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EVALUATION OF ESSENTIAL OIL YIELD AND CHEMICAL COMPONENTS OF SELECTED BASIL CULTIVARS

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ABSTRACT

The essential oils yield and the chemical compositions of essential oils of broad and narrow basil varieties were assessed for two years. The essential oil was obtained via hydrodistillation and analyzed using gas chromatography. The essential oil extraction yield varied among basil cultivars. The oil extraction yield for broad leaf basil cultivars, Large Leaf Basil and Genovese, was 1.3% and 2.1% respectively, while narrow leaf basil cultivars, Finissimo Verde a Palla and Larosa Emanuele-Sementi, had oil extraction yields of 1.5% and 1.8%, respectively. Regarding the active substances of basil oil, linalool and eugenol were dependent on the cultivar and year of cultivation. In the broad leaf basil cultivars the concentration of linalool remained constant during the two cultivation years, while eugenol content varied from the first to the second year of cultivation. In narrow leaf basil the content of linalool content fluctuated with cultivation year, while eugenol was the same in both experimental years. The broad leaf basil cultivar had on average for both cultivation years 3.8 mg g^{-1} linallol and 0.5 mg g^{-1} eugenol and the narrow leaf basil had 2.8 mg g^{-1} linallol and 0.7 mg g^{-1} eugenol.

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Introduction

The sweet basil (*Ocimum basilicum* L.) belongs to the *Lamiaceae* family and the *Ocimum* genus. It is an annual herbaceous and aromatic plant which yields many essential oils (9). It is believed to have originated in India and introduced to Europe in the 12th century. Today basil is cultivated and distilled for oil in many countries like France, Belgium, Hungary and the USA (3, 5). The basil essential oil has antimicrobial and insecticidal activities which in combination with its pleasant aroma make basil essential oil a major aromatic agent in various industries such as food, pharmaceutical, cosmetic, and aromatherapy industries (1, 10).

The wide distribution throughout the world and the production of new cultivars through breeding programs have resulted in a great variation in the essential oil composition among basil cultivars currently on the international market (13).

Intensive investigations around the world have revealed that the concentration of the main active substances of basil oil, estragol, linalool, 1-8-cineol, methyl chavicol, camphor, eugenol, and geraniol, varies among basil chemotypes (4, 6, 7). According to Petropoulos and Vlachou (8) the majority of Greek basil population belongs to European chemotype (sweet basil, Genovease).

There are reports concerning the activity of basil essential oil from Greek populations as an herbicide (11), and its effect on physical and chemical soil properties (2); however, little attention has been given on essential oil yield extraction from Greek basil populations. The aim of this work was to study the variations of essential oils extraction yield and the chemical composition of basil oil between broad and narrow leaf basil cultivars.

Materials and Methods

An experiment was conducted for two years at the Technological Education Institute of Larissa, Laboratory of aromatic and medical plants. The trial comprised two broad basil cultivars, *Ocimum basilicum var. latifolia* (cv. Genovese and Large Leaf Basil) and two narrow leaf basil cultivars, *O. basilicum var. minima* (cv. Finissimo Verde a Palla and Larosa Emanuele-Sementi), which were used for volatile oil isolation.

Plant husbandry

The two basil cultivars were produced in hot frame and were transplanted in field blocks (2 × 1 m) at the Technological Education Institute of Larissa. The soil was loamy sand, with pH 6.5, organic mater 1.6% and field holding capacity 40%. Plants were irrigated according to local common practices, and were fertilized with nitrogen, phosphorus and potassium. Annually, 100 kg N, 60 kg P_2O_5 and 100 kg K_2O per hectare were used for plant fertilization.

Sample preparation

At the beginning of the blossom period, the aboveground part of the plants, at 10 cm above the soil surface, were harvested. Soon afterwards, plant leaves without stalks and inflorescences were collected. Dry matter content was estimated after weighting plant tissues immediately after removal (fresh mass)

and air dried at room temperature in a well aerated room until weight remained constant (dry mass).

Extraction and analysis of basil essential oils

A sample (10 g) of air dried basil leaf and inflorescences were used for essential oil distillation. Basil oil was extracted by hydrodistillation using an Electrothermal type (UK) apparatus. The duration of this procedure was 2 hours. The yield (v/w) of the obtained essential oil was expressed as a percentage of absolute dry weight.

The volatile constituents of basil oils were analyzed using GC/MS instrument (Agillent 7890 type gas chromatograph and Agillent 5975 mass-selective detector). Helium was used as carrier gas at a flow rate of 3 ml min^{-1}. Separation of oil substances was done with a DB-WAX capillary column (30 m × 0.25mm; film thickness 0.25 μm).

Essential oil solution (1 μl) in methanol was injected using split mode (split ratio 100:1) and analyzed with the column held to 40°C for 3 min, raised initially to 185°C with 15°C min^{-1} heating ramp and then to 250°C at the rate of 10°C min^{-1}, isothermal at this temperature for 5 min. Injector and interface temperature was set at 300°C and 230°C, respectively. The electron impact mass spectra were collected at an ionization voltage of 70 eV over the *m/z* range 30–450.

The components of oil samples were identified with the use of commercial standard eugenol (99.8% pure) and linalool (98.5% pure). Compounds were further identified using their MS data compared to the NIST mass spectral library.

The experiment was a randomized block design with two cultivars with four replications per cultivar.

Statistical analysis

Data were analyzed using the SPSS statistical package. Analysis of variance was used to assess treatment effects. Mean separation was done using the standard error of differences at the 5% level, when significant differences between varieties were found.

Results and Discussion

There were not significant differences in the weight of green and dry drug between the broad and narrow leaf basil varieties (**Fig. 1**).

The average fresh weight of the basil varieties Genovese, Large Leaf Basil, Finissimo Verde a Palla and Larosa Emanuele-Sementi studied was 888 g m^{-2}, 800 g m^{-2}, 690 g m^{-2} and 730 g m^{-2} respectively. The dry weight was 130 g m^{-2} for Genovese, 210 g m^{-2} for Large Leaf Basil, 100 g m^{-2} for Finissimo Verde a Palla and 120 g m^{-2} for Larosa Emanuele-Sementi (**Fig 1**).

On average for both cultivation years, the yield in green drug of broad leaf basil cultivars was 8430 kg ha^{-1} and that of the dry drug was 1672 kg ha^{-1}. For narrow leaf basil cultivars the yield in green and dry drug was 7076 kg ha^{-1} and 1070 Kg ha^{-1} respectively. In addition, the ratio green to dry drug was 5:1 for broad leaf basil and 6.5:1 for narrow leaf basil cultivar. According to Dachler and Pelzman (4) the green to dry drug ratio in basil varied from 7:1 to 8:1. In our experiment, broad leaf basil had better yield in dry drug, while the ratio green to dry drug for the narrow leaf basil was similar to that in international literature (12). The better production in dry drug was probably due to the climatic conditions of the cultivation area.

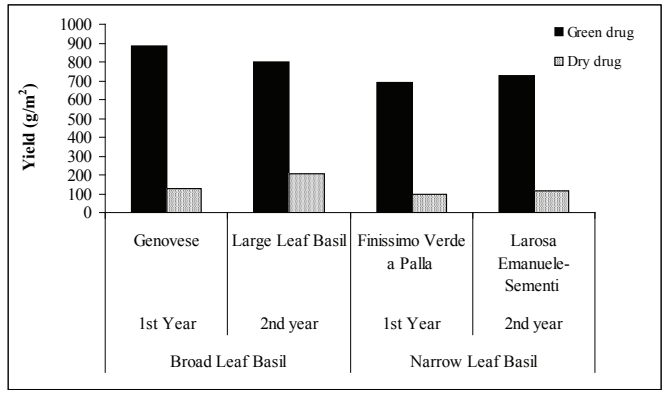

Fig. 1. Evaluation of green and dry drug weight in basil varieties.

The essential oil yield differed between varieties. The two broad leaf basil varieties, Large Leaf Basil and Genovese, had the lowest (1.3%) and highest content in oil (2.1%) respectively, of all the varieties evaluated. The narrow leaf basil variety Finissimo Verde a Palla had oil content equal to 1.5%, while for Larosa Emanuele-Sementi variety the oil content was 1.8% (**Fig. 2**).

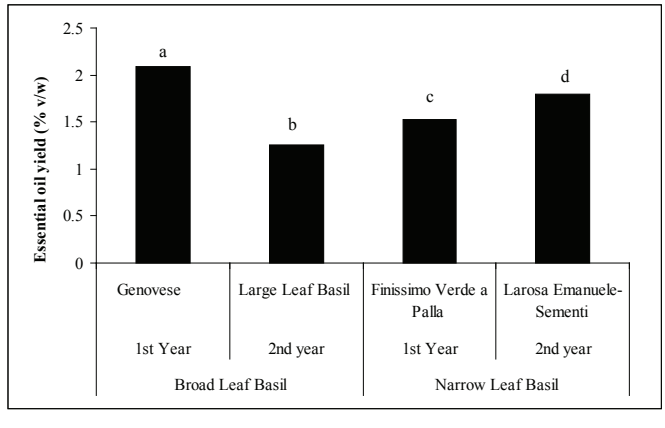

Fig. 2. Evaluation of essential oil yield in two basil varieties (broad and narrow leaf basil) and two years. The letter above each bar gives differences between varieties according to Duncan test ($P < 0.001$, sed = 0.01).

In the first experimental year the broad leaf basil had higher yield in essential oil (2.1%) compared to narrow leaf basil. In contrast, in the second year the narrow leaf basil was more productive in essential oil extraction yield (1.8%) compared to broad leaf basil. In this study the production of essential oils varied with the year of cultivation and basil variety. According to Hanus *et al*. (6), Dachler and Pelzman (4), and Marquard and Kroth (7), the concentration of basil essential oil depends on many factors such as plant genotype and variety, year of culture, date of sowing and harvest time. Hanus et al. (6)

reported variations in the basil oil extraction yields between 0.04 to 0.7%, while Dachler and Pelzman (4) from 0.5 to 1.5%.

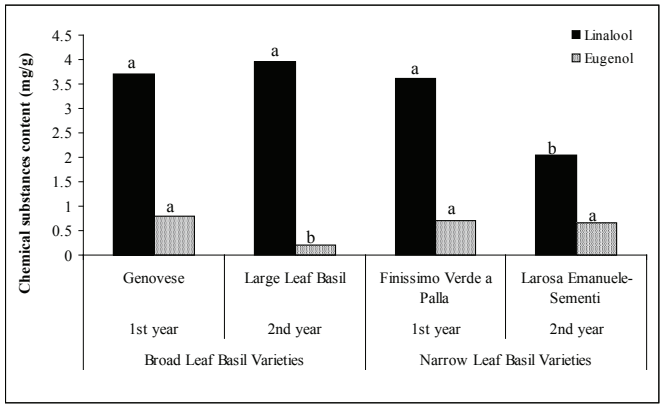

Fig. 3. Differences in linalool and eugenol content in dry drug among basil varieties in two years. The letter above each bar gives differences between varieties according to Duncan test ($P < 0.001$, sed = 0.2).

The percentage of oil yield found in this study was higher (1.3 - 2.1%) than previously reported extractions due to plant tissues (e.g. leaf and inflorescence without shoots) which were selected for oil extraction (12).

The basil essential oil extraction yield is considerably important in the international market. According to international standards, a product is commercially acceptable when its essential oil consecration is higher than 0.4% (6, 7).

The main active substances (linalool and eugenol) content of basil varieties showed remarkable differences which depended on the variety. Linalool was in greater content in dry basil drug than eugenol in both years and all varieties. Furthermore, in broad leaf basil the concentration of linalool remained constant with time, while eugenol content varied from the first to the second year of cultivation. Concerning narrow leaf basil, the content of eugenol was the same in both experimental years. In contrast, the linalool content fluctuated with cultivation year (**Fig. 3**). Generally, the broad leaf basil cultivar had a greater content of linalool (3.8 mg g^{-1}) and lower content of eugenol (0.5 mg g^{-1}) than narrow leaf basil cultivar which had linalool content 2.8 mg g^{-1} and eugenol 0.7 mg g^{-1}.

Conclusions

The production of basil dry drug and essential oil extraction yield depends on the variety, year and area of cultivation. The broad leaf basil had greater dry drug yield than narrow leaf basil in both years. On average, broad and narrow leaf basil cultivars had the same essential oil yield in both years (broad leaf basil 1.67%; narrow leaf basil 1.66%). Linalool exhibited the highest concentration followed by eugenol in all basil varieties and both experimental years. The mean concentration of linalool for both experimental years was higher in broad leaf basil compared to narrow leaf basil. However, eugenol had a higher mean concentration for both years in narrow leaf basil than in broad leaf basil varieties.

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