# EFFECTS OF FOLIAR FERTILIZATION ON ESSENTIAL OIL COMPOSITION AND ANTIOXIDANT ACTIVITIES OF TWO VARIETIES OF FENNEL

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**Abstract**

Volatile oils extracted from various species of aromatic and medicinal plants establish a certain category of raw materials of high interest both in food industry and in cosmetics or perfumery. The chemical compounds from essential oils composition are much more appreciated, compared to synthetic substances, whereas natural extractions are associated with a much lower number of risk factors for population health. This research is topical at international level, because there is a lack of scientific data that would highlight as clearly as possible the influence of some factors on the quality of the essential oil obtained from aromatic and medicinal plants. The aim of this research was to determine the influence of foliar fertilizers on essential oil composition and antioxidant activities of two varieties of *Foeniculum vulgare* Mill., var. *vulgare* and var. *dulce*. The field research was established in USV Iasi Research Station – Ezăreni Farm, on May 2021, using a randomized block design with three replications. The data obtained in this research showed a significant influence of foliar treatments on the major components of the essential oil of bitter fennel and sweet fennel seeds. The main components of the oil extracted from fennel seeds were: anethole, fenchone, estragole, anisaldehyde and α-pinene. Also, by using complex foliar treatments (macronutrients, micronutrients and various amino acids) the specific synthesis processes of secondary metabolites (phenolic components, flavonoids) can be positively influenced.

**Key words**: chemical composition, sweet fennel, bitter fennel, foliar fertilization, antioxidant activity

*Foeniculum vulgare* Mill. is an aromatic and medicinal plant species, being part of *Apiaceae (Umbelliferae)* botanical family. Since ancient times, this plant has been used as medicine, and can be used as a remedy for a wide range of medical conditions.

Volatile oils extracted from various species of aromatic and medicinal plants form a certain category of raw materials of high interest both in food industry and in cosmetics. The chemical compounds from essential oils composition are much more appreciated, compared to synthetic substances, whereas natural extractions are associated with a much lower number of risk factors for population health.

## MATERIALS AND METHOD

**Plant material**

Two fennel varieties, var. *vulgare* and var. *dulce*, used in this experiment were obtained from the University of Life Sciences „Ion Ionescu de la Brad", Iași and from National Agricultural Research and Development Institute Fundulea.

**Field experiment**

The field research was located in USV Iasi Research Station – Ezăreni Farm. A randomized block design with three replications was used in the experiment. The field experiment was established in May 2021 using 2 months old fennel seedlings. The harvesting was on October 2021, when plants reached ripe stage – the primary umbels were fully matured and the seeds were brownish. The row spacing was 50 cm, and 30 cm between plants on the row.

**Foliar fertilization**

The research included the usage of three foliar fertilizers, with different chemical composition: AA+M+µ (NPK, micronutrients and amino acids), M+µ (NPK and micronutrients), AA (Organic foliar fertilizer with animal hydrolyzed proteins - 20 amino acids). The treatments were applied one time, at the same development stage - fulfillment of inflorescence rachis.

**Essential oil extraction**

After harvesting, the fennel seeds were separated from the umbels and then were shade-exsiccated for 14 days. Dried seeds (500 g/sample) were distilled for 3 hours using the hydro distillation method in a Clevenger type apparatus, according to European Pharmacopeia (European Pharmacopeia, 2005). The collected volatile oil was then dried using anhydrous sodium sulfate and kept at 4°C, prior to chromatographic analysis.

**Volatile oil GC-MS analysis**

Chemical composition of essential oils was analyzed using gas chromatographic – mass spectrometry method. The GC-MS analysis was carried out using GC-MS Triple Quad (Agilent) apparatus, with helium 5.0 as carrier gas. The oils were diluted in methanol

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of analytical purity, and then 1 µl of each sample were injected into the system. The column temperature was first programmed at 60°C for 2 minutes, then gradually raised to 240°C at 10°C/min and kept for 10 minutes. The essential oil chemical components were identified by comparison with those saved in NIST MS-08 database.

**Seed extracts preparation**

Prior the antioxidant analysis, determination of polyphenols and flavonoids, the seeds were ground and then the extracts (5 g/ sample) were prepared by incubation with 95 mL ethanol (80%). After 24 hours, the seeds extracts were filtered. The analyzes were carried out in triplicate using a UV/Vis spectrophotometer DLAB PS-V1100.

**Antioxidant activity**

In order to determine the antioxidant activity, 0,1 mL of seed extract was incubated with 2.9 mL of alcoholic solution od DPPH (60 µM) for 180 minutes. The absorbance of the samples (optical density OD) was read at 515 nm.

The DPPH radical scavenging activity was expressed as ratio (% inhibition) to the maximum absorbance of the DPPH solution.

**Total phenolic content (TPC)**

The quantification of total polyphenols was performed using Folin-Ciocalteu method (Lobiuc A., *et al*, 2017). The absorbance was read at 760 nm using the spectrophotometer, with the control sample consisting of the same reagents and the seed extract being replaced by an equal volume of solvent. The results were expressed as mg gallic acid (GAE) $g^{-1}$ sample.

**Total flavonoid content (TFC)**

Total flavonoid content analysis was performed using aluminium chloride method (Lobiuc A., *et al*., 2017). The seed extract was mixed with $NaNO_2$ (5%) and $AlCl_3$ (10%). Subsequently incubation, the absorbance was read at 510 nm. The results were reported as quercetin (QE) $g^{-1}$ seed sample.

**Statistical analysis**

All results were referred as mean of three replications. Data were analyzed by analysis of variance (ANOVA) and in order to assess the difference between treatment means, the Tukey test was performed. Statistical analysis was achieved using IBM SPSS 28.0.

## RESULTS AND DISCUSSIONS

### Climate conditions

In 2021, the highest temperature value was registered in July, 22.2°C, and in February was reported the lowest value, -0.8°C. The values were related to the multiannual average, with slight differences.

The year 2021 was defined by a higher rainfall regime (with an annual sum of 691.8 mm) compared with the multiannual average sum for Iași region (517.8 mm). The climatic conditions for year 2021 are shown in *table 1*.

Table 1

**Climatic conditions for 2021 in Iași Region**

| Month | Average Precipitations (mm) | Average Air Temperature (°C) |
|---|---|---|
| **January** | 26.8 | -0.7 |
| **February** | 22.8 | -0.8 |
| **March** | 65.5 | 3.1 |
| **April** | 56.4 | 7.8 |
| **May** | 86 | 14.9 |
| **June** | 107.4 | 19.8 |
| **July** | 80.3 | 22.2 |
| **August** | 155.6 | 20.9 |
| **September** | 12.4 | 14.4 |

### Influence of foliar fertilizers on the chemical composition of fennel essential oil

The chemical composition of essential oils extracted from the two fennel varieties (var. *dulce* and var. *vulgare*) are presented on *table 2*. The chemical elements are indexed according to their elution time from chromatographic column.

Following the GC-MS analysis, 15 chemical components were identified in the volatile oil extracted from sweet fennel and bitter fennel fruits.

The main components of the oil extracted from *Foeniculum vulgare* var. *vulgare* were: anethole (70.49% - 76.21%), fencone (12.03% - 14.25%), estragole (3.45% - 4.19%), anisaldehyde (1.05% - 5.51%) and α-pinene (2.08% - 3%).

The major components of the volatile oil of *Foeniculum vulgare* var. *dulce* were: anethole (64.89% - 74.65%), fencone (12.43% - 15.34%), estragole (5.69% - 7.79%) and α-pinene (2.14% - 5.61%).

The data obtained in the research showed a significant influence of foliar treatments on the major components of the essential oil of bitter fennel and sweet fennel seeds.

### Influence of foliar treatments on antioxidant activity

Alcoholic extracts from fennel seeds contain high amounts of phenolic components, most of them also having the ability to reduce free radicals. The main such compounds are caffeoylquinic acid, rosmarinic acid, 1,5 dicaffeoylquinic acid, hyperoside. Hydroxycinnamic acid derivatives and glycosides have also been identified. (Parejo I.V. *et al*, 2004)

From the results presented on *figure 1*, it is positive that foliar fertilization has a significant influence on the free radical scavenging activity of fennel seeds extracts. *Foeniculum vulgare* var. *vulgare* established a lower antioxidant activity, compared to *Foeniculum vulgare* var. *dulce* where the data recorded higher values (over 50%).

A clear influence of foliar treatments can also be observed, in the case of sweet fennel, all values being improved by using different nutrients. Statistically significant differences were obtained with fertilizers AA+M+µ (22%) and M+µ (21%).

In bitter fennel plants, the usage of foliar fertilizers based on AA+M+µ and M+µ, contributed to an increase in antioxidant activity, while the value obtained in case of the treatment with amino acids presented a lower value and similar to blank value (35.62 % inhibition against DPPH solution).

Table 2

**Main fennel essential oil compounds (%)**

Means with the same lower-case letter are not significantly different at P < 0.05 according to Tukey's test

| Fennel variety | var. *vulgare* | | | | var. *dulce* | | | |
|---|---|---|---|---|---|---|---|---|
| **Compound** | **blank** | **AA** | **AA+M+µ** | **M+µ** | **blank** | **AA** | **AA+M+µ** | M+µ |
| **1R-α-pinene** | 2.71$^{a}$ | 2.08$^{a}$ | 2.62$^{a}$ | 3.00$^{a}$ | 3.93$^{b}$ | 5.61$^{c}$ | 2.14$^{a}$ | 4.31$^{b}$ |
| **camphene** | 0.12$^{ab}$ | 0.05$^{a}$ | 0.07$^{a}$ | 0.07$^{a}$ | 0.07$^{a}$ | 0.11$^{ab}$ | 0.05$^{a}$ | 0.10$^{ab}$ |
| **β-pinene** | 0.22$^{a}$ | 0.2$^{a}$ | 0.25$^{a}$ | 0.28$^{a}$ | 0.33$^{a}$ | 0.46$^{ab}$ | 0.23$^{a}$ | 0.36$^{a}$ |
| **β-terpinene** | 0.09$^{a}$ | 0.07$^{a}$ | 0.06$^{a}$ | 0.09$^{a}$ | 0.09$^{a}$ | 0.13$^{a}$ | 0.09$^{a}$ | 0.12$^{a}$ |
| **α-phellandrene** | 0.05$^{a}$ | 0.30$^{b}$ | 0.71$^{c}$ | 0.18$^{b}$ | 1.74$^{a}$ | 2.23$^{b}$ | 1.40$^{a}$ | 1.74$^{a}$ |
| **D-limonene** | 1.12$^{b}$ | 0.61$^{a}$ | 0.77$^{a}$ | 0.74$^{a}$ | 0.86$^{ab}$ | 1.10$^{b}$ | 0.68$^{a}$ | 1.10$^{b}$ |
| **β-phellandrene** | 0.43$^{a}$ | 0.37$^{a}$ | 0.31$^{a}$ | 0.35$^{a}$ | 0.50$^{a}$ | 0.64$^{a}$ | 0.53$^{a}$ | 0.66$^{a}$ |
| **γ-terpinene** | 0.62$^{b}$ | 0.15$^{a}$ | 0.25$^{a}$ | 0.16$^{a}$ | 0.58$^{a}$ | 0.70$^{ab}$ | 0.45$^{a}$ | 0.66$^{ab}$ |
| **β- cymene** | 0.27$^{a}$ | 0.69$^{ab}$ | 0.58$^{ab}$ | 0.95$^{b}$ | 0.79$^{a}$ | 0.81$^{a}$ | 0.64$^{a}$ | 0.78$^{a}$ |
| **L-fenchone** | 14.25$^{b}$ | 12.03$^{a}$ | 12.92$^{a}$ | 13.68$^{ab}$ | 12.43$^{a}$ | 15.02$^{b}$ | 12.82$^{a}$ | 15.34$^{b}$ |
| **alcanfor** | 0.29$^{a}$ | 0.25$^{a}$ | 0.26$^{a}$ | 0.29$^{a}$ | 0.24$^{a}$ | 0.29$^{a}$ | 0.26$^{a}$ | 0.31$^{a}$ |
| **4-terpineol** | 0.07$^{a}$ | 0.07$^{a}$ | 0.06$^{a}$ | 0.06$^{a}$ | 0.04$^{a}$ | 0.08$^{a}$ | 0.07$^{a}$ | 0.09$^{a}$ |
| **estragole** | 3.65$^{a}$ | 4.19$^{ab}$ | 3.56$^{a}$ | 4.14$^{ab}$ | 6.03$^{ab}$ | 7.79$^{b}$ | 5.69$^{a}$ | 7.38$^{b}$ |
| **anethole** | 75.46$^{b}$ | 74.80$^{b}$ | 76.21$^{b}$ | 70.49$^{a}$ | 72.14$^{b}$ | 64.89$^{a}$ | 74.65$^{b}$ | 66.81$^{a}$ |
| **p-anisaldehyde** | 1.34$^{a}$ | 4.15$^{b}$ | 1.38$^{a}$ | 5.51$^{b}$ | 0.22$^{a}$ | 0.15$^{a}$ | 0.29$^{a}$ | 0.23$^{a}$ |
| **Total identified** | **99.90** | **99.81** | **99.91** | **99.99** | **99.99** | **99.98** | **99.99** | **99.99** |

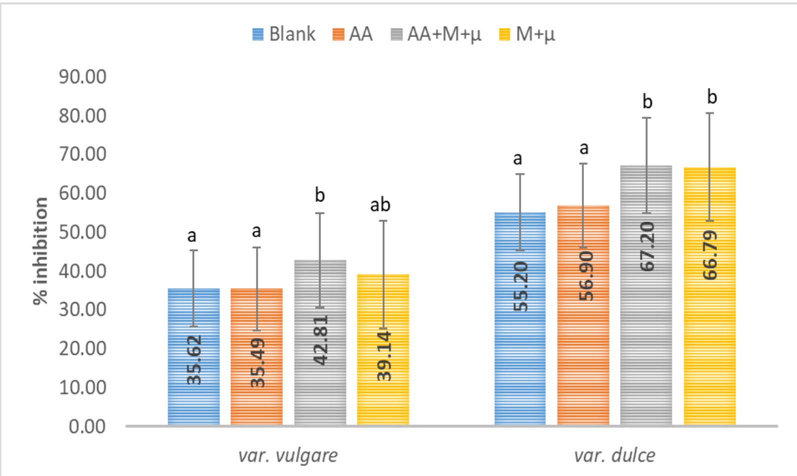


Figure 1 **Antioxidant activity of ethanolic extracts from fennel seeds**

Means with the same lower-case letter are not significantly different at P < 0.05 according to Tukey's test

**Influence of foliar fertilizers on total phenolic content (TPC)**

Phenolic compounds from plant substrates are secondary metabolites that have different roles, such as protection against the actions of biological factors, structural and signaling roles between organism cells. The synthesis processes of these chemical substances can be modified under the action of the compounds present in the structure of the fertilizers (Veberic R., 2016).

Fertilization has a stimulating effect on the content of polyphenolic compounds in case of *Foeniculum vulgare* Mill. This improvement of components with antioxidant actions may be due to the influence of fertilizers in achieving the biosynthesis of flavonoids and polyphenols through the acetate and shikimic acid pathway. (Sousa C. *et al*, 2008)

The synthesis of phenolic components in both fennel varieties was significantly influenced by using foliar treatments (*figure 2*). The data in case of var. *vulgare* were increased with all three fertilizers, the results showed statistically significant differences for treatment with AA+M+µ (10.71 mg gallic acid/g extract) with an increase of 27.5%, compared to blank, where phenolic content was 8.40 mg GAE. Also, at var. *dulce*, all treatments induced an important improvement in the content of phenolic components of the ethanolic extracts, the differences compared to the control being statistically significant, P <0.05.

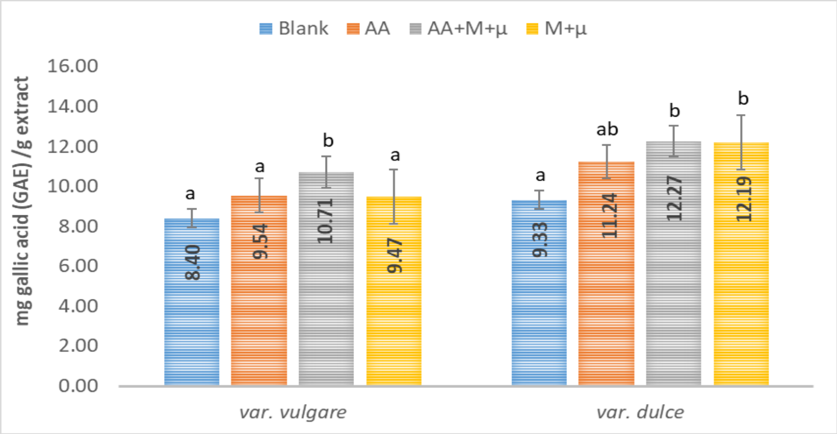


Figure 2 **Total phenolic content of ethanolic extracts from fennel seeds**
Means with the same lower-case letter are not significantly different at P < 0.05 according to Tukey's test

**Influence of foliar treatments on total flavonoid content (TFC)**

From data showed in *figure 3*, the total flavonoid content of the ethanolic extracts of var. *vulgare* was lower (14.76 – 18.62 mg quercetin/g extract) in comparison with var. *dulce*, where the flavonoid compounds registered values between 19.41 mg QE and 23.68 mg quercetin/g extract.

The application of foliar fertilizers significantly influenced the synthesis of flavonoids in bitter fennel plants, the differences being statistically significant (P<0.05) in case of the treatments with amino acids (AA) and complex fertilizer (amino acids, macroelements and microelements - AA+M+µ).

For sweet fennel plants, the most important effect was identified with AA+M+µ fertilization, with a positive difference of 21%, compared to the reference plants. Higher values were also achieved when fertilizing with macronutrients and micronutrients, with a total flavonoid content of 21.53 mg quercetin/g extract.

By using some additions such as amino acids and other stimulants, the ability to synthesize beneficial substances by the plants, as well as the physiological activities, can be improved. It can also increase the rate of some metabolic transformations, including the synthesis processes of secondary metabolites. However, nitrogen fertilization has as main result the reduction of phenolic content in plant substrates. This is explained by the competition that occurs between the synthesis reactions of secondary components and the basic processes for obtaining the proteins and essential amino acids in growth and development of plants (Koricheva J., 1998).

So, the increase of the concentration of polyphenolic compounds, as well as the antioxidant activity of ethanolic extracts of both fennel varieties, by using a treatment with AA+M+µ, is due to the combined action of the amino acids and especially of the microelements, and to a minor extent of macronutrients nitrogen, phosphorus and potassium.

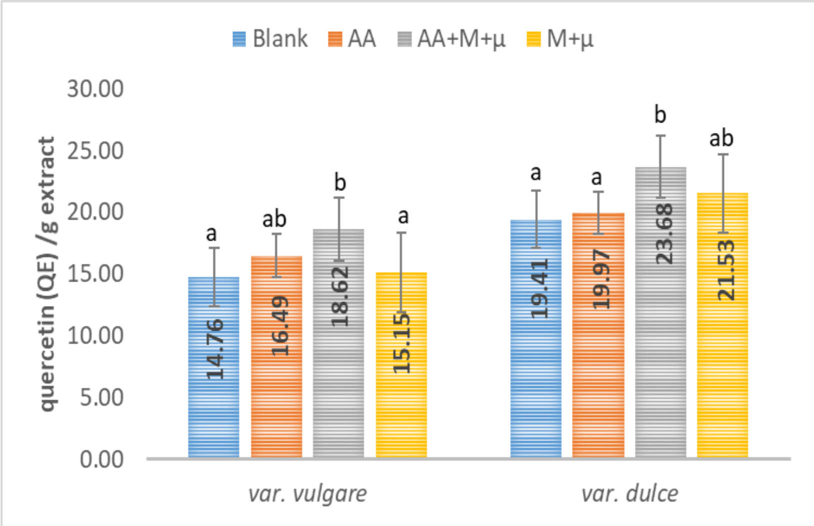


Figure 3 **Total flavonoid content of ethanolic extracts from fennel seeds**
Means with the same lower-case letter are not significantly different at $P < 0.05$ according to Tukey's test

The results of this research can also be correlated with those obtained in other studies.

Damjanović A.M. *et al*, (2005) determined the main chemical compounds in the composition of fennel essential oil, from Montenegro: trans-anethole (62.0%), fencone (20.3%), estragole (4.90%) and limonene (3.15%).

Anwar F. *et al*, (2009) determined the main components of fennel essential oil: trans-anethole (69.87%), fencone (10.23%) and estragole (5.45%), with a total of 23 chemical compounds identified.

Olgun Ç. *et al*, in 2017, identified, as the main component in the volatile extract of *Foeniculum vulgare* Mill., anethole in proportion of 80%.

Certain phenylpropanoic compounds were also identified in the composition of the volatile oil: p-methoxy-phenyl-acetone, anisic acid, anisic ketone, dihydroanethole, anisic aldehyde and p-methoxy-1-phenyl-1-propanol (Anka Z.M. *et al*, 2019).

## CONCLUSIONS

Based on the data showed in this research, it can be concluded that all antioxidant activity values were improved by using the foliar treatments. Also, the synthesis of phenolic and flavonoids components in plants from both fennel varieties was significantly influenced by the application of foliar fertilizers.

Furthermore, the chemical composition of the essential oil of sweet and bitter fennel seeds is substantially influenced through use of foliar nutrients, due to the combined action of the amino acids and especially of the microelements.

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