

## Research on the antimicrobial activity of the plant species *Foeniculum vulgare*

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### Abstract

The process of minimizing the manifestation of storage diseases of fruits by using treatments with biological products is a basic premise in the development of organic agriculture. On 01.03.2022, inoculations were made on a culture medium established using a proprietary recipe that supports the growth and development of any living organism. The culture medium represents the physical and chemical support necessary for the growth and development of any living organism, including pathogenic ones. It also must respond, through its composition, to the nutritional and hormonal requirements of the organism in question. The experiment consisted of a micro dilution of fennel essential oil (*Foeniculum vulgare* Mill.) in the composition of biopreparations, made according to a proprietary recipe, that were used to determine the minimum inhibitory concentration (MIC). MBC corresponds to the minimum concentration of sample that can kill the microorganism. The same micro dilution experiment derived from the MIC determination was used.

**Keywords:** *Foeniculum vulgare*; Biological agriculture; Pathogen; Biopreparations; Minimum inhibitory concentration.

### 1. Introduction

Fennel (*Foeniculum vulgare* Mill.), family Apiaceae (Umbelliferae) is an annual, biennial or perennial aromatic plant. The plant is native to southern Europe and the Mediterranean region [5, 3]. Fennel is commercially cultivated in Romania and grows wild in many areas of the country. There are two types of fennel in Romania: sweet fennel, *Foeniculum vulgare* Mill. subsp. *vulgare*, and bitter fennel, *Foeniculum vulgare* subsp. *piperitum* [7]. The plant is used as a spice, but also as an important ingredient in various kinds of medicine around the world. Most parts of fennel are edible (bulbs, leaves, stems and fruits). Fennel seeds and essential oil are used as flavoring agents in food products for appetizing, as a digestive aid, liqueurs, bread, cheese and as an ingredient in cosmetic and pharmaceutical products. Fennel infusions are the classic decoction for breastfed babies to prevent flatulence and colic spasms [2, 10, 11, 14]. The plant parts have been extensively investigated for several medicinal and therapeutic activities and have been reported to possess carminative, antioxidant, antibacterial, antifungal and mosquito repellent properties [8, 9].

Research has shown that the volatile oil of fennel is a mixture of many different constituents, and the main ingredients are: anethole (40–70%), fenchone (1–20%), and estragole (2–9%). [1, 12]. In the essential oil of sweet fennel, the content of fennel usually does not exceed 5%, while in the bitter types, its content can reach up to 20%. In the oil of sweet fennel, the content of anethole reaches 84–90%, while its proportion in bitter fennel is about 61–70% [13]. Hydrodistillation (HD), steam distillation or solvent extraction methods of essential oils have some disadvantages, such as thermal decomposition of the extracts, and their contamination with solvent or solvent residues. Pollution of residual plant material with solvent can also be an environmental problem [16]. It is important to develop more efficient methods for standardizing and quantifying essential oil extraction. New eco-friendly techniques, such as supercritical fluid extraction, ultrasound and microwave-assisted techniques, represent potential solutions to reduce energy

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consumption [15], and/or to solve environmental problems related to residues [6]. Recently, microwave-assisted hydrodistillation (MWHd) has become a widely used method for obtaining essential oils from various medicinal plants due to its advantages (e.g., more efficient heating, shortened extraction time) compared to classical HD. Heating technology is based on the molecular movements of polar molecules and ions inside the solvent and plant matrix; it is strongly influenced by the dielectric constants of the solid-liquid-vapor system, developed through the evolution of the process. The heating method achieves a more homogeneous temperature distribution in the plant powder suspension [4].

Thus, fennel oil extracted by SDE and SFE showed similar compositions, with trans-anethole, estragole and fenchone as the main components. On the other hand, thymol and p-cymene, the most abundant compounds in thyme leaves, showed large differences, with generally higher amounts of monoterpenes obtained by SDE. The most important odorants of fennel seeds determined by gas chromatography-olfactometry (GC-O) showed similar patterns when SDE and SFE were applied. Trans-anethole (anise, licorice), estragole (anise, licorice), fenchone (mint, camphor, warm aroma) and 1-octen-3-ol (mushroom) were the most intense odor compounds detected in fennel extracts [13].

## 2. Material and methods

The plant material consists of samples of *Foeniculum vulgare* plants from SCDL Buzău, essential oils were extracted from plants harvested in different phases of vegetation (vegetative stage, beginning of flowering stage, full flowering stage).

The antimicrobial activities of fennel essential oils, extracted at three developmental stages of the plants were carried out using the disk diffusion technique against three bacterial strains *Monilinia fructigena*, *Venturia inaequalis*, (Figures 1 and 2).

Thus, three recipes for biopreparations were created that included essential oils of fennel extracted at different stages of the plant's development.

### 2.1. Operational method

The primary screening of the antimicrobial activity of the samples studied was evaluated by the disk diffusion method according to previously published. Briefly, the culture suspension of each species was inoculated into the optimal culture medium [17]. Subsequently, sterile paper discs with a diameter of 6 mm, soaked with 10  $\mu$ L of essential oil (mixed with 5% DMSO) from the three phenological stages, were deposited on each plate. Chloramphenicol (30  $\mu$ g) was used as a positive control for bacteria and nystatin (100UI) was used as a positive control for yeast and fungi, while DMSO (10  $\mu$ L; 5%) was used as a negative control. The plates were incubated under the following growth conditions; 37  $^{\circ}$ C for 24 hours, 25  $^{\circ}$ C for 48 hours and 25  $^{\circ}$ C for 72 hours, for bacteria, yeast and fungi, respectively. After incubation, inhibitory diameters were measured in millimeters and results are expressed as means  $\pm$  standard deviation of three replicates.

In order to determine the minimum inhibitory concentration MIC, a micro dilution experiment of the mixture was used. MBC corresponds to the minimum concentration of sample that can kill the microorganism. The same micro dilution experiment derived from the MIC determination was used. After incubation, 10  $\mu$ L of each tube that did not show visible growth was subcultured onto Tryptone Soy Agar (Biokar, Beauvais, France) and incubated at 37 $^{\circ}$ C for 24 hours, and the lowest concentration that did not show any growth on the medium was considered as the minimum bactericidal concentration (MIC).



**Figures 1 and 2** Pathogens *Monilinia fructigena* and *Venturia inaequalis* (own source)

The results regarding the *Foeniculum vulgare* species also involved the preparation of three solutions each from the same base to which different essential oils were added, extracted as follows:

FV1 – vegetative stage, FV2- Beginning of flowering stage, FV3- full flowering stage.

The basic formula used was made of: Surfactant consistency 35%, Base Organic lavender 48.8%, fennel water 15 %, oil fennel essential oil 15 drops, cosgard 6 drops for a total volume of 50 ml.

Tests were carried out regarding the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC), they were performed to highlight the possible bactericidal and/or bacteriostatic effects of essential oils, and the results were recorded in tables 1 and 4.

The data resulting from the measurements were statistically analyzed using ANOVA software.

### 3. Results and discussion

**Table 1** Results on the MIC and MBC of essential oils extracted from *Foeniculum vulgare* in percentages (v/v) at three developmental stages of the plant against the pathogen *Monilinia fructigena* (Brown rot).

ID no.	<i>Monilinia fructigena</i>	CIM and CBM results of fennel essential oils % (v/v)		
		R1	R2	R3
1	CIM	1	0.50	0.50
2	CBM	2	0.50	0.50

Where R1 is FV1-Vegetative stage, R2 is FV2-Beginning of flowering stage R3 is FV3-Full flowering stage

Table 1 includes results on the MIC and MBC of essential oils extracted from *Foeniculum vulgare* in percentages (v / v) at three developmental stages of the plant used against the pathogen *Monilinia fructigena* (Brown rot).

- MIC - minimum inhibitory concentration and MBC - minimum bactericidal concentration

The MIC and CBM of the essential oils extracted from *Foeniculum vulgare* in percentages (v/v) from fennel harvested at all three stages of plant development (vegetative stage, beginning of flowering stage, full flowering stage) and applied against the pathogen *Monilinia fructigena* (Brown rot), are expressed as the average zone of inhibition of the pathogen compared to the fungicide boscalid 4 (µg/ml).

The results were highly significant as antibacterial against *Monilinia fructigena*, tested in comparison with this fungicide ( $p \leq 0.05$ ), taking into account that the essential oil was in pure form and boscalid was at a concentration of 30 µg/disc.

**Table 2** Results of the average values of MIC and MBC of essential oils extracted from *Foeniculum vulgare* in percentages (v/v) in three developmental stages of the plant used against the pathogen *Monilinia fructigena*

ID. No.	<i>Monilinia fructigena</i>	Average value	Average report%	Average difference	General average
1	CIM	0.67	80.00	- 0.16	0.83
2	CBM	1.00	120.00	0.17	

In Table 2 are the results of the average values of MIC and MBC of the essential oils extracted from *Foeniculum vulgare* in percentages (v/v) in three stages of development of the plant used against the pathogen *Monilinia fructigena*. Where the average values MIC (0.67): the minimum concentration at which the essential oil inhibits the growth of the fungus. A lower value indicates a higher antimicrobial efficiency. MBC (1): the concentration at which the essential oil completely kills the fungus. Usually equal to or greater than the MIC. The fact that the difference between the MIC (0.67) and MBC (1) is relatively small suggests that the oil has a fungicidal, not just fungistatic, effect.

**Table 3** ANOVA test results on the MIC and MBC of essential oils extracted from *Foeniculum vulgare* in percentages (v/v) at three developmental stages of the plant used against the pathogen *Monilinia fructigena*

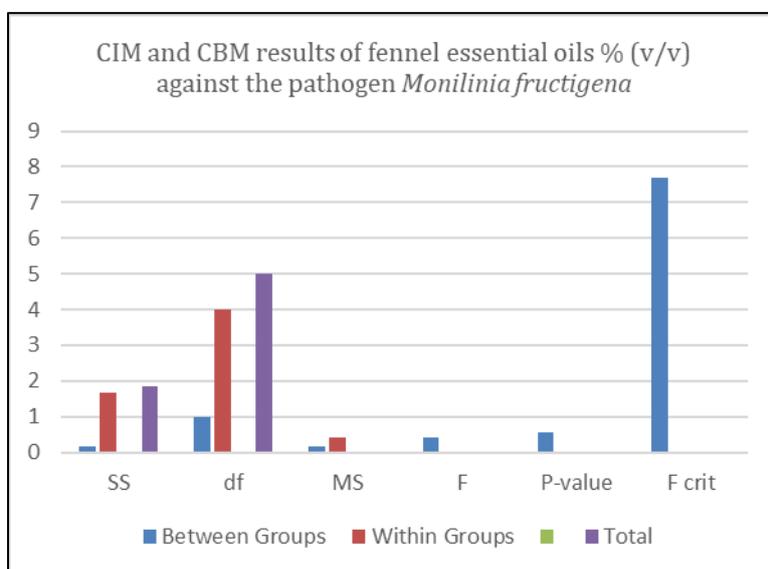
ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	0.166666667	1	0.166667	0.4	0.561438	7.708647
Within Groups	1.666666667	4	0.416667			
Total	1.833333333	5				

Table 3 has the results of the ANOVA test on the MIC and MBC of the essential oils extracted from *Foeniculum vulgare* in percentages (v/v) in three developmental stages of the plant (vegetative stage, beginning of flowering stage, full flowering stage) used against the pathogen *Monilinia fructigena*.

The results of the ANOVA analysis showed that the calculated F factor 0.400 < critical F 7.71, in this case, there is no significant difference between the groups.

The results suggest that, although slight variations in antibacterial activity (MIC/MBC) were recorded depending on the developmental stage of fennel, these variations are not statistically significant.

In other words, the essential oil largely retains its antibacterial effectiveness throughout stages FV1, FV2, FV3.



**Figure 3** Spatial representation of the results regarding the MIC and MBC of the essential oils extracted from *Foeniculum vulgare* against the pathogen *Monilinia fructigena*

Figure 3 is a spatial representation of the results regarding the MIC and MBC of the essential oils extracted from *Foeniculum vulgare* for the pathogen *Monilinia fructigena*. The overall mean = 0.83, this represents the average of the observed values (MIC and MBC), for all three stages of development of plants (vegetative stage –FV1, beginning of flowering stage-FV2, full flowering stage-FV3). A low value suggests a good treatment efficacy, reflecting the overall effectiveness of the oils - that is, a low concentration is needed to inhibit or kill the pathogen. Correlation ratio = 0.30, this is a weak positive correlation between two variables (probably between developmental stage and the antifungal effect of the oil). The value of 0.30 indicates: there is a weak, but not solid or significant, tendency for the effect to change depending on the developmental stage of the plant; The oil maintains a relatively constant efficiency, regardless of the stage.

**Conclusion:** Although there is a small variation between the antifungal effects of fennel oil depending on the plant stage (correlation of 3.30), this is not strong enough to consider that the developmental stage significantly influences its effectiveness.

In addition, the low average (0.83) indicates an effective treatment overall, and the DL of 1.46 confirms that the differences between stages are not statistically significant. It indicates a very good antifungal potential of fennel essential oils, with a predominantly fungicidal action.

**Table 4** Results on the MIC and MBC of essential oils extracted from *Foeniculum vulgare* in percentages (v/v) in three developmental stages of the plants, used against the pathogen *Venturia inaequalis*.

ID no.	<i>Venturia inaequalis</i>	CIM and CBM results of fennel essential oils % (v/v)		
		R1	R2	R3
1	CIM	1	0.50	0.50
2	CBM	1	1	0.50

Where R1 corresponds to FV1-Vegetative stage, R2 corresponds to FV2-Beginning of flowering stage, R3 corresponds to FV3-Full flowering stage

- MIC - minimum inhibitory concentration and MBC - minimum bactericidal concentration

Table 4 presents the results of the MIC and MBC of the essential oils extracted from *Foeniculum vulgare* in percentage (v/v) in three stages of development of the plants used against the pathogen *Venturia inaequalis*. The results obtained in the case of *Foeniculum vulgare* are expressed as the mean inhibition zone of pathogen by the all three biosolutions with essential oil extracted from fennel harvested in three stages of development of plants (vegetative stage –FV1, beginning of flowering stage–FV2, full flowering stage–FV3), compared to the fungicide Score 4 (µg/ml), and were highly significant as antibacterial effect against *Venturia inaequalis* pathogen which causes the disease called scab, tested in comparison to this fungicide (p ≤ 0.05).

**Table 5** Results of average values regarding the MIC and MBC of essential oils extracted from *Foeniculum vulgare* in percentages (v / v) in three developmental stages of the plants against the pathogen *Venturia inaequalis*.

ID. no	<i>Monilinia fructigena</i>	Average value	Average report%	Average difference	General average
1	CIM	0.67	88.89	- 0.08	0.75
2	CBM	0.83	111.11	0.08	

In table 5 we have the results of the average values regarding the MIC and MBC of the all three essential oils extracted from *Foeniculum vulgare* in percentages (v/v) added in biosolutions and used against the pathogen *Venturia inaequalis* where the average values, MIC = 0.67 mg/ml, the minimum concentration that inhibits the growth of the fungus. A value below 1 mg/mL suggests good antifungal efficiency. MBC = 0.83 mg/ml, the concentration at which the fungus is destroyed completely. The relatively small difference between MBC and MIC suggests that the essential oil is almost fungicidal, not just fungistatic.

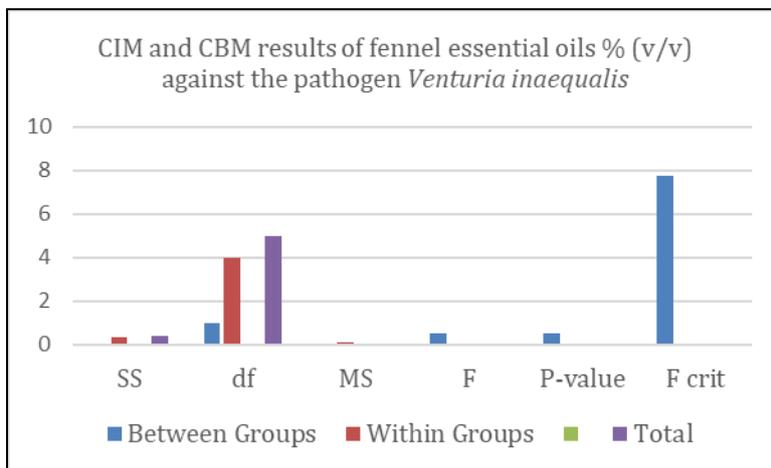
**Table 6** ANOVA test results on MIC and MBC of essential oils extracted from *Foeniculum vulgare* in percentages (v/v) in three developmental stages of plants, added in biosolutions and used against the pathogen *Venturia inaequalis*.

ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	0.041667	1	0.041667	0.5	0.518519	7.708647
Within Groups	0.333333	4	0.083333			
Total	0.375	5				

Hypotheses: H<sub>0</sub> - (null hypothesis): all groups average are equal; H<sub>1</sub> - (alternative hypothesis): at least one group has a different average.

Table 6 contains the results of the ANOVA test on the MIC and MBC of the essential oils extracted from *Foeniculum vulgare* in percentages (v/v) in three stages of development of the plants, prepared on biosolutions and used against the pathogen *Venturia inaequalis*. Since the calculated F (0.5) < critical F (7.7) it follows that the null hypothesis is not rejected, i.e. all group means are equal.

**Conclusion:** There is not enough evidence to state that there are significant differences between the means of the groups analyzed. Therefore, from a statistical point of view, the means can be considered equal at the chosen significance level ( $\leq 0.05$ ).



**Figure 4** Spatial representations of the MIC and MBC of essential oils extracted from *Foeniculum vulgare* in percentages (v / v) in three developmental stages of the plants used against the pathogen *Venturia inaequalis*

In figure 4 we have the spatial representation of the MIC and MBC of the essential oils extracted from *Foeniculum vulgare* in percentages (v/v) in three stages of development of plants and used against the pathogen *Venturia inaequalis* where Overall mean = 0.75mg/ml, this is a relatively low value for the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC). This means that the fennel oil was effective against *Venturia inaequalis*, requiring a low dose to inhibit or kill the fungus. This average reflects the overall effectiveness of the fennel oils tested. It can be used as a comparative value between treatments or phenological stages. Correlation ratio = 0.33, this value indicates a weak positive correlation between variables - most likely between the plant development stage (FV1; FV2; FV3) and the antifungal effect of the oil. This suggests that: there is a tendency for effectiveness to change slightly depending on the stage of the plant, but the influence is not strong, so the fennel essential oil has a relatively constant effect against the pathogen regardless of the time of harvest. Fennel essential oil (*Foeniculum vulgare*) exhibits effective antifungal activity against *Venturia inaequalis*, with an average concentration of 0.75mg/ml – a low value, indicating effectiveness. The weak positive correlation (0.33) shows that the developmental stage of the plant has a small influence on the effectiveness of the oil, and the differences between stages are not statistically significant (DL=0.65). Therefore, fennel oil retains its antifungal potential throughout the plant's development.

#### 4. Conclusion

The results suggest that, although slight variations in antibacterial activity (MIC/MBC) were recorded depending on the developmental stage of fennel, these variations are not statistically significant.

In other words, the essential oil largely retains its antibacterial efficacy throughout the FV1, FV2, FV3 stages. Although there is a small variation in the antifungal effects of fennel oil depending on the plant stage (correlation of 3.30), this is not strong enough to consider that the developmental stage significantly influences efficiency. In addition, the low average (0.83) indicates an effective treatment overall, and the DL of 1.46 confirms that the differences between stages are not statistically significant. It indicates a very good antifungal potential of fennel essential oils, with a predominantly fungicidal action.

Since calculated F (0.5) < critical F (7.7) it follows that the null hypothesis is not rejected, i.e. all group means are equal. There is not enough evidence to state that there are significant differences between the averages of the groups analyzed. Therefore, from a statistical point of view, the averages can be considered equal at the chosen significance level ( $\leq 0.05$ ). The study highlighted the effectiveness of fennel oil in combating the pathogen *Venturia inaequalis*, responsible for the occurrence of smut. The minimum concentration (MIC) and minimum bactericidal concentration (MBC) values recorded a low overall average of 0.75 mg/ml, indicating a high antifungal potential. The correlation ratio of 0.33 suggests a weak influence of the plant development stage on the effectiveness of the treatment, and the experimental limit difference (DL) of 0.65 confirms that the variations between the three stages (FV1; FV2; FV3) are not statistically significant. The results of the ANOVA analysis indicate that the values of the minimum inhibitory concentration and the minimum

bactericidal concentration of the essential oils from *Foeniculum vulgare* do not vary significantly between the three developmental stages ( $F$  calculated = 0.00 <  $F$  critical = 7.7), regarding their effectiveness against the pathogen *Monilinia*. Therefore, the harvest phase of the plant does not significantly influence the antifungal activity of the tested oil. Although there is a small variation between the antifungal effects of fennel oil depending on the stage of the plant (correlation of 3.30), this is not strong enough to consider that the developmental stage significantly influences the effectiveness. In addition, the low average (0.83) indicates an effective treatment overall, and the DL of 1.46 confirms that the differences between stages are not statistically significant. It indicates a very good antifungal potential of fennel essential oils, with a predominantly fungicidal action. Fennel essential oil has consistent and high antifungal efficacy against storage pathogens, regardless of the time of harvest (vegetative stage, early flowering, full flowering).

From a statistical and practical point of view, any stage can be chosen for extraction, without loss of anti-fungal efficiency. Therefore, fennel essential oil can be considered a viable natural alternative in phytosanitary protection strategies against *Venturia inaequalis*.

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## Compliance with ethical standards

### Disclosure of conflict of interest

No conflict of interest to be disclosed.

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