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IN VITRO ANTIMICROBIAL ACTIVITY OF DIFFERENT ESSENTIAL OILS FROM *Lavandula* sp.

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Abstract

The objective of this work was to highlight the antimicrobial properties of lavender essential oil (EO) obtained from the George 90 cultivar, which is the newest lavender cultivar approved in Romania for oil production. In order to highlight its antimicrobial properties, the essential oil obtained from the George 90 cultivar was tested in comparison with two other essential oils (EOs) obtained from the species Lavandula latifolia and Lavandula angustifolia, these two lavender species being the parents of the new George 90 cultivar. Three food pathogenic fungi (Aspergillus brasieliensis ATCC 16404*, Fusarium oxysporum and Penicillium expansum), two food spoilage bacteria (Bacillus cereus and Bacillus subtilis) and six species of phytopathogenic molds (Alternaria sp., Botrytis cinerea, Fusarium culmorum FC46, Fusarium oxysporum f. sp. radicis lycopersici ZUM2407 (FORL), Macrophomina phaseolina and Sclerotinia sclerotiorum) were used in this study. In all the interactions with phytopathogenic agents and bacteria studied, a close relationship was observed between the antifungal potential and the dose of essential oils tested. Thus, as expected, increasing the dose of lavender essential oil is directly proportional to the inhibition effectiveness of the tested microorganisms.*

Key words*: antimicrobial properties, essential oil, new lavender cultivar.*

INTRODUCTION

Lavender is part of the Laminaceae family, Lamiales order, it originates in SW-SE Europe, being a thermophilic subshrub that grows in the form of a 40-1.60 cm tall bush, with a diameter of 100-150 cm. The leaves are persistent, linear, 2-6 cm long and have a silver-gray color in the cold weather and silver-green in the hot weather. In the months of May-June-July, depending on the climate, it emits flower stalks of 40-60 cm, finished with spikes of bluish-violet flowers of ± 8-10 cm, very pleasantly scented.

The essential oils obtained from *Lavandula* × *intermedia*, which result from natural crosses between *Lavandula latifolia* and *Lavandula angustifolia* species, contain a mixture of monoterpenes present in both parental lines, and are mainly utilized in personal care and hygiene products including soaps, shampoos, mouth washes, and industrial and household cleaners, among others (Sarker et al., 2012).

Essential oil obtained from *L.* × *intermedia* has been widely investigated and is known to vary in composition (Balajan and Pirbalouti, 2015), but according to our knowledge, no documented reports on variation of chemical composition of the essential oils from different populations of *L.* × *intermedia* leaves and flower cultivated in Romania are available.

The George 90 cultivar comes from a foreign lavender variety, which was acclimatized in Romania, being registered in 2017 in the official Catalog of the State Institute for the Testing and Registration of Varieties, within the Ministry of Agriculture and Rural Development in Romania. Regarding the phenological observations and biometric determinations specific to the lavender culture, the characteristics of the cultivar George 90 are highlighted in comparison with *L. latifolia* and *L. angustifolia,* these two lavender species being considered its parents. The George 90 lavender cultivar presents the following characteristics, which result from the technical examination report:

- plant growth type is stretched;
- the plant size is very large;
- the intensity of the green color of the foliage is medium;

- the gray intensity of the foliage is low;
- the bearing of the external flower stems, at full bloom, is spread out;
- density at full bloom is medium;
- the length of the flowering stalk, including the spike is very long;
- intensity of the green color of the stalk is medium;
- side branches, above the foliage, at the stem level are present;
- the number of side branches, at the stem level is small;
- the length of the largest branch, including the spike is very long;
- the spike has a cylindrical shape, with a large number of flowers and vertices, many flowers on the terminal verticil and a very long distance between vertices;
- regarding the flower, it can be observed that the calyx color is greenish, the calyx pubescence is strong represented and the corolla color is purple.

There are various studies that shows that lavender essential oils present antibacterial (Danh et al., 2012; Gismondi et al., 2021; Jianu et al., 2013; Man et al., 2019; Mesic et al., 2021; Valkova et al., 2021) and antifungal activity (Behmanesh et al., 2015; Císarová et al., 2016; Dhaouadi et al., 2018; Erdogan et al., 2016; Schroder et al., 2018; Zuzarte et al., 2012). For example, a study conducted by Ciocarlan et al. (2021) showed that *L. angustifolia* L. essential oil presented good antibacterial activity at 300 µg/mL concentration against *Erwinia carotovora*, *Bacillus subtilis*, *Xanthomonas campestris*, *Pseudomonas fluorescens*, and at 150 µg/mL concentration against *Candida utilis* and *Erwinia amylovora*. Another study conducted by Badr et al. (2021) determined the antimicrobial activity of *Lavandula spica* essential oil on different microorganisms (Gram-positive and Gram-negative bacteria, fungi and yeast), and the results showed a minimum inhibitory concentration (MIC, mg/L) of 3150 against *Salmonella typhimurium* and 3000 against *Staphylococcus aureus*. Regarding *Candida albicans*, lavender essential oil presented EC_{50} (mg/L) of 561.26. The *in vitro* antifungal activity was conducted using *Aspergillus flavus* and *Aspergillus niger* fungi and the results showed values of EC_{50} of 1265.38 and 1492.93 respectively (Badr et al., 2021). Jeddi et al. (2023) also studied the antimicrobial activity of *L. angustifolia* essential oil. Therefore, several microorganisms were tested (*Micrococcus luteus*, *Bacillus cereus*, *Salmonella enterica*, *S. aureus*, *Klebsiella aerogenes*, *Escherichia coli*, *Candida tropicalis* and *C. albicans*) and the results showed good activity of the tested essential oil, with inhibition zones varying between 19.1 to 13.4 for bacteria and between 24.10 and 19.05 for fungi. Essential oil extracted from *L. latifolia* L. was tested for its antimicrobial activity against five phytopathogenic fungi by Al-Ansari et al. (2021). The results showed that the essential oil presented inhibitory activity, with MIC (µg/mL) values of 2.5 for *A. flavus*, >10 for *Aspergillus nidulans*, 0.125 for *Trichophyton mentagrophytes*, 5 for *Leptosphaeria maculans*, 2.5 for *Rhizoctonia solani* and >10 for *Fusarium oxysporum*. Antimicrobial activity of essential oil obtained from flowers and leaves of *Lavandula officinalis* was determined by Martucci et al. (2015). The results of their study showed a MIC (µg/mL) of 2000 on *E. coli* and of 1000-1200 on *S. aureus*. Sumalan et al. (2020) determined the antifungal activity of lavender essential oil on *Penicillium digitatum*, with results showing a minimum fungicidal dose of 350 µl.

MATERIALS AND METHODS

Materials

The tested essential oils were obtained by hydrodistillation from *L. latifolia* (LL), *L. angustifolia* (LA) and *L.* × *intermedia* George 90 cultivar (GE).

Lavender EOs exhibits high volatility at ambient temperature as well as a good solubility in organic solvents and alcohol. EOs can also be entrained by water vapor despite the fact that they have low solubility in water. The lavender EOs used in this scientific work were transparent and had a strong smell and flavor. Two approaches have been used during the experiments. The studied essential oils were tested in terms of antimicrobial activity on both food pathogenic strains (*Aspergillus brasieliensis* ATCC 16404, *Fusarium oxysporum*, *Penicillium expansum*, *Bacillus subtilis*, *Bacillus cereus*) and phytopathogenic

strains (*Alternaria* sp., *Botrytis cinerea*, *Fusarium culmorum* FC46, *Fusarium oxysporum* f. sp. *radicis lycopersici* (FORL) ZUM2407, *Macrophomina phaseolina*, *Sclerotinia sclerotiorum*).

Methods

A GC–MS/MS TRIPLE QUAD Agilent 7890 A equipment (Santa Clara CA, USA) was used in the analysis of the essential oil's chemical composition according to the method presented by Schroder et al. (2022).

In order to determine the antifungal activity of the food pathogenic strains, the fungi were cultivated on Potato Dextrose Agar (purchased from Scharlau) for 7 days at 25°C, and then they were used in the experiments as a form of spore suspension, which was obtained in aseptic conditions, having a concentration of 10^6 spores/ml. The working method used was the disc diffusion method. Briefly, on solidified culture media, filter paper discs (Φ = 6 mm) were placed and essential oil was distributed in quantities varying between 5 and 10 µl/disc in order to achieve the specific testing quantity per plate (20, 25, 30, 35, 40, 45 µl). The plate was then inoculated in the center with 2 µl of spore suspension. The plates thus obtained were incubated at 25°C for 7 days, and the colony growth determination was realized by measuring the colony diameters at the end of the incubation period. The degree of inhibition was expressed as a percentage and calculated using the formula:

$$Inhibition\ degree, \% = \frac{A_c - A_i}{A_c} \times 100$$

where A_c represents the average for Control colonies and A_i represents the average for sample colonies.

For antibacterial activity determination, the used bacterial strains were cultivated on Nutrient Agar (purchased from Scharlau) culture media for 24 h at 35°C and then used. Briefly, on the solidified culture media 100 µl of bacterial suspension was distributed using a Drigalski spatula, and the plates were left at rest for 30 minutes to facilitate the microorganism incorporation. Filter paper discs (Φ = 6 mm) were then applied, and the essential oil quantity was distributed equally on the discs, varying between 2.5 and 10 µl/disc, the function of the necessary quantity for testing. The plates were then incubated for 24h at 35ºC, and the antibacterial activity was evaluated by assessment of the zone of inhibition in the Petri dish.

The lavender EOs were also analyzed for their antifungal activity against phytopathogenic fungi. Potato-Glucose-Agar medium (purchased from Roth) was used, for refreshing the phytopathogenic cultures and evaluation of lavender oils' antifungal activity. For the antifungal evaluation, test plates were prepared by inoculating the fungi one cm away from the edge of the Petri, using mycelial plugs of 6 mm in diameter. Diametrically opposite, 1 cm away from the edge of the Petri plates, one well was made in each plate, using a sterile corkborer, in which different doses (20, 30, 40 µl) of lavender EO were introduced. Control plates were similarly prepared, for each phytopathogenic fungi. However, no EOs were added in the wells of the control plates. Both test and control plates were incubated at 25°C. At different incubation times, after 5, 7, and 12 days respectively, biometric measurements were made to determine the mycelial growth in the test plates, and control cultures. The antifungal activity of the EOs was quantified as efficacy (E%) to inhibit or limit the growth of the studied phytopathogens. The following formula was used for the calculation:

$$E\% = \frac{R_C - R_I}{R_C} \times 100$$

Where R_C represents the radius of the mycelial colony in the Control plate, and R_I represents the radius of the mycelial colony, grown in the test plate, towards the essential oil.

RESULTS AND DISCUSSIONS

Morphological characteristics

Lavender is a thermophilic species, sensitive to excess water, therefore demanding of heat, loving direct sunlight, warm, stony, and calcareous soils but rich in macroelements. In our country, thanks to the soils rich in macro-microelements and minerals, lavender has adapted perfectly even to the frosty weather conditions in winter.

Following the phenological observations and biometric determinations, the characteristics of the cultivar George 90 were highlighted in comparison with its parents, namely *L. latifolia* and *L. angustifolia,* and the results are shown in Table 1, expressed as the average of the 5-year measurements (respectively 2017, 2018, 2019, 2020 and 2021).

Table 1. Characteristics of tested lavender varieties (average of 5 years)

Phenological characteristics determined	*Lavandula latifolia*	*Lavandula angustifolia*	George 90 lavender cultivar
Plant height (cm)	131	75	129
Inflorescence length (cm)	12.5	10	12
Flower stem length (cm)	69	43	70
The number of vertices on the floral spike (cm)	84	70	88
Plant diameter in the 5th year of cultivation (cm)	131	75	129
Variety/Production of inflorescences/ha (kg)	15730	6435	18590
Variety/oil content %/ ha	169.8	49	324.8

Lavender has an important ornamental interest, being very loved for the color and fragrance of the flowers, but at the same time appreciated for the persistence of the foliage. The ornamental lavender has many cultivars, which have special ornamental characteristics: richer flowering, flowers more colorful or with a different color than the type species - red, white, and dark blue. Figure 1 shows the appearance of a flowering lavender field from the George 90 lavender cultivar.

Figure 1. The appearance of flowering lavender field belonging to the George 90 cultivar

Chemical composition of the essential oils

Following a study on *L.* × *intermedia* var. George 90 essential oil that was obtained from a lavender plantation in Suceava county, Romania, the chemical composition and the proportion of their compounds were analyzed by Gas Chromatography-Mass Spectrometry (GC–MS), and the major identified compounds are shown in Table 2.

Table 2. Chemical composition of the essential oils

Major Components	*Lavandula angustifolia*	*Lavandula latifolia*	George 90 cultivar
Eucalyptol (%)	14.49	18.04	15.62
Camphor (%)	13.33	11.43	11.49
B-Linalool (%)	31.59	28.39	31.95
P-Menth-1-en-4-ol (%)	6.92	8.63	9.32

It can be observed that eucalyptol, camphor, and B-linalool are the three major components of the studied essential oils. The highest percent of eucalyptol was obtained from *L. latifolia* at 18.04%, while the highest content of camphor was obtained for *L. angustifolia* at 13.88 %. Shunying et al. (2005) studied the chemical composition and antimicrobial activity of the essential oils obtained from *Chrysanthemum indicum*, which showed greater bacteriostatic activity due to the higher percentage of camphor identified in the oil obtained from the processed flowers.

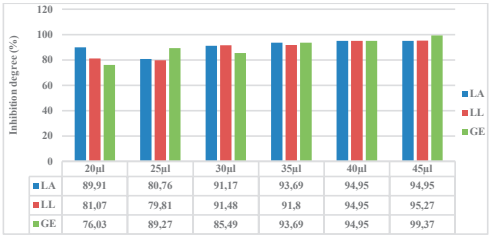
Linalool is an unsaturated monoterpene alcohol with the specific odor description; “light and refreshing, floral-woody, with a faint citrusy note” (Kamatou and Viljoen, 2008). The highest content in B-linalool was found George 90 cultivar with 31.95%. Linalool is also the principal component of many essential oils known to exhibit several biological activities such as antibacterial and antiplasmodial effects (van Zyl et al., 2006).

The p-Menth-1-en-4-ol, also known as terpinen-4-ol or 1-para-menthen-4-ol, belongs to the class of organic compounds known as menthane monoterpenoids is one of the major compounds identified in the studied lavender EOs, which is present in highest quantity in George 90 cultivar, respectively 9.32%.

Antifungal activity against food pathogenic fungi

The amounts of lavender oil tested showed an inhibitory effect on the growth of *Fusarium oxysporum* (Figure 2).

At a concentration of 20 µl of lavender oil/Petri plate, it is observed that all three oils inhibit the growth of the fungus, the strongest degree of inhibition being manifested by LA (89.91%), followed by LL (81.07%) and GE (76.03%).



	20µl	25µl	30µl	35µl	40µl	45µl
LA	89,91	80,76	91,17	93,69	94,95	94,95
LL	81,07	79,81	91,48	91,8	94,95	95,27
GE	76,03	89,27	85,49	93,69	94,95	99,37

Figure 2. Inhibition degree of tested essential oils against *Fusarium oxysporum*

There is a tendency in increasing the degree of inhibition with the increase in the amount of tested lavender oil. When using 40 µl of lavender oil per plate, the degree of inhibition is of 94.95% for all tested lavender oil (Figure 1), while at a quantity of 45 µl per plate, the highest inhibition degree was obtained for the GE essential oil. The aspect of the plates inoculated with the fungus *Fusarium oxysporum* in the presence of the tested essential oils, at the end of the incubation period is presented in Figure 3.

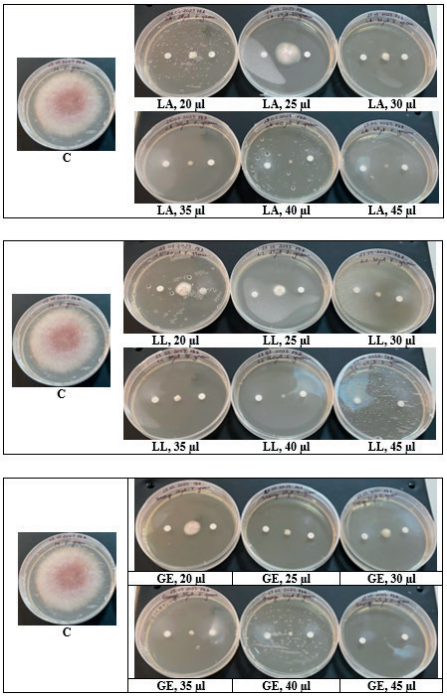
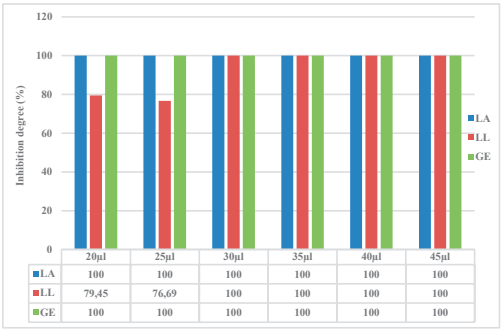


Figure 3. Appearance of plates inoculated with the fungus *Fusarium oxysporum* in the presence of the tested essential oils

Figure 4 shows the fact that LA, as well as GE samples, had a strong antifungal effect on *Aspergillus brasiliensis* ATCC 16404 fungus, showing a degree of inhibition of 100% for tested quantities. Regarding the antifungal potential of LL essential oil, it can be observed that for 20 µl and 25 µl per plate, the degree of inhibition varies in the range of 79.45-76.69%, while for higher quantities, the degree of inhibition was of 100%.



	20µl	25µl	30µl	35µl	40µl	45µl
LA	100	100	100	100	100	100
LL	79,45	76,69	100	100	100	100
GE	100	100	100	100	100	100

Figure 4. Inhibition degree of tested essential oils against *Aspergillus brasiliensis* ATCC 16404

In Figure 5, the appearance of plates inoculated with the *Aspergillus brasiliensis* ATCC 16404 fungus in the presence of the tested essential oils, at the end of the incubation period, can be seen.

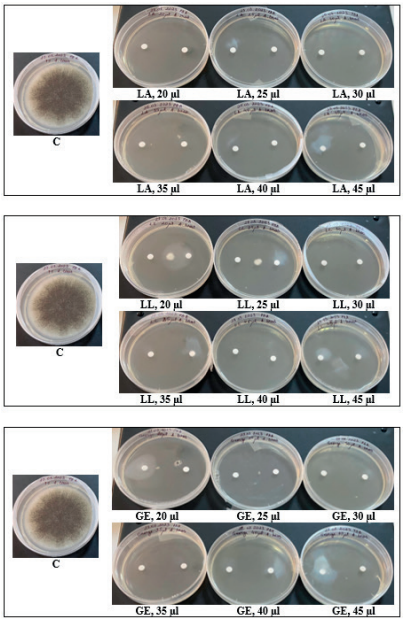
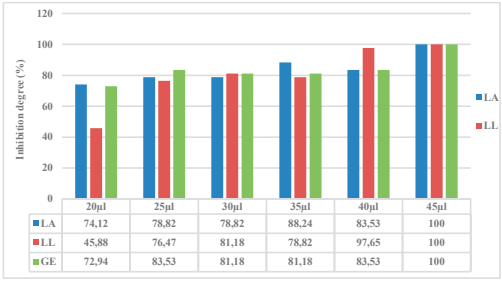


Figure 5. Appearance of plates inoculated with the fungus *Aspergillus brasiliensis* ATCC 16404 in the presence of the tested essential oils

In the case of the *Penicillium expansum* fungi, it was observed that the degree of inhibition is maximum (100%) when 45 µl of essential oil was applied, for all tested essential oils, which denotes a higher natural resistance of this fungus (Figure 6).



	20µl	25µl	30µl	35µl	40µl	45µl
LA	74,12	78,82	78,82	88,24	83,53	100
LL	45,88	76,47	81,18	78,82	97,65	100
GE	72,94	83,53	81,18	81,18	83,53	100

Figure 6. Inhibition degree of tested essential oils against *Penicillium expansum*

In Figure 7, the appearance of the plates inoculated with the *Penicillium expansum* fungus in the presence of the tested essential oils, at the end of the incubation period, can be seen.

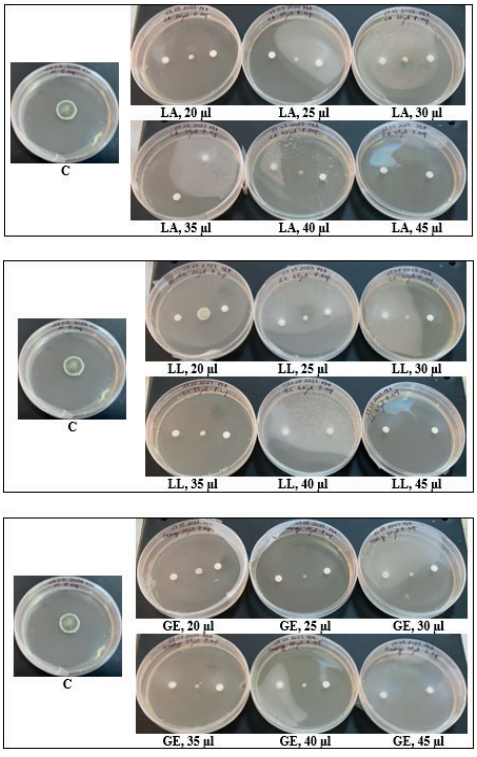


Figure 7. Appearance of plates inoculated with the fungus *Penicillium expansum* in the presence of the tested essential oils

Antibacterial activity against food pathogenic bacteria

Regarding the antibacterial activity of the studied essential oils, several observations were made during the experiments. It was shown that all tested samples showed an antibacterial effect when 2.5 µl of essential oil was applied in the presence of *Bacillus cereus* strain but not on *Bacillus subtilis*. The antibacterial effect on the two *Bacillus* strains tested increased as the amount of lavender essential oil was increased, and the strongest antibacterial effect was shown by LL samples, the diameters of inhibition zones measured being clearly larger than those recorded for LA and GE essential oils. The results are presented in Table 3 and the aspect of the plates inoculated with the tested bacterial strains in the presence of the studied essential oils is presented in Figure 8.

Tabel 3. Antibacterial activity of the tested essential oils

Bacterial strain	Essential oil quantity	Diameter of inhibition zone (cm)		
		LA	LL	GE
Bacillus cereus	2.5 µl	0.65	1.10	0.73
	5 µl	0.83	1.35	1.03
	7.5 µl	1.43	1.63	1.35
	10 µl	1.63	1.80	1.50
Bacillus subtilis	2.5 µl	0	0	0
	5 µl	1.25	0	1.03
	7.5 µl	2.43	2.40	1.60
	10 µl	3.60	4.08	3.68

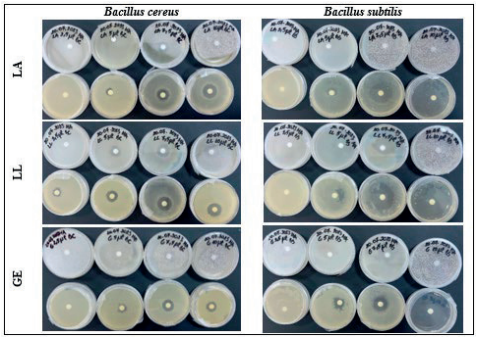


Figure 8. The appearance of plates inoculated with the two bacteria under study in the presence of the tested essential oils

Antifungal activity against phytopathogenic fungi

Regarding the antifungal activity against phytopathogenic fungi, a first visual analysis was made after 3 days of incubation. No microbial growth was observed in the test plates and the fungal radius in the Control plates was also

reduced. Therefore, incubation was continued at 25°C, and the first biometric measurements were collected after 5 days of incubation. Studies have shown the potential of lavender essential oil to inhibit phytopathogenic fungi, regardless of the lavender species from which the oil was extracted (Table 4).

Table 4. Biometry of phytopathogenic fungi grown in the presence of lavender extracts after 5 days of incubation at 25°C

Variant	Dose of EOs	Alt.	B.c.	*F.c.*	*FORL*	*M.p.*	*S.s.*
		Colony radius (mm)					
Control	-	17	58	31	30	60	55
LA	20 µl	3	0	0.5	5	7	5
	30 µl	0.5	0	0	2	2	1
	40 µl	0	0	0	1.5	0.5	0
LL	20 µl	0	1	0	8	4	2
	30 µl	0	0.5	0	3	3	0
	40 µl	0	0	0	2	2	0
GE	20 µl	0	0	0	3	5	0
	30 µl	0	0	0	2	1	0
	40 µl	0	0	0	1.5	0	0

Legend: LA = *Lavandula angustifolia* essential oil, LL = *Lavandula latifolia* essential oil, GE = essential oil of lavender George 90 cultivar, EOs = essential oils, Alt. = *Alternaria* sp., B.c. = *Botrytis cinerea*, F.c. = *Fusarium culmorum* FC46, FORL = *Fusarium oxysporum* f.sp. *radicis lycopersici* ZUM2407, M.p. = *Macrophomina phaseolina*, S.s. = *Sclerotinia sclerotiorum*.

After 7 days of incubation, the antifungal efficacy of the EOs was calculated. The inhibition of the *Alternaria* sp. phytopathogen was maintained in the first 7 days of incubation in the presence of lavender EOs, however after prolonged incubation, up to 12 days, the inhibitory efficacy was reduced (Figure 9).

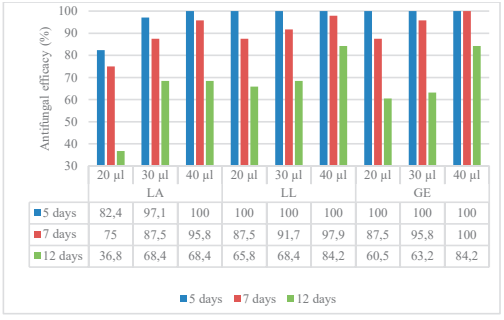


Figure 9. *In vitro* antifungal efficacy of the lavender EOs against *Alternaria*, after 5 to 12 days of incubation

The best results were obtained when using the EO of lavender cultivar George 90. All tested doses of GE completely inhibited the growth of the phytopathogen in the first 5 days of incubation. After 7 days, only the highest tested dose was able to maintain a complete fungal growth inhibition, while after 12 days of incubation, the efficacy of *Alternaria* sp. growth inhibition was reduced to 84.2%, when 40 µl dose of GE was used (Figure 10).

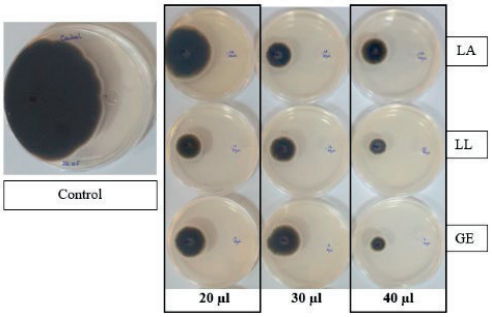


Figure 10. Inhibitory activity of lavender EOs against *Alternaria* sp. phytopathogen, after 12 days of incubation at 25°C

Against the *Botrytis cinerea* phytopathogen, the best results were also obtained when using GE essential oil (Figure 11). The EO of lavender cultivar George 90 completely inhibited the growth of grey mold during the 12 days of incubation at 25°C, for all tested doses (Figure 11). The *L. angustifolia* EO (LA) completely inhibited *B. cinerea* growth during the first 7 days of incubation, at all doses (20-40 µl). After 12 days, the complete inhibition was maintained only when using the 30 and 40 µl EO/plate, while at the lower dose (20 µl LA/plate), the antifungal efficacy was reduced to 99.2% compared to the Control culture (Figure 12). The *L. latifolia* EO (LL) at the highest tested dose (40 µl/plate) showed complete inhibition of *B. cinerea* fungal growth during the first 12 days of incubation. However, lower dose of LL against this mold is fungistatic. The lower doses of LL, 20 and 30 µl EO/plate, revealed an antifungal efficacy of 98.3% and 99.2% respectively after 7 days of incubation. The inhibitory activity decreased with time, and after 12 days of incubation the efficacy was reduced to 94.2% at the dose of 20 µl EO/plate, and at 98.3% when 30 µl EO/plate were used. However, the highest dose of 40 µl EO/plate completely inhibited the growth of *B. cinerea* (Figure 13).

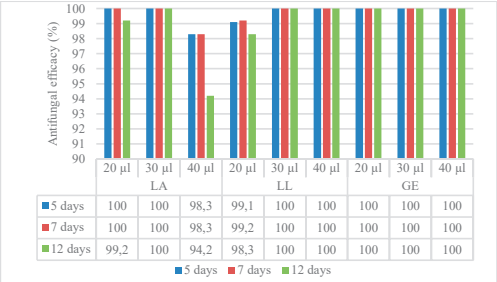


Figure 11. *In vitro* antifungal efficacy of lavender EOs against *Botrytis cinerea* phytopathogen

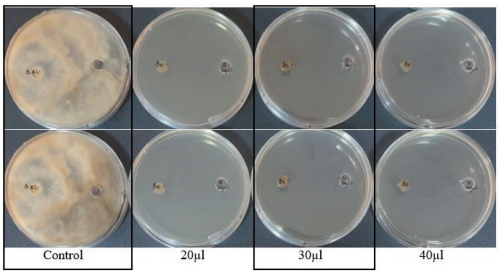


Figure 12. The inhibitory activity of the GE essential oil against *Botrytis cinerea*, after 7 days (upper line) and 12 days (bottom line) of incubation at 25°C

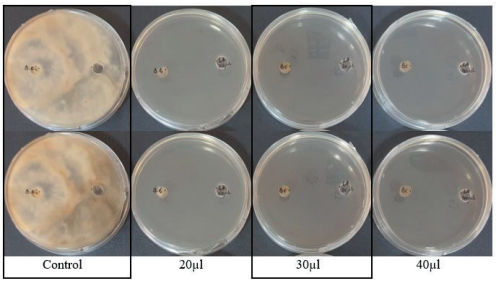


Figure 13. The inhibitory activity of the LA essential oil against *Botrytis cinerea*, after 7 days (upper line) and 12 days (bottom line) of incubation at 25°C

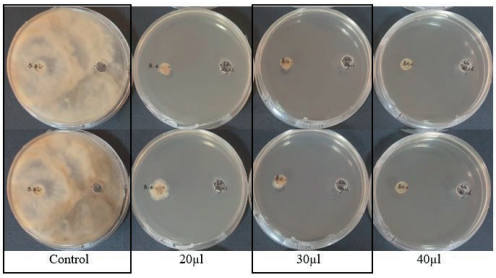


Figure 14. The inhibitory activity of the LL essential oil against *Botrytis cinerea*, after 7 days (upper line) and 12 days (bottom line) of incubation at 25°C

All tested doses of EOs completely inhibited *Fusarium culmorum* FC46 growth in the first 5 days of incubation (Figure 15).

Figure 15. *In vitro* antifungal efficacy of lavender EOs against *Fusarium culmorum* FC46 phytopathogen

The *L. latifolia* EO (LL) maintained a complete inhibition of *F. culmorum* FC46 after 12 days of incubation, however the antifungal efficacy decreased when lower doses were used. At 20 and 30 µl LL/plate, the efficacy against *F. culmorum* FC46 decreased to 71.7% and 73.3% respectively (Figure 16).

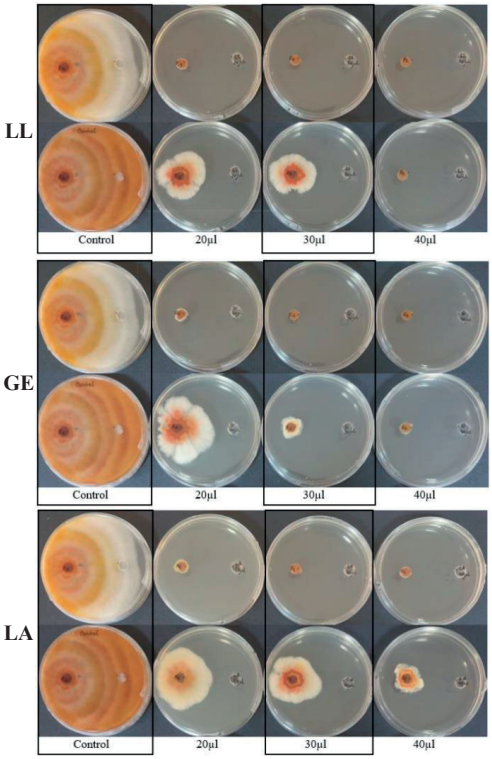
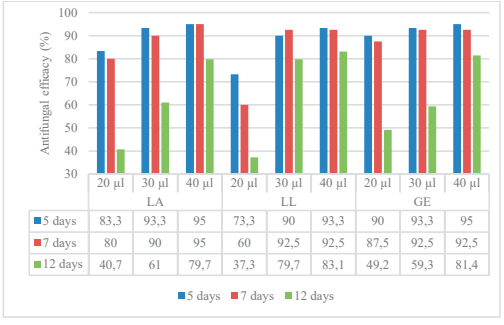


Figure 16. Inhibitory activity of EOs against *F. culmorum*, after 7 days (upper line) and 12 days (bottom line) of incubation at 25°C

The GE essential oil, maintained a complete inhibition of *F. culmorum* FC46 growth in the first 5 days of incubation at all tested doses, as well as after 7 days of incubation, at the dose of 30 and 40 µl EO/plate. After 12 days of incubation, the fungistatic efficacy of this essential oil began to decrease, up to 99.2% at 40 µl GE/plate and to 90% at 30 µl GE/plate, respectively. At 20 µl GE/plate the efficacy considerably dropped down to 53.3% fungal inhibition (Figure 15). The LA essential oil completely inhibited the growth of *F. culmorum* FC46 only during the first 7 days of incubation, when applied in 30 and 40 µl dose. After 12 days of incubation the antifungal efficacy decreased to 81.7% at 40 µl LA/plate, 66.7% when using 30 µl LA/plate, respectively 61.7% at 20 µl LA/plate (Figure 15).

The *F. oxysporum* f.sp. *radicis lycopersici* ZUM2407 (FORL) phytopathogen was less sensitive to the applied lavender EOs treatment. When using doses of 30 µl and 40 µl EOs/plate, no significant differences were observed between treatments after 5 and 7 days of incubation. However, after 12 days of incubation, the antifungal efficacy decreased considerably (Figure 16). Thus, at the highest dose tested (40 µl EO/ plate), LL revealed 83.1% antifungal efficacy (Figure 17), GE revealed 81.4% efficacy, while LA revealed 79.7% antifungal efficacy against FORL (Figure 18).

The growth of *Macrophomina phaseolina* phytopathogen was inhibited by the tested lavender essential oils only during the first 5 days of incubation (Figure 20).



	20 µl	30 µl	40 µl	20 µl	30 µl	40 µl	20 µl	30 µl	40 µl
	LA			LL			GE		
5 days	83,3	93,3	95	73,3	90	93,3	90	93,3	95
7 days	80	90	95	60	92,5	92,5	87,5	92,5	92,5
12 days	40,7	61	79,7	37,3	79,7	83,1	49,2	59,3	81,4

5 days 7 days 12 days

Figure 17. *In vitro* antifungal efficacy of lavender EOs against FORL phytopathogen

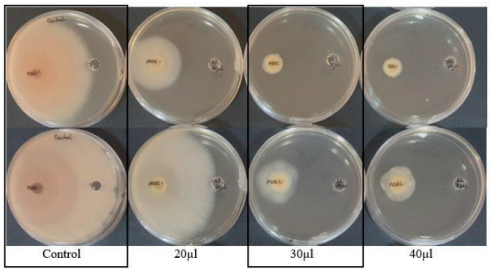


Figure 18. Inhibitory activity of LL essential oils against FORL, after 7 days (upper line) and 12 days (bottom line) of incubation at 25°C

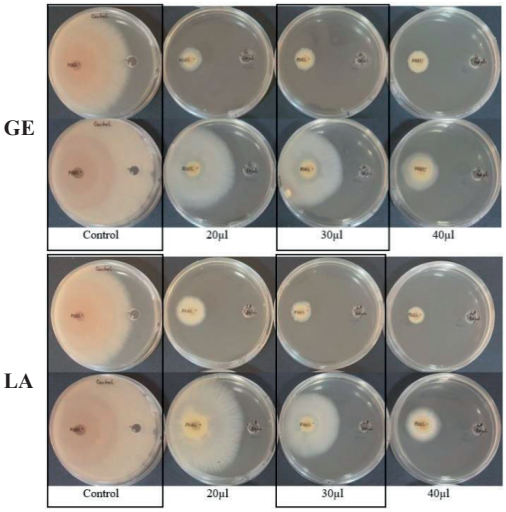
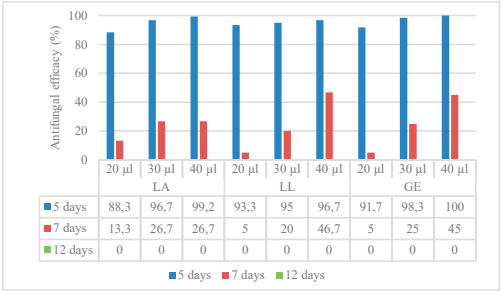


Figure 19. Inhibitory activity of GE and LA essential oils against FORL, after 7 days (upper line) and 12 days (bottom line) of incubation at 25°C



	20 µl	30 µl	40 µl	20 µl	30 µl	40 µl	20 µl	30 µl	40 µl
	LA			LL			GE		
5 days	88,3	96,7	99,2	93,3	95	96,7	91,7	98,3	100
7 days	13,3	26,7	26,7	5	20	46,7	5	25	45
12 days	0	0	0	0	0	0	0	0	0

5 days 7 days 12 days

Figure 20. *In vitro* antifungal efficacy of lavender EOs against *Macrophomina phaseolina* phytopathogen

When using GE essential oil in a dose of 40 µl / plate, the fungal growth was completely inhibited in the first 5 days of incubation.

The antifungal efficacy considerably decreased to 45%, after 7 days of incubation, while after 12 days, GE completely lost its inhibitory effect. However, the GE essential oil was the only one inhibiting the pigmentation of the pathogen (Figure 21).
Similar antifungal results were observed when testing the LA and LL essential oils, with the difference that the fungal pigmentation was not inhibited by this EOs.

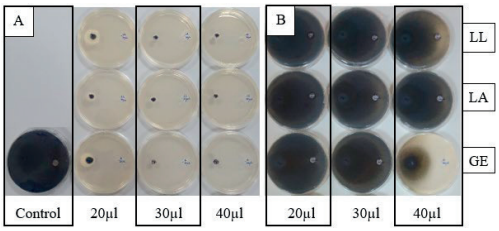


Figure 21. The antifungal activity of the lavender EOs against *Macrophomina phaseolina* after 5 days (A), respectively 12 days (B) of incubation at 25°C

GE lavender essential oil showed very good inhibitory activity against *Sclerotinia sclerotiorum* (Figure 22).

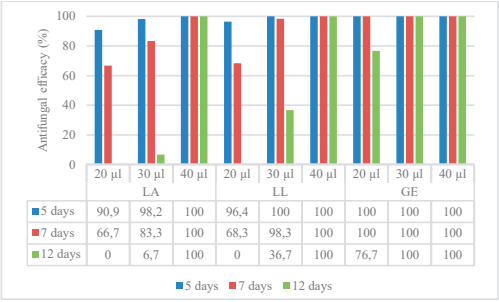


Figure 22. *In vitro* antifungal efficacy of lavender EOs against *Sclerotinia sclerotiorum* phytopathogen

Compared to the other tested lavender essential oils, GE completely inhibited the growth of the pathogen during the first 12 days of incubation, at both 30 and 40 µl/plate. Moreover, when using the dose of 20 µl GE/plate, the pathogen was completely inhibited during the first 7 days of incubation, while after 12 days the efficacy decreased to 76.7%, which is well above the inhibitory potential of the other two tested lavender oils (Figure 23).

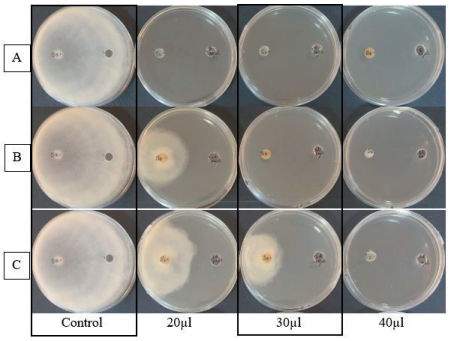


Figure 23. The antifungal activity of GE (A), LL (B) and LA (C) lavender essential oils against *Sclerotinia sclerotiorum*, after 7 days of incubation at 25°C

The antifungal effects of LA and LL essential oils against *S. sclerotiorum*, were similar compared to each other. However, LL was slightly more effective.

CONCLUSIONS

The highest sensitivity in terms of the antifungal activity of lavender essential oils was recorded by the fungus *Aspergillus brasiliensis* ATCC 16404, its growth being completely inhibited (100%) when adding to the plate an amount of 20 µl LA or GE essential oil. The fungi *Penicillium expansum* and *Fusarium oxysporum* showed greater resistance, the highest degree of inhibition being obtained when 45 µl of essential oil was added to the plate, thus obtaining an inhibition rate of 94.95% for LA, 95.27% for LL and 99.37% for GE, and in the case of *Penicillium expansum* the inhibition rate was 100% for all tested oils. Regarding the antibacterial activity, a complete inhibition of the development of the two bacteria under study was not observed in the case of the tested essential oils, the bacteria *Bacillus cereus* being less sensitive to their presence compared to *Bacillus subtilis*. The halo formed around the essential oil-soaked disc increased as the amount of applied essential oil increased. Its largest diameters were measured for the amount of 10 µl of essential oil LL, namely 1.80 cm in the case of *Bacillus cereus* bacteria and 4.08 cm in the case of *Bacillus subtilis* bacteria.

In all interactions with phytopathogenic agents, a close relationship was observed between the antifungal potential and the dose of essential oil tested. Thus, as expected, increasing the dose of lavender essential oil is directly proportional to the effectiveness of inhibiting phytopathogenic fungi. The three tested lavender essential oils inhibited the growth of the analyzed phytopathogenic fungi. At 40 µl EO/plate, all tree tested lavender EOs completely inhibited the growth of the *Botrytis cinerea*, *Fusarium culmorum* FC46 and *Sclerotinia sclerotiorum* phytopathogens in the first 7 days of incubation. Against *Alternaria* sp., only GE lavender essential oil maintained a complete fungal inhibition at 40 µl EO/plate in the first 7 days of incubation. *Macrophomina phaseolina* and *Fusarium oxysporum* f.sp. *radicis lycopersici* ZUM2407 phytopathogens were less sensitive to lavender essential oil treatments. Their inhibition was possible only in the first 5 days of incubation.

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