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SENSITIVITY OF *Fusarium solani* ISOLATED FROM ASPARAGUS ON ESSENTIAL OILS

WRAŻLIWOŚĆ *Fusarium solani* WYIZOLOWANEGO ZE SZPARAGA NA OLEJKI ETERYCZNE

Abstract: The purpose of this study was to evaluate the antifungal properties of sage oil, clove oil and basil oil against *Fusarium solani* isolated from white asparagus spears (*Asparagus officinalis L.*). A dual culture plate method was used for the assessment of the inhibitory effects of essential oils and volatile components on mycelium, inoculated into a PDA medium. The culturing process was conducted for 9 days at a temperature of 26°C and the fungal linear growth was measured every 1–3 days. The conidial germination of the fungus in the presence of oils was evaluated by microscope method. The results show differences in the fungistatic activity of oils, dependent on the type and dose of the oil. Generally the oils had a higher impact against the fungal spores than on mycelium growth of *F. solani*, however the type of oil and dose determined different amounts. The conidial germination was most inhibited by the basil oil (98.9% maximum inhibition), followed by the clove and sage oils at a concentration of 4% (respectively 89.9% and 85.4% maximum inhibition). In contrast, the oils had much less of an impact on the linear mycelial growth of *F. solani*. Use of the highest dose of clove oil reduced the linear growth of mycelium by 29.9%, sage oil by 14.8% and basil oil by 4%. Furthermore, gas metabolites limited the growth of *F. solani*. The degree of inhibition of mycelium amounted to 17.1% for clove oil, 15.5% for sage oil and 9.3% for basil oil used in the maximum concentration 4.0%. Based on these results, clove oil was the greatest inhibitor of mycelial growth and sporulation of *F. solani*.

Keywords: *Fusarium solani,* asparagus, essential oils, antifungal activity

Introduction

Fungi of the genus *Fusarium* are geographically widespread and occur as saprophytic organisms in the soil. They have the ability to produce highly toxic secondary metabolites (fumonisins, moniliformins, beauvericin) and therefore are classified as opportunistic organisms for plants and people [1–4].

Several pathogenic *Fusarium* species cause disease in asparagus plants (*Asparagus officinalis* L.), such as *Fusarium* stem and crown rot (FCRPR). These diseases can occur in asparagus seedlings, however they are most typically observed in mature plants

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[1, 3, 5–7]. Both white and green asparagus are easily infected by the different *Fusarium* species. Researchers have shown that the presence of *Fusarium* ranges based on region, with some regions only reporting occasional presence and others a more dominant presence. Infection of asparagus plants depends particularly on environmental conditions, agronomic practices and asparagus cultivar susceptibility [2, 5, 8–10]. This concerns mainly *F. proliferatum*, *F. oxysporum*, *F. culmorum, F. solani* and *F. redolens* [1, 3, 5, 11–15]. There have been many different approaches used towards minimizing the damage caused by *Fusarium* crown and root rot. These have included incorporating organic matter into the soil, improving soil fertility, applying arbuscular mycorrhizae (*Glomus intraradices*), chemical control and biological control (*Trichoderma* sp.) [14, 16–19].

In recent years there has been increased interest in the possibility of using natural products such as plant extracts or essential oils as biological agents of plant protection and plant disease control. The application of essential oils in particular has been evaluated as a potentially effective and safe alternative to chemicals. Plants have a limitless ability to synthesize secondary metabolites (*eg* phenols, flavones, flavonoids, alkaloids, tannins) which operate as the plant's defence mechanism against pathogenic microorganisms. Based on this process, certain essential oils in liquid phase and/or vapour phase can be used to delay or inhibit the growth of pathogenic and/or toxin producing fungi [20–27]. Many essential oils have been demonstrated to have biological activity on many fungal plant microorganisms, including *A. flavus*, *A. niger*, *Mucor* sp., *Rhizoctonia* sp. *Fusarium* sp. Some studies suggest that within an experimental system the extent of inhibition of mycelial growth and spore germination depends on the concentration of essential oils [20, 21, 28, 29].

The aim of this study was to investigate the antifungal activity of three essential oils (sage oil, basil oil and clovebud oil) against the mycelial growth and spore germination of *Fusarium solani*.

Materials and methods

The research material was the strain of *Fusarium solani* isolated from the spears of white asparagus plants (*Asparagus officinalis* L.) exhibiting signs of dry rot. The essential oils (EOs) used were sage oil (*Salvia sclarea* L.) and basil oil (*Ocimum basilicum* L.), both from plants of the *Lamiaceae* family, and clovebud oil (*Eugenia caryophyllus*) from a tree of the *Myrtaceae* family [30]. The antimicrobial effect of the tested oils (in both liquid and vapour phase) was observed by employing various antifungal assays. The sensitivity of spores and mycelium of *F. solani* to EOs was examined using the medium poisoning method (method I), disc volatilization method (method II) and cavity slide technique (method III) with a few modifications [23, 27, 29, 30–33].

I Poisoning method. The experiments were conducted on a Potato dextrose agar (PDA) medium. Fungal mycelial discs (8 mm in diameter) were cut with a sterile cork borer from the periphery of 7 day-old pure culture of *F. solani* and were placed at the centre of the medium containing the essential oils at varying concentrations (0.5%,

1.0%, 2.0%, 4.0%). Initially, in order to enhance the oil solubility, the oils were dissolved with 0.5% dimethyl sulfoxide (DMSO). In the controls tests, EOs were replaced with 0.5% DMSO solution and inoculated following the same procedure.

II Volatilization method. In order to investigate the effects of the volatile fraction of the essential oils, petri dishes containing the PDA medium were inoculated with the fungal mycelial discs (8 mm in diameter). In contrast, the suspension of each EO was not added into the medium but rather dripped onto the sterilized filter disc (6 cm in diameter) positioned on the covers of the Petri dishes. A blank sterilized filter disc with no oil treatment was used as the control. After applying the EOs, the filter discs were then immediately inverted on top of the lid and hermetically sealed with parafilm to prevent any leakage of essential vapour. All plates (from methods I and II) were incubated in the dark at 24 ± 2°C for 9 days until the mycelium in the control plates reached the edge of the plates. The radial mycelia growth was observed and measured in two perpendicular diameters at intervals of 1–2 days. The experiment was performed in four replicates, where one repeat was represented by one plate containing the growth medium with one mycelia disc. The antagonistic activity of the EOs was evaluated using the growth rate index (GRI) and additionally evaluated using the percentage of mycelial growth inhibition in comparison to the control [33].

III Cavity slide technic. The evaluation of fungistatic activity of the EOs was also carried out on the basis of spore germination of *F. solani*. Conidia of *F. solani* were harvested from the 7 day-old culture grown in PDA medium using sterile water containing 0.5% Tween 80. The conidia suspensions were then filtered through sterile gauze to remove hyphae and adjusted to a concentration of $1.65 \cdot 10^6$ spores/cm^3 using a haemocytometer Thoma. Subsequently aliquots of 30 mm^3 of the essential oil solutions at different concentrations were mixed with 30 mm^3 of the spore suspensions in a cavity slide. Then they were incubated in a moist chamber at 24 ± 2°C for 18 hours. Microscopic observation and enumeration showed varying degrees of spore germination as well as no germination. The data evaluated were the rate of conidia germination and the percentage of spore germination inhibition [34].

All experiments were conducted in triplicate and the means were statistically compared using two-way analysis of variance with a Tukey's composition test. The differences between the means were considered significant for values of $p \leq 0.01$. The data are presented as mean values ± standard deviation calculated from four determinations.

Results and discussion

The essential oils were individually tested against *Fusarium solani*. The mycelial growth and spore germination of *F. solani* was affected differently by each of the three essential oils. The inhibition effect depended on the type of oil, the amount of essential oil, the incubation period and the susceptibility of the spores and mycelia of the fungus.

All amounts of the essential oil significantly restricted the spore germination of *F. solani* with the highest inhibition activity detected for 4% concentration. Basil oil

exhibited a statistically significant negative effect on the conidia, with a germination rate of 0.64 to 16.80 compared to the control germination rate of 58.62 (Table 1).

Table 1

Effect of essential oils on the spore germination (SG) of *F. solani* (means and standard deviation ± SD)

Treatment	Clovebud oil		Sage oil		Basil oil		Mean	
Control	58.62 ± 1.48	A	58.62 ± 1.48	A	58.62 ± 1.48	A	58.62	A
0.5%	26.62 ± 1.00	B	34.29 ± 4.36	B	16.80 ± 5.43	B	25.90	B
1.0%	23.19 ± 3.77	C	27.17 ± 4.77	C	5.55 ± 1.44	C	18.64	C
2.0%	14.14 ± 2.61	D	17.28 ± 3.57	D	2.37 ± 0.65	D	11.26	D
4.0%	5.90 ± 2.16	E	8.56 ± 1.84	C	0.64 ± 1.11	E	5.03	E
Mean	25.69	B	29.18	A	16.79	C		

Values followed by a same alphabetic letter are not significantly different (capital letters $p \leq 0.01$).

All concentrations of the basil oil inhibited *F. solani* spores from 71.33% to 98.90%. Clovebud had a moderate effect with a maximum degree of inhibition reaching 89.93% for 4% concentration. Sage oil had the lowest effect with a maximum degree of inhibition reaching 85.99% for 4% concentration. (Fig. 1).

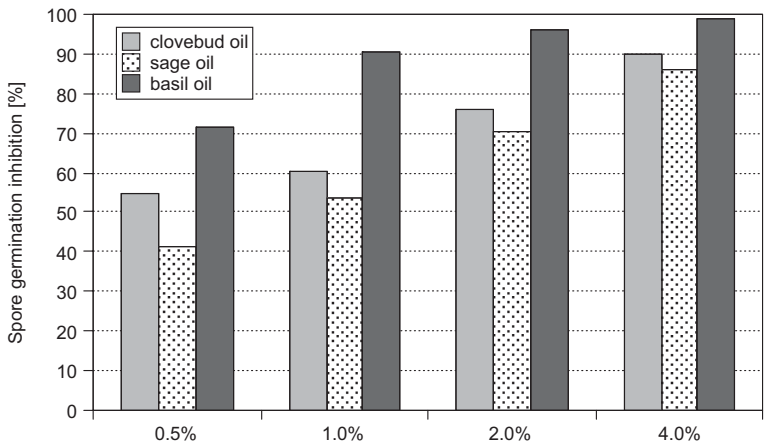

Fig. 1. Percentage inhibition of spore germination of *F. solani* at different concentrations of essential oils

In contrast, the essential oils showed a much smaller effect on the linear mycelial growth of *F. solani.* Growth inhibition was significantly influenced by the essential oil concentration and the incubation time. Increased concentration of the essential oils resulted in a gradual decrease in the linear growth of the mycelium. The clovebud oil at 4% concentration resulted in the lowest GRI value, followed by the sage oil with

a moderate GRI value, and the basil oil with the highest GRI value. At 0.5% EO concentration, the GRI ranged from 59.76 (clovebud oil) to 63.60 (basil oil), while at 4% EO concentration it ranged from 45.25 (clovebud oil) to 62.79 (basil oil) – compared to the control GRI of 64.62 (Table 2).

Table 2

Effect of essential oils (liquid phase) on the growth rate index (GRI) of *F. solani*

Treatment	Clovebud oil		Sage oil		Basil oil		Mean	
Control	64.62 ± 0.71	A	64.62 ± 0.71	A	64.62 ± 0.71	A	64.62	A
0.5%	59.76 ± 0.52	B	62.79 ± 0.68	B	63.60 ± 0.41	B	62.05	B
1.0%	58.74 ± 1.18	C	59.72 ± 0.51	C	63.21 ± 1.31	C	60.60	C
2.0%	53.22 ± 0.61	D	57.73 ± 0.58	D	62.24 ± 0.38	D	57.73	D
4.0%	45.25 ± 0.85	E	55.05 ± 1.54	E	62.79 ± 0.81	E	54.36	E
Mean	56.32	C	59.98	B	63.29	B		

Values followed by a same alphabetic letter are not significantly different (capital letters $p \leq 0.01$).

The highest degree of inhibition of mycelium was obtained for clovebud oil (29.96%), followed by sage oil (14.79%). Basil oil had the least impact on the inhibition of mycelium at only 4.01% (Table 2, Fig. 2).

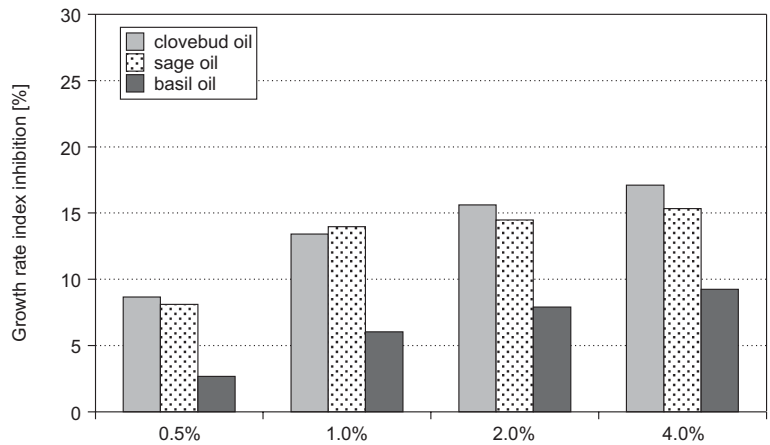

Fig. 2. Growth inhibition of *F. solani* by essential oils (liquid phase) at different concentrations

Results obtained from the volatilization method indicated that gas metabolites also significantly limited the growth of tested *F. solani*. The growth rate index was similar to that obtained for the oils applied in the liquid phase. The lowest GRI at 4% EO concentration was found for clovebud oil (52.79), followed by sage oil (53.93) and finally for basil oil (28.02) (Table 3).

Table 3

Effect of essential oils (vapour phase) on the growth rate index (GRI) of *F. solani*

Treatment	Clovebud oil		Sage oil		Basil oil		Mean	
Control	63.69 ± 0.62	A	63.69 ± 0.62	A	63.69 ± 0.62	A	63.69	A
0.5%	58.16 ± 0.31	B	58.52 ± 0.96	B	62.23 ± 0.47	B	59.63	B
1.0%	55.15 ± 0.75	C	54.78 ± 0.65	C	60.08 ± 0.55	C	56.67	C
2.0%	53.74 ± 0.24	D	54.46 ± 0.98	D	58.88 ± 0.85	D	55.69	Dd
4.0%	52.79 ± 0.24	E	53.93 ± 0.75	E	58.02 ± 0.22	E	54.91	Ee
Mean	56.71	B	57.08	B	60.58A	A		

Values followed by a same alphabetic letter are not significantly different (small letters $p \leq 0.05$); capital letters $p \leq 0.01$).

It was observed that the degree of inhibition of mycelium amounted to 17.1% for clovebud oil, 15.5% for sage oil and 9.3% for basil oil at the maximum concentration of each oil. However, the EOs in at 4% concentration volatile phase resulted in a two times smaller growth inhibition value than the EOs in 4% concentration liquid phase (Figs. 2, 3).

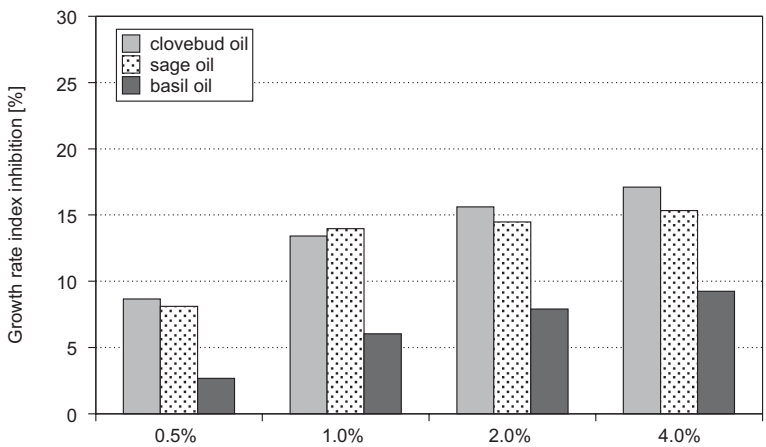

Fig. 3. Growthinhibition of *F. solani* by essential oils (vapour phase) at different concentrations

Environmental factors such as temperature, light, day length change during the vegetation period, as well as genetic factors, organ age impact on the seasonal quantitative variations in plant components [35–38].

Previous research has shown that the antifungal activity of EOs is not caused by major compounds, but is rather the result of a synergistic or antagonistic effect of different compounds present in the mixture, even if in minor percentages [36, 39, 40]. Dzamić el al [41] reported that there is a relationship between the high presence of linalyl acetate and linalool in sage oil and observed moderate antifungal activity. Some

studies suggest that the extent of inhibition of fungal growth and mycotoxin production (aflatoxin B1, G2, fumonisins, deoxynivalenol) depends on the concentration of essential oil [23, 31, 42–44].

The antimicrobial properties of sage oil have been attributed to the presence of the major oxygenated monoterpenes 1,8-cineole and camphor [36, 37, 45–48], thujone [45, 47], linalool and linalyl acetate [40, 41, 49, 50]. However Pinto et al [47] demonstrated that thujone content may not be related to the inhibition of fungal development.

Some studies found that sage oil was active in inhibiting the growth of pre- and post-harvest phytopathogenic and saprophytic fungi belonging to the genera *Fusarium*, *Aspergillus*, *Penicillium*, *Alternaria*, *Trichoderma*, *Gliocladium* [41, 43, 45, 47, 48]. Among the *Fusarium* sp. the most sensitive to sage oils were *Fusarium solani*, *F. equiseti* and *F. verticillioides* whereas *F. tricinctum*, *F. subglutinans* were the least sensitive [45]. According to Gomori et al [31] clary sage inhibited the growth of *F. culmorum* by 73.7%, *F. graminearum* by 9.26% and *A. parasiticus* by 28,05%. In another study, sage oil caused a total inhibition of *F. graminearum* growth [46]. Furthermore, Snieskiene et al [23] observed that volatile fractions of sage essential oils had the strongest fungicidal effect, after three days of incubation, on *F. sambucinum*, *F. culmorum*, *F. oxysporum* and *A. alternata* (reduction of mycelium growth amounted to more than 90%).

The high inhibition results of clove oil could be attributed to its major aromatic component, eugenol, which is known to inhibit fungal growth and fungal spore production [27, 39, 51–54]. Similarly, the main components of basil oil are eugenol, 1,8-cineole and linalool, which may exhibit antagonistic properties against certain fungi [42, 55].

Eugenol may cause morphological malformation in fungal cell, dissolve fat of fungal cell walls and therefore can interfere with the permeability of cells, destroying conidia and fungal hyphae. The inhibition levels of *F. oxysporum* by clove oil fractions containing a high content of eugenol ranged between 84.44–100% [53]. Velluti et al [51] observed that clove oil reduced *F. verticillioides*, *F. proliferatum* and *F. graminearum* colony growth by about 62%. Cosic et al [52] indicated in their results that clove oil had a stronger impact than sage oil on mycelium growth of *Fusarium* species. Sameza et al [27] showed that clove oil could significantly inhibit the mycelia growth and spore germination of *R. stolonifer* and *F. solani.* The oil caused a complete inhibition of fungal growth in all tested doses and a complete inhibition of spore germination but only at a dose greater than 31.2 ppm for *R. solani* and 250 ppm for *F. solani* [27].

These results are also in accordance with those obtained by Beg et al [56] for *A. alternata* and *F. chlamydosporum*. Their microscopic observations showed 20–40% lysis of conidia after 72 h of incubation at 5% concentration. However at higher clove oil concentration (10%), up to 20% of conidia were lysed after 24 h of incubation. Clove oil and eugenol considerably reduced the quantity of ergosterol, which is an important component of fungal cell membrane responsible for maintaining the consistency, integrity and functionality of the cells. Clove oil suppressed the growth of pathogenic fungi *Aspergillus* sp., *Fusarium* sp. and *Alternaria* sp. by a range of 41% to 65% [57].

Hussain et al [35] reported that basil essential oils and linalool possessed strong antifungal activity against *A. niger*, *F. solani* and moderate activity against *M. mucedo*, *R. solani*. It has been further observed that essential oils from winter and autumn crops demonstrated greater activity which might be attributed to be a high content of linalool and other oxygenated compounds [35]. Other studies have shown a significant impact of linalool [24] on mycelial growth of *A. niger*, *F. moniliforme,* and to a lesser extent, on *A. flavus*, *P. roquefortii* as well as an impact of eugenol [58] against *Rhizoctonia* sp., *Alternaria* sp.

The antifungal mechanisms of essential oils depend on their components and their the lipophilic nature. Therefore, volatile phenolic compounds (carvacrol, eugenol, thymol) may interfere with cell wall enzymes (enzyme inhibition), possibly through reaction with sulfhydryl groups or through interactions with proteins. They may change the cell permeability, membrane fluidity and disintegrate fungal hyphae [42, 59–62].

In this study, all of the essential oils investigated exhibited inhibitory effects on mycelial growth and the fungal spore production of *F. solani.* However, the extent of inhibition was significantly dependent upon the concentration of EOs, the type of oil, and phase type (volatile or liquid phases).

Conclusions

All three of the essential oils demonstrated antagonistic effects to a greater or lesser extent on *Fusarium solani*. Depending on the type of oil and the dose, they had a stronger effect on spore germination (even in small doses) than on mycelium growth of *F. solani*. The inhibitory effect of the essential oils increased with the increase of their concentration. In general, all amounts of the tested essential oils significantly restricted the spore germination and mycelial growth of *F. solani* with the maximum activity detected at 4% EO concentration. Additionally, it was noted that basil oil was the greatest inhibitor of spore germination (98.90%), whereas clove budoil was the greatest inhibitor of mycelial growth (29.96% in liquid phase and 17.10% in gaseous phase). The data obtained in volatilization methods established that all three of the oils in gaseous phase had fungistatic effect on *F. solani* but not to a visible degree – particularly when compared to the liquid phase data in the poisoning method. Taking into consideration both the germination of conidia and mycelial growth, the findings indicated that clove oil possesses the strongest inhibitory activity against *F. solani* isolated from white asparagus.

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WRAŻLIWOŚĆ *Fusarium solani* WYIZOLOWANEGO ZE SZPARAGA NA OLEJKI ETERYCZNE

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Abstrakt: Celem badań była ocena przeciwgrzybicznych właściwości olejku z szałwii, olejku goździkowego i olejku bazyliowego wobec *Fusarium solani* wyizolowanego z pędów szparaga (*Asparagus officinalis* L.) Ocenę właściwości antagonistycznych testowanych olejków i ich metabolitów gazowych wobec grzybni przeprowadzono metodą hodowlano-płytkową na podłożu PDA. Hodowlę prowadzono przez 9 dni w temp. 26^{o}C, dokonując pomiarów co 1–3 dni. Natomiast zarodnikowanie grzyba w obecności olejków oceniono metodą mikroskopową. Przeprowadzone badania wykazały różnice w aktywności metabolicznej testowanych olejków. W zależności od rodzaju olejku i jego dawki, stwierdzono większą aktywność wobec zarodników grzybów niż na wzrost grzybni *F. solani*. Największy wpływ na zdolność kiełkowania zarodników wykazał olejek bazyliowy, następnie kolejno olejek goździkowy i olejek szałwiowy w stężeniu 4%. Stopień zahamowania kiełkowania zarodników wyniósł odpowiednio: 98,9%, 89,9% oraz 85,4%. Natomiast zdecydowanie mniejszy wpływ wykazały na wzrost grzybni *F. solani*. Zastosowanie największej dawki olejku goździkowego ograniczyło tempo wzrostu grzyba o 29,9%, olejku szałwiowego o 14,8% a olejku bazyliowego tylko o 4,0%. Również metabolity gazowe ograniczały wzrost grzybni *F. solani*. Stopień zahamowania wzrostu wynosił odpowiednio 17,1% dla olejku goździkowego, 15,5% dla olejku szałwiowego i 9,3% dla bazyliowego. Biorąc pod uwagę analizowane czynniki, olejek goździkowy okazał się największym inhibitorem kiełkowania zarodników i wzrostu grzybni *F. solani.*

Słowa kluczowe: *Fusarium solani*, szparagi, olejki eteryczne, aktywność przeciwgrzybowa