inhibition of mycelial growth of *Fusarium* species.

Treatments	Means
T1 350 µl limonene	1.717146 a
T2 400 µl limonene	1.693551 a
T3 450 µl limonene	1.850651 a
C2-T1	2.903072 b
C2-T2	3.013036 bc
C2-T3	3.178050 bc
C1-T1	3.407806 c
C1-T2	3.421662 c
C1-T3	3.444731 c

Mean ± SEM comparisons were performed using a mixed model with repeated measures (species). Same letters are not significant at $P\leq$ 0.05. Treatments: T1: 350 µl limonene, T2: 400 µl limonene, T3: 450 µl limonene, C1: the pathogen without limonene and with 1 ml EtOH (solvent used to solubilize limonene), C2: without limonene and without EtOH. Day of measurement: every 24 h until the control treatment reached the margin of the Petri dish (4-7 d). Same letters are not significant at $P\leq$ 0.05.

TABLE 3. Summary of regression coefficients, and *P-values* for each *Fusarium* species.

Species	Treatment Coeff.	*P-value*
F. avenaceum	-0.28	0.002
F. cerealis	-0.07	0.2896
F. culmorum	0.22	0.0243
F. graminearum	0.19	0.1206
F. poae	0.12	0.1061
F. pseudograminearum	0.1	0.3248
F. subglutinans	0.18	0.3024

Statistical analyses were performed using partitioned linear regression. Significant *P-values* at $P<0.05$.

Greenhouse assay

The greenhouse assay carried out with wheat cultivar *T. aestivum* L. var. Apogee in pots in the greenhouse showed significant effects on the severity of *F. poae* and the antifungal action of limonene. Significant interaction between *F. poae* and limonene was observed for treatments T1 and T3, but not for treatment T2 (Tab. 4).

TABLE 4. Analysis of variance to detect the effect of limonene on *Fusarium* severity (%) under greenhouse conditions.

T1			
Source of variation	**Chisq.**	**Df.**	**Pr (>Chisq.)**
F. poae (F)	42.6444	1	6.5650e-11*
Limonene (L)	12.8992	1	0.0003*
F x L	9.6913	1	0.0018*
T2			
Source of variation	**Chisq.**	**Df.**	**Pr (>Chisq.)**
F. poae (F)	88.7826	1	<2e-16*
Limonene (L)	0.8796	1	0.3483
F x L	0.0296	1	0.8634
T3			
Source of variation	**Chisq.**	**Df.**	**Pr (>Chisq.)**
F. poae (F)	41.341	1	1.278e-10*
Limonene (L)	11.877	1	0.0005*
F x L	10.283	1	0.0013*

Treatments. T1: *F. poae* and limonene applied at the same time. T2: Limonene applied 24 h before *F. poae*. T3: *F. poae* applied 24 h before treatment with limonene. Chisq: Wald type II Chi-square tests. Df: degrees of freedom*.

The severity of the disease caused by *F. poae* on wheat showed significant differences in T2 with respect to C0, C1, C3, and T1 and T3. Treatment T2 exhibited 60% pathogen severity, whereas C0, C1, and C3 showed values below 15% (7%, 12%, and 12%, respectively). Treatments T1 and T3 reflected values of 20% severity and had significant differences compared to C2, which had 60% severity (Figs. 1A-E).

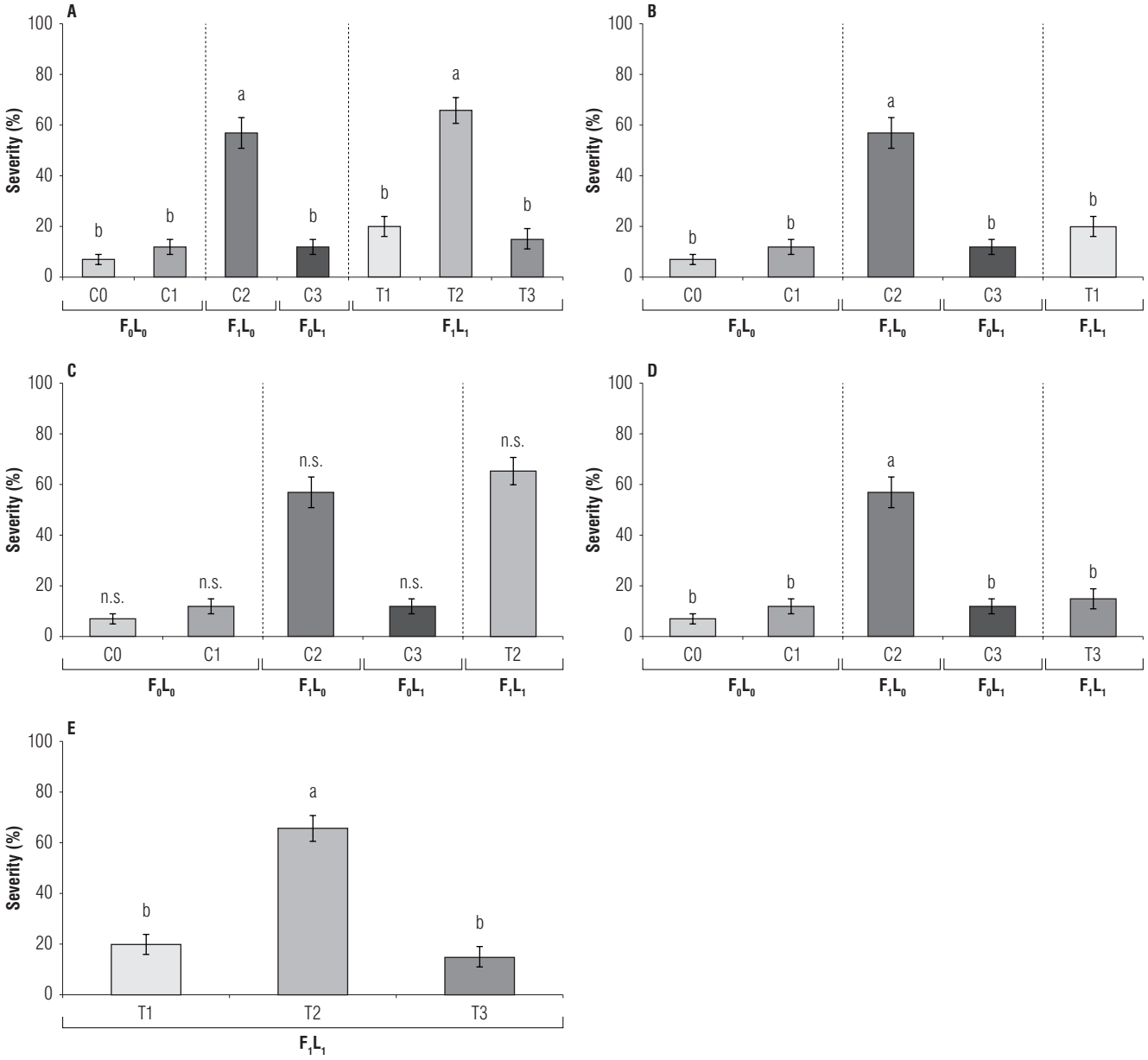

FIGURE 1. Disease severity (%) under greenhouse conditions. A) Disease severity (%) registered after different treatments on wheat spikes (cv. Apogee), B) comparison between the time of application of *F. poae* and limonene treatments (T1, T2, and T3). Treatments: T1, *F. poae* and limonene applied at the same time; T2, Limonene applied 24 h before *F. poae*; T3, *F. poae* applied 24 h before treatment with limonene. C) Comparison of T1, T2 (D), and T3 (E) regarding control treatments (C0-C1), *F. poae* treatment (C2), and limonene treatment (C3), respectively. Controls: C0, the pathogen inoculated with a solution without limonene and with ethanol; C1, the pathogen inoculated with a solution without limonene and without ethanol (only water); C2, only the pathogen inoculated; and C3, only the limonene solution applied. Values are means ± SEM, comparisons were performed using Tukey's test. Data with the same letters are not significantly different at $P \leq 0.05$.

Discussion

Cereal production worldwide is widely influenced by FHB. There are various strategies associated with its management, among which are agronomic practices that aim to reduce the concentration of the inoculum in the system. These include zero tillage system with crops that are not hosts of the pathogen (Shude *et al*., 2020), the use of wheat varieties that are less susceptible and the application of fungicides, primarily from the triazole group, which provide incomplete control of the fungus (Tini *et al*., 2020). Currently, essential oils are considered an alternative to chemically synthesized fungicides, as they are environmentally friendly and exhibit low toxicity (Taheri *et al*., 2023).

In this work, we used limonene, a well-studied terpene and the main component of essential oils derived from citrus fruits, which exerted *in vitro* inhibition of the mycelial growth of all *Fusarium* species tested, showing its effective fungistatic action on pathogens. The results obtained are consistent with findings of Achimón *et al*. (2022), who used essential oils from lemon, orange, and grapefruit peels, with limonene being the component responsible for the antifungal activity against *Rhizoctonia solani* and *Sclerotium rolfsii*. Similarly, Guédez *et al*. (2014) showed that the action of orange essential oil (*Citrus sinensis* L.) had inhibitory effects greater than 80% on postharvest fungi of *Carica papaya*. Recently, Jian *et al*. (2023) showed that the limonene formulation known as Wetcit® is a promising alternative to synthetic fungicides to control *F. graminearum* growth and deoxynivalenol (DON) production.

Few studies have been carried out using essential oils on extensive crops to control fungal pathogens, and there are no records of studies on the action of *F. poae*. The severity of *F. poae* on *T. aestivum* cultivar Apogee was decreased by 80% due to the action of limonene. Similar results were reported by Jian *et al*. (2023), who showed the effective action of limonene against *F. graminearum in vitro*. Likewise, Perczak *et al*. (2019) showed the antifungal activity of essential oils (derived from different plant materials used for cosmetic and research purposes), against *F. graminearum* and *F. culmorum,* and they were effective in reducing the production of toxins in seeds. The effect observed was mainly noted in treatments in which limonene was applied simultaneously with and after the pathogen.

Conclusion

Results presented here indicate that limonene was an effective antifungal against *Fusarium* sp in in vitro assays. Additionally, limonene showed potential to control FHB caused by *F. poae* in wheat plants. Further studies are necessary to explore the use of limonene in controlling FHB in extensive crops such as *Triticum aestivum* L. Nevertheless, this work presents promising results for the control of this disease using limonene or essential oils rich in limonene.

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Conflict of interest statement

The authors declare that there is no conflict of interests regarding the publication of this article.

Author's contributions

SLB designed the experiments, performed statistical analysis, data interpretation, and writing. CM developed bioassays and assays, and data interpretation. FSF and CM did data interpretation. MVM designed the experiments and participated in data interpretation. All authors reviewed the final version of the manuscript.

Literature cited

Achimón, F., Leal, L. E., Pizzolitto, R. P., Brito, V. D., Alarcón, R., Omarini, A. B., & Zygadlo, J. A. (2022). Insecticidal and antifungal effects of lemon, orange, and grapefruit peel essential oils from Argentina. *AgriScientia, 39*, 71–82. https://doi.org/10.31047/1668.298x.v39.n1.33777

Bai, G., & Shaner, G. (1994). Scab of wheat: Prospects for control. *Plant Disease, 78*(8), 760–766. https://doi.org/10.1094/PD-78-0760

Bates, D., Mächler, M., Bolker, B., & Walker, S. (2015). Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*, *67*(1), 1–48. https://doi.org/10.18637/jss.v067.i01

Brennan, J. M., Leonard, G., Fagan, B., Cooke, B. M., Ritieni, A., Ferracane, R., Nicholson, P., Simpson, D., Thomsett, M., & Doohan, F. M. (2007). Comparison of commercial European wheat cultivars to *Fusarium* infection of head and seedling tissue. *Plant Pathology*, *56*(1), 55–64. https://doi.org/10.1111/j.1365-3059.2006.01458.x

Castañares, E., Dinolfo, M. I., Moreno, M. V., Berón, C., & Stenglein, S. A. (2013). *Fusarium cerealis* associated with barley seeds in Argentina. *Journal of Phytopathology, 161*(7-8), 586–589. https://doi.org/10.1111/jph.12097

Castañares, E., Dinolfo, M. I., Ponte, E. M., Pand, D., & Stenglein, S. A. (2016). Species composition and genetic structure of *Fusarium graminearum* species complex populations affecting the main barley growing regions of South America. *Plant Pathology*, *65*(6), 930–939. https://doi.org/10.1111/ppa.12470

Castañares, E., Wehrhahne, L., & Stenglein, S. A. (2012). *Fusarium pseudograminearum* associated with barley kernels in Argentina. *Plant Disease, 96*(5), 763–763. https://doi.org/10.1094/PDIS-01-12-0050-PDN

Champeil, A., Fourbel, J. F., Doré, T., & Rossignol, L. (2004). Influence of cropping system on *Fusarium* head blight and mycotoxin levels in winter wheat. *Crop Protection*, *23*(6), 531–537. https://doi.org/10.1016/j.cropro.2003.10.011

Dhifi, W., Bellili, S., Jazi, S., Bahloul, N., & Mnif, W. (2016). Essential oils' chemical characterization and investigation of some biological activities: A critical review. *Medicines*, *3(4)*, Article 25. https://doi.org/10.3390/medicines3040025

Dinolfo, M. I., Martínez, M., Castañares, E., Vanzetti, L. S., Rossi F., Stenglein S. A., & Arata A. F. (2022). Interaction

of methyl-jasmonate and *Fusarium poae* in bread wheat. *Fungal Biology*, *126*(11-12), 786–792. https://doi.org/10.1016/j.funbio.2022.10.002

Di Rienzo J. A., Casanoves, F., Balzarini, M. G., Gonzalez, L., Tablada, M., & Robledo, C. W. (2015). Grupo Infostat, FCA, Universidad Nacional de Río Cuarto. https://www.infostat.com.ar

Duschatzky, C. B., Martinez, A. N., Almeida, N. V., & Bonivardo, S. L. (2004). Nematicidal activity of the essential oils of several Argentina plants against the root-knot nematode. *Journal of Essential Oil Research, 16*(6), 626–628. https://doi.org/10.1080/10412905.2004.9698812

Guédez, C., Cañizalez, L., Avendaño, L., Scorza, J., Castillo, C., Olivar, R., Méndez, Y., & Sánchez, L. (2014). Actividad antifúngica del aceite esencial de naranja (*Citrus sinensis* L.) sobre hongos postcosecha en frutos de lechosa (*Carica papaya* L.). *Revista de la Sociedad Venezolana de Microbiología*, *34*, 82–86. http://saber.ucv.ve/ojs/index.php/rev_vm/article/view/7998

Gupta, A., Jeyakumar, E., & Lawrence, R. (2021). Journey of limonene as an antimicrobial agent. *Journal of Pure and Applied Microbiology, 15*(3), 1094–1110. https://doi.org/10.22207/JPAM.15.3.01

Hartmans, K. J., Diepenhorst, P., Bakker, W., & Gorris, L. G. M. (1995). The use of carvone in agriculture: Sprout suppression of potatoes and antifungal activity against potato tuber and other plant diseases. *Industrial Crops and Products*, *4*(1), 3–13. https://doi.org/10.1016/0926-6690(95)00005-W

Huang, Y., Ho, S. H., Lee H. C., & Yap, Y. L. (2002). Insecticidal properties of eugenol, isoeugenol and methyleugenol and their effects on nutrition of *Sitophilus zeamais* Motsch. (Coleoptera: Curculionidae) and *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae). *Journal of Stored Products Research*, *38*(5), 403–412. https://doi.org/10.1016/S0022-474X(01)00042-X

Jian, Y., Chen, X., Ma, H., Zhang, C., Luo, Y., Jiang, J., & Yin, Y. (2023). Limonene formulation exhibited potential application in the control of mycelial growth and deoxynivalenol production in *Fusarium graminearum*. *Frontiers in Microbiology, 14*, Article 1161244. https://doi.org/10.3389/fmicb.2023.1161244

Jones, J. D. G., & Dangl, J. L. (2006). The plant immune system. *Nature*, *444*, 323–329. https://doi.org/10.1038/nature05286

Lenth, R. V. (2018). Emmeans: Estimated marginal means, a.k.a. least-squares means, R package version 1.3.0. https://CRAN.R-project.org/package=emmeans

Li, G., Boontung, R., Powers, C., Belamkar, V., Huang, T., Miao, F., Baenziger, P. S., & Yan, L. (2017). Genetic basis of the very short life cycle of 'Apogee' wheat. *BMC Genomics*, *1*8, Article 839. https://doi.org/10.1186/s12864-017-4239-8

Li, J. L., Liu, X. Y., Xie, J. T., Di, Y. L., & Zhu, F. X. (2015). A comparison of different estimation methods for fungicide EC50 and EC95 values. *Journal of Phytopathology*, *163*, 239–244. https://doi.org/10.1111/jph.12312

Marei, G. I. K., Rasoul, M. A. A., & Abdelgaleil, S. A. M. (2012). Comparative antifungal activities and biochemical effects of monoterpenes on plant pathogenic fungi. *Pesticide Biochemistry and Physiology, 103*(1), 56–61. https://doi.org/10.1016/j.pestbp.2012.03.004

Martínez, M., Arata, A., Dinolfo, M. I., Lázaro, L., Welin, B., & Stenglein, S. (2024). Evaluation of PSP1 biostimulant on *Fusarium graminearum*-wheat pathosystem: Impact on disease parameters, grain yield, and grain quality. *Pest Management Science*, *80*(7), 3578–3589. https://doi.org/10.1002/ps.8062

Nogueira, M. S., Decundo, J., Martinez, M., Dieguez, S. N., Moreyra, F., Moreno, M. V., & Stenglein, S. A. (2018). Natural contamination with mycotoxins produced by *Fusarium graminearum* and *Fusarium poae* in malting barley in Argentina. *Toxins, 10*(2), Article 78. https://doi.org/10.3390/toxins10020078

Pawar, V. C., & Thaker, V. S. (2007). Evaluation of the anti-*Fusarium oxysporum* f. sp. *cicer* and anti-*Alternaria porri* effects of some essential oils. *World Journal of Microbiology and Biotechnology*, *23*, 1099–1106. https://doi.org/10.1007/s11274-006-9339-6

Perczak, A., Gwiazdowska, D., Marchwińska, K., Juś, K., Gwiazdowski, R., & Waśkiewicz, A. (2019). Antifungal activity of selected essential oils against *Fusarium culmorum* and *F. graminearum* and their secondary metabolites in wheat seeds. *Archives of Microbiology, 201*, 1085–1097. https://doi.org/10.1007/s00203-019-01673-5

Pérez-Mosqueda, L. M., Trujillo-Cayado, L. A., Carrillo, F., Ramirez, P., & Muñoz, J. (2015). Formulation and optimization by experimental design of eco-friendly emulsions based on d-limonene. *Colloids and Surface B: Biointerface. 128*, 127–131. https://doi.org/10.1016/j.colsurfb.2015.02.030

Ravichandran, C., Badgujar, P. C., Gundev, P., & Upadhyay, A. (2018). Review of toxicological assessment of *d*-limonene, a food and cosmetics additive. *Food and Chemical Toxicology*, *120*, 668–680. https://doi.org/10.1016/j.fct.2018.07.052

R Core Team. (2022). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org

Shude, S. P. N., Yobo, K. S., & Mbili, N. C. (2020). Progress in the management of Fusarium head blight of wheat: An overview. *South African Journal of Science*, *116*(11/12), 1–7. https://doi.org/10.17159/sajs.2020/7854

Taheri, P., Soweizy, M., & Tarighi, S. (2023). Application of essential oils to control some important fungi and bacteria pathogenic on cereals. *Journal of Natural Pesticide Research, 6*, Article 100052. https://doi.org/10.1016/j.napere.2023.100052

Thakur, M., & Sohal, B. S. (2012). Role of elicitors in inducing resistance in plants against pathogen infection: A review. *Biochemistry, 2013*, Article 762412. https://doi.org/10.1155/2013/762412

Tini, F., Beccari, G., Onofri, A., Ciavatta, E., Gardiner, D. M., & Covarelli, L. (2020). Fungicides may have differential efficacies towards the main causal agents of Fusarium head blight of wheat. *Pest Management Science, 76*(11), 3738–3748. https://doi.org/10.1002/ps.5923

Tsao, R., & Zhou, T. (2000). Antifungal activity of monoterpenoids against postharvest pathogens *Botrytis cinerea* and *Monilinia fructicola*. *Journal of Essential Oil Research*, *12*(1), 113–121. https://doi.org/10.1080/10412905.2000.9712057

Ünal, M. U., Ücan, F., Şener, A., & Dinçer, S., (2012). Research on antifungal and inhibitory effects of DL-limonene on some yeasts. *Turkish Journal of Agriculture and Forestry, 36*(5), 576–582. https://doi.org/10.3906/tar-1104-41

Wiese, M. V. (1987). *Compendium of wheat diseases* (2nd ed.). The American Phytopathological Society Press, St Paul. https://archive.org/search.php?query=external-identifier%3A%22urn%3Aoclc%3Arecord%3A742800815%22

Xu, X. (2003). Effects of environmental conditions on the development of *Fusarium* ear blight. *European Journal of Plant Pathology, 109*, 683–689. https://doi.org/10.1023/A:1026022223359

Zadoks, J. C., Chang, T. T., & Konzak, C. F. (1974). A decimal code for the growth stages of cereals. *Weed Research*, *14*(6), 415–421. https://doi.org/10.1111/j.1365-3180.1974.tb01084.x

Zaker, M. (2016). Natural plant products as eco-friendly fungicides for plant diseases control - a review. *The Agriculturists, 14*(1), 134–141. https://doi.org/10.3329/agric.v14i1.29111

Zhou, H., Tao, N., & Jia, L. (2014). Antifungal activity of citral, octanal and α-terpineol against *Geotrichum citri-aurantii*. *Food Control, 37*, 277–283. https://doi.org/10.1016/j.foodcont.2013.09.057