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Agronomic performance and essential oil composition of *Ocimum basilicum* L.: Effect of genotype and date of harvest

A. Vazquez[(1)], E. Sanchez*, C. van Baren, D. Frezza***

* *Cátedra de Horticultura, Facultad de Agronomía de la Universidad de Buenos Aires, Av. San Martin 4453 C1417DSE, Buenos Aires, Argentina.*

** *Cátedra de Farmacognosia, Facultad de Farmacia y Bioquímica de la Universidad de Buenos Aires, Junin 954, 2° piso, C1113AAD, Buenos Aires, Argentina.*

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Abstract: An experiment was conducted to assess the agronomic performance and essential oil composition of *Ocimum basilicum* L. (basil) with two genotypes during autumn-winter cycle, in a hydroponic system in greenhouse. Genotypes (i.e. green and purple) were provided to study productive parameters. Two harvest dates and both genotypes formed treatments to investigate oil composition and its stability. One pruning was made before the last harvests. Results of fresh and dry weight (g. plant^{-1}), absolute growth rate (g. day^{-1}) and relative growth (g. g^{-1}.day^{-1}) and yield (g. plant^{-1}) showed great differences in comparison with the optimal growing season values. Although pruning encourages new growth, it was strongly reduced in purple basil. Essential oil composition varied for both genotypes and between harvest dates. Linalool prevailed at the first harvest date whereas methyl-eugenol increased towards the second harvest date, and significantly in purple basil. Radiation and temperature data showed a downward trend during the cycle which influenced biomass production and essential oil composition. Green basil had better productive behavior than the purple variety. Essential oil stability between harvest dates varied for both genotypes. Pruning strongly affected purple basil growth which altered essential oil composition. The findings presented in this study confirm that it is possible to grow basil in autumn-winter season in greenhouse. Although yield slightly decreases in comparison with optimal growing season, high quality aromatic plants can be obtained.

1. Introduction

Sweet Basil (*Ocimum basilicum* L.) is an annual herbaceous crop cultivated mainly for culinary purposes. There is a significant demand by consumers seeking fresh and high quality herbs all year round. The volatile oils in basil are responsible for its characteristic aroma (Fischer *et al.,* 2011) and flavor as a condiment, which along with color and freshness determine its commercial value. Essential oil aromatic compounds and productive behavior are affected by the environment, genotype and agronomic techniques. Some chemotypes from different geographic origins have been classified based on the aroma profiles of essential oil (Suppakul *et al.,* 2003). Another classification of *O. basilicum* cultivars has been made according to morphology, height, leaf color, dimension and flower color (Darrah, 1980). Yield and essential oil composition are remarkably variable between purple and green genotypes (Marotti *et al.,* 1996; Sajjadi, 2006) which also differ in biomass yield (Hochmuth and Leon, 1999). Basil is cultivated under a range of conditions but temperate climates are the most suitable for the crop. Chang *et al.* (2005) stated that the maximum dry matter content was obtained with temperatures of 30°C. Putievsky (1983) reported that increasing daytime temperatures between 21°C and 30°C enhanced plant height. Light influences essential oil composition and productive behavior. When the irradiance level decreases the methyl-eugenol content increases, plants are smaller, have thinner leaves, less dry and fresh weight, sprouts and foliar area, whereas with high irradiance levels, linalool, eugenol and the total content of essential oil rise and photosynthesis and growth rate increase. Under high irradiance conditions, more photosynthates are biosynthesized and a greater amount of secondary metabolites accumulate (Chang *et al*., 2008). Although this aromatic crop is grown in open field and greenhouse conditions, hydroponic basil cultivation in a protected environment is an efficient commercial alternative, most importantly for those areas with limited agricultural soils and dependant on irrigation (Hassanpouraghdam *et al*., 2010). The benefits of this system include high quality plants, rapid growth, off-season and

[(1)] Corresponding author: avazquez@agro.uba.ar

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all-year-round production, maximizing the benefits for producers. On the other hand, in a pure hydroponic system the nutritive solution is recycled, reducing the environmental impact and with minimal groundwater contamination (Resh, 2001). Nutritive solution management is important to obtain plants with high yield and good quality.

Studies regarding electrical conductivity demonstrated that the highest fresh weight (g.plant^{-1}) was obtained with 1.5 ds.m^{-1} and it did not affect essential oil concentration (Carrasco and Izquierdo, 1996), while values above 3 ds.m^{-1} affected plant growth. Considering environmental conditions, the production system and the different varieties of *Ocimum basilicum* L., the aim of this work was to evaluate the agronomic performance and essential oil composition of green and purple genotypes during the autumn-winter cycle.

2. Materials and Methods

The trials were carried out in the experimental fields of the Horticultural Department of Agriculture College of the University of Buenos Aires, in a polyethylene-metallic greenhouse. Seeds of two varieties of basil, purple and green (*Ocimum basilicum* var. Violeto and *Ocimum basilicum* var. Genovese) were obtained from Zorzi, di Hortus sementi SRL. Seeds were sown, at the beginning of autumn to finish the crop cycle in winter, in expanded polystyrene growing trays on a soilless media mix (vermiculite, peat moss, perlite and fertilizer NPK with micro elements 1.3 g l^{-1}, pH 5.5-6.5, with fine structure).The trays were located in a hydroponic floating system until the seedlings had two to three pairs of unfolded leaves. Plants were transplanted into a closed hydroponic NFT (Nutrient Film Technique) system. A low polyamide tunnel was built to avoid frost damage and it was used from late afternoon to early morning each day. The nutrient solution was composed of Ammonium Nitrate 5.625 g, Potassium Nitrate 75 g, Calcium Nitrate 93.75 g, Mono Potassium Phosphate 28.13 g, Magnesium Sulphate 33.75 g and micro elements 18.75 c.c. Crop density was 25 pl. m^{-2}. Electrical conductivity and pH of the nutrient solution were measured three times a week. Environmental temperature (°C), radiation (W.m^{-2}), relative humidity and nutrient solution temperature (°C) were measured using a data logger (Hobo). Thermal time was calculated using base temperature for basil (T_{base}=10.9°C),

A. Plant growth

Fresh and dry, aerial and root plant weight (g.plant^{-1}), number of leaves, root density (g.cm^{-3}), plant height (cm), absolute growth rate (AGR g.d^{-1}), relative growth rate (RGR g.g^{-1}.d^{-1}) and yield (g. m^{-2}) were measured throughout the cycle.

B. Identification and quantification of volatile oils

Oil extraction. Essential oil analysis was carried out with leaves harvested on two harvest dates with an interval of 37 days between them for both genotypes. Basil samples were collected during a period of 92 days in the autumn-winter season and two harvests were made with a pruning between them: sample 1 (5 days after transplant), sample 2 (13 days after transplant), sample 3 (21 days after transplant), sample 4 (29 days after transplant), sample 5 (first harvest and 50 days after transplant), pruning, sample 6 (second harvest and 92 days after transplant).

Fresh leaf material (250 g per sample) was subjected to a 2-h water distillation using a Clevenger type apparatus where material and distilled water where located. A refrigerant attached to the distillation balloon allowed accumulation and separation of the essential oil from the condensed mixture. The oils obtained were dried over anhydrous sodium sulfate.

Identification of volatile oils. The essential oils were analyzed by CG-FID-MS, with Perkin Elmer GC equipment model Clarus 500. Chromatograph operating conditions with CG-FID-MS were: Helium as a carrier gas at a constant flow rate of 1.87 ml/min, and an auto sampler connected to an injector split (Split rate: 1:100) in turn connected to a flux divisor of two fused silica capillary column (polar and no polar). The temperature parameters were T. initial: 90°C; ramp (3°C /min); T. final: 225°C (15 min); T. injector: 255°C; T. detector: 275°C; final run time 70 min; mass range scanned 40-400 m/z. The injected samples consisted of 0.2 µl in a dilution of 10% ethanol.

The components of the essential oil were identified by comparing their retention times obtained from the two columns of different polarity with those of authentic samples and/or data in the literature, and comparison with the mass spectra in the database of the Pharmacognosy Department of the University of Buenos Aires and other commercial sources. The relative percentage amounts of the volatile oil constituents were evaluated from total peak area (TIC).

Statistical analysis. The experiment was conducted in a complete randomized block design repeated over time, with three replications. The treatments for growth stage were green genotype and purple genotype. The treatments for essential oil analysis were according to harvest date (first and second) and genotype: green genotype, 92 days after transplant (DAT); purple genotype, 92 DAT; green genotype, 50 DAT; purple genotype, 50 DAT. Data was analyzed by ANOVA and means were compared by Tukey test at the 0.05 probability level.

3. Results and Discussion

Plant growth

Fresh and dry aerial weight. The aerial fresh weight was significantly different between genotypes ($p<0.0001$) and harvest dates ($p<0.0001$). On the third sample date, the differences between genotypes began to be greater. On the fifth sample date the difference for green genotype climbed up to a 47%, a result that coincided with other authors who obtained 91% more biomass for green genotypes than purple (Neikin and Schuch, 2010).

The data from the present study contrast with a crop

grown in optimum season, as reported by some authors for green basil with values between 64.44 and 110.33 g.plant^{-1} (Benito and Chiesa, 2000; Carrasco *et al.,* 2007) and fresh weights of 57.84 g.plant^{-1} and 41.50 g.plant^{-1} for purple basil (Krizaj, 2010). Differences in aerial dry weight between genotypes ($p<0.0001$) and date of harvest ($p<0.0001$) were significant. Aerial dry weight followed the same trend as aerial fresh weight (Fig. 1). The values recorded for 50 DAT (Table 1) were lower than those registered in the same productive system in optimum season, with 10% fewer days of cycle; green and purple genotype with 6.72 g.plant^{-1} and 4.26 g. plant^{-1} (Krizaj, 2010) respectively. An increase of 92% and 184% compared to values reported in this experiment.

Fresh and dry root weight. There were statistical differences in fresh and dry root weight (Table 1) between genotypes (p_{fresh}= 0.0007 and p_{dry} = 0.0024) and date of harvest ($p_{fresh}<0.0001$ and $p_{dry}<0.0001$).

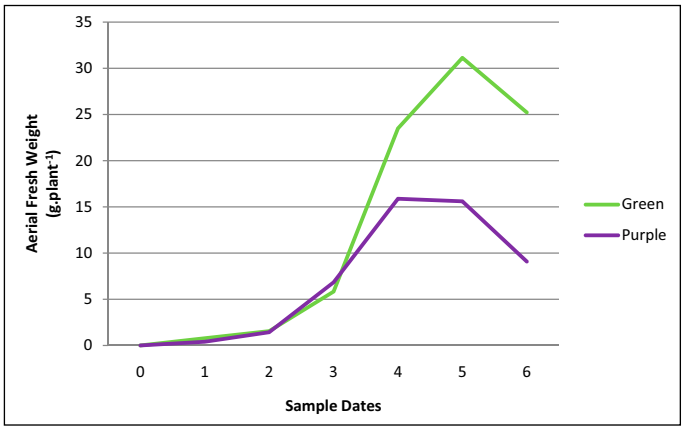

Fig. 1 - Fresh aerial weight (g.plant^{-1}) over the whole growth cycle (5th sample date = first harvest; 6th sample date = second harvest) for both genotypes.

Table 1 - Growth parameters for both genotypes 50 days after transplant. Average values and standard error

Growth parameters (g.plant^{-1})	Genotype	
	Green	Purple
Aerial fresh weight	31.14±0.23	16.5±2.18
Root fresh weight	18.13±0.23	6.5±1.80
Aerial dry weight	3.5±0.01	1.5±0.10
Root dry weight	0.7±0.02	0.43±0.12

Aerial and root dry matter

Aerial dry matter differed significantly between genotypes ($p=0.0339$) and sample dates ($p=0.0169$). The difference increased greatly for green basil at the third sample date. At the first harvest (50 DAT), dry matter percentage for purple basil was 19% lower than the green basil value (Table 2). The data obtained was similar to other authors' results, in protected environments and soilless systems, for green (11.67%) and purple basil (9.85%) (Cenóz and Burgos, 2010). These authors also concluded that dry matter (%) was effectively reduced in protected environment systems compared with other systems (Cenóz and Burgos, 2010). However, other experiments did not show significant differences in dry matter between genotypes in optimum season for NFT system (Krizaj, 2010). The present study was carried out in autumn-winter and both genotypes performed differently which would suggest a noticeably genotypic effect when the season is not optimal. Root dry weight was not significantly different between dates of harvest and genotypes.

Table 2 - Aerial and root dry matter values 50 days after transplant for both genotypes. Mean values and standard error

Genotype	Areal dry matter (%)	Root dry matter (%)
Green	11.24±0.1	3.86±0.05
Purple	9.26±1.92	7.33±3.68

Root density, plant height, leaf number and leaf apparition rate

Root density was significantly different between genotypes ($p<0.0001$) and sample dates ($p<0.0001$). Root density was markedly higher in purple basil at all sample dates except sample date 2, however root weight (dry and fresh) were not higher due to the high content of water and the lower percentage of dry matter. Significant differences were detected for plant height values between sample dates ($p<0.0001$) and genotypes ($p<0.0115$). From the third sample date, plant height was markedly higher for green basil until the end of the study. In optimal season studies (Benito and Chiesa, 2000) with similar growing cycle duration, taller plants were obtained with 133% more height in 56 days (79.8 cm). Leaf number also showed significant differences between sample dates ($p<0.0001$), however this difference was not significant between genotypes. In a shorter cycle (10% fewer days) in optimum season, 124% more leaves were obtained (120 leaves.plant^{-1})(Krizaj, 2010); 208% more leaves.plant^{-1} in NFT in greenhouse (Carrasco *et al.,* 2007). Parameter values are presented in Table 3. Leaf apparition rate 50 DAT was 1.074 leaves.day^{-1} for green and 21% lower for purple basil (0.84 leaves.day^{-1}).

Absolute and relative growth rates

Green basil maintained a higher absolute growth rate (AGR) over almost all the cycle period, and reached the maximum (1.86 g.day^{-1}) on the fourth sample date (29 DAT) as did purple basil (0.74 g.day^{-1}). On the contrary, the highest relative growth rate (RGR) for both genotypes was reached with the first sample date (0.2 g.g^{-1}.d^{-1}). Both genotypes presented a downward trend over the study pe-

Table 3 - Growth parameters at each sample date for both genotypes. Mean values and standard error

Parameter	Genotype	1	2	3	4	5
Root density (g.cm^{-3})	Green	3.7±0.6	3.1±1.7	1.1±1	1.1±5	1.2±1.1
	Purple	4.4±0.0	2.9±1.3	1.2±4.3	1.3±0.01	1.2±1
Height (cm)	Green	10.6±0.4	7.7±1.5	13.7±3.5	25.6±3.8	34.3±1.2
	Purple	10.1±1.6	9.8±1	15.7±2.5	22.5±0.5	23.3±0.5
Leaves Number	Green	4±0.5	11±3.6	25±4.7	44±10.9	54±1.5
	Purple	5±1.1	10±3.7	21±11.9	43±1.5	42±1.7

riod (Figs. 2 and 3). Results obtained 50 DAT were notably low, supporting another author's findings (Krizaj, 2010). As expected, plant response to decreasing radiation and temperature was translated into lower parameter values in comparison with those of an optimal season. In general, until the fourth sample date, temperature and radiation allowed moderate photosynthesis and growth. From the beginning of the study, radiation declined 50% (from 406 W.m^{-2} to 197 W.m^{-2}).

Pruning between the first and second harvest stressed plants, however it caused a different effect in purple basil, which had a slower regrowth compared with the green variety. Purple genotype showed a lower leaf apparition rate

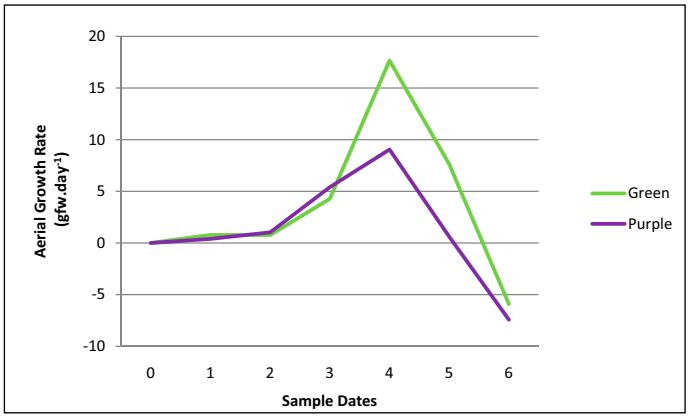

Fig. 2 - Absolute growth rate (AGR) for aerial fresh weight (g fw.day^{-1}) for both genotypes during the cycle. Values represent the mean.

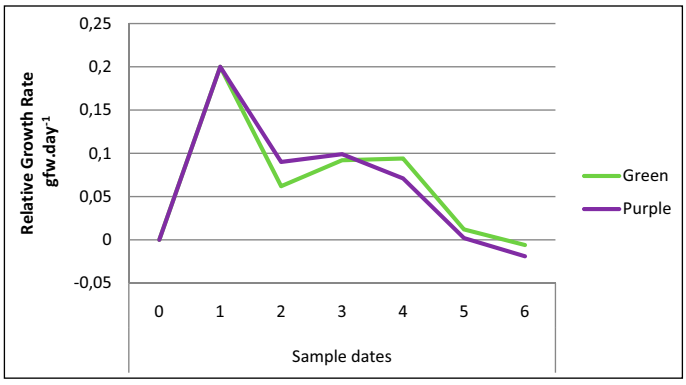

Fig. 3 - Relative growth rate (RGR) for aerial fresh weight (g fw.g^{-1}. day^{-1}) for both genotypes during the cycle. Values represent the mean.

than the green genotype during the cycle. This was added to the adverse environmental conditions after pruning, which led to a slower recovery. Average temperature during the study was inferior to optimal for the species and showed a downward trend, furthermore it was outside the range for maximum dry matter accumulation. The data revealed a genotypic effect in growth response in the autumn-winter season that does not occur in an optimal season (Krizaj, 2010). The contrast in behavior between genotypes was noted when environmental conditions began to be adverse. Relative humidity, nutritive solution temperature, electrical conductivity and nutritive solution pH did not show great variations that could influence growth parameters. Although thermal time 50 DAT was 50% lower than thermal time achieved in optimal season for the same duration and crop conditions (Krizaj, 2010), it was possible to grow basil under protection in the autumn-winter season.

Biomass yield: descriptive analyses

Biomass yield for green basil, 50 DAT, was 778.5 g.m^{-2} and 412.5 g.m^{-2} for purple basil. In contrast, greater values were obtained in an optimal season in open field, with yields between 1000 and 1500 g.m^{-2} for green genotype (Gill and Randhawa, 1996), 29% and 92% more respectively than found in the present investigation. Also, in greenhouse and NFT system and in optimal season, 86% and 151% more biomass yield was obtained for green and purple basil respectively (Krizaj, 2010). At the fifth sample date (first harvest) fresh weight yield per square meter was 778.5 g.m^{-2} for green basil and 47% lower (412.5 g.m^{-2}) for purple basil. At the second harvest date, after pruning, green and purple genotype yielded 630.5 g.m^{-2} and 227 g.m^{-2} respectively.

Volatile oil analysis

Essential oil composition and genotype effect. GC-MS analyses identified 32 aromatic compounds in green and 30 aromatic compounds in purple basil. In both cases the identified compounds account for 94% of the total. The composition is expressed relative to 100%, as each peak has an area and the total of areas is 100%. The essential oils from *O. basilicum* show significant differences between genotypes: linalool (p= 0.0001), eugenol (p=0.0457), methyl- eugenol (p=0.0001), alpha transbergamotene (p= 0.0251), 1.8 cineol (p= 0.0113) and tau cadinol (p= 0.0253). The other components were not consid-

ered in the statistical analyses as they were only detected in some samples and with extremely low values. Values for the main compounds are shown in Figure 4.

Date of harvest effect

The mean percentages of linalool (p=0.0001) and methyl-eugenol present significant differences between dates of harvest (p=0.0019) whereas eugenol, alpha trans-bergamotene, 1.8 cineol and tau cadinol values did not show significant variation between dates.

The mean contents for linalool and methyl eugenol for both genotypes varied as follows: linalool from 41.8% at the first harvest date to 25.7% at the second; methyl-eugenol from 5.78% at the first harvest date to 18.78% at the second. Linalool significantly decreased at the second harvest while methyl eugenol increased, however the content was lower than linalool as seen in figures 5 and 6.

Different radiation led to essential oil variations. It could be that the highest radiation level before the first harvest led to higher rates of linalool. Radiation decreased over the period of the experiment: at the second harvest date lower levels of methyl- eugenol were found. This might be explained by the fact that in this moment radiation was lower. Unlike other authors' findings (Chang *et al.,* 2008), in this study no differences in eugenol values between dates of harvest were found. Temperature influences aromatic compounds and metabolic activity of plants. The effect of temperature at 25°C, in which the highest contents of linalool, 1.8 cineol and eugenol are obtained, was reported (Chang *et al.,* 2005). Although is known that geranial pyrophosphate is precursor of both linalool and 1.8 cineol, and the enzymes linalool synthetase and 1.8 cineol synthetase were identified, the environmental effects on the enzymes activity is not clear at the moment. (Chang *et al.,* 2005). In this study, linalool percentages in the two successive harvests (36.6% and 22.7%) were higher than values reported by other authors in hydroponic systems (Fernandes *et al.,* 2004). Higher radiation and moderate temperatures before the first harvest date might explain the higher content of linalool for both genotypes.

Eugenol is metilated to methyl- eugenol by the enzyme eugenol-O-methyl-transferase (Lewinsohn *et al.,* 2000;

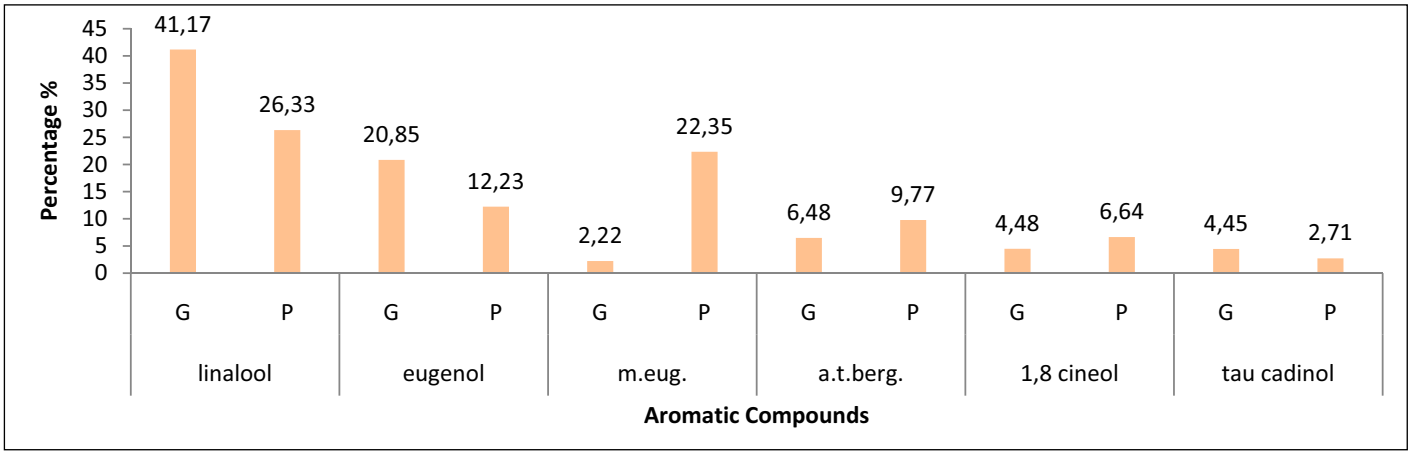

Fig. 4 - Percentage composition of the main aromatic compounds of basil essential oil for both genotypes (G= green, P= purple). Mean value of the two harvest dates (Methyl eugenol= m.eug, Alpha-trans-bergamotene= a.t.berg.).

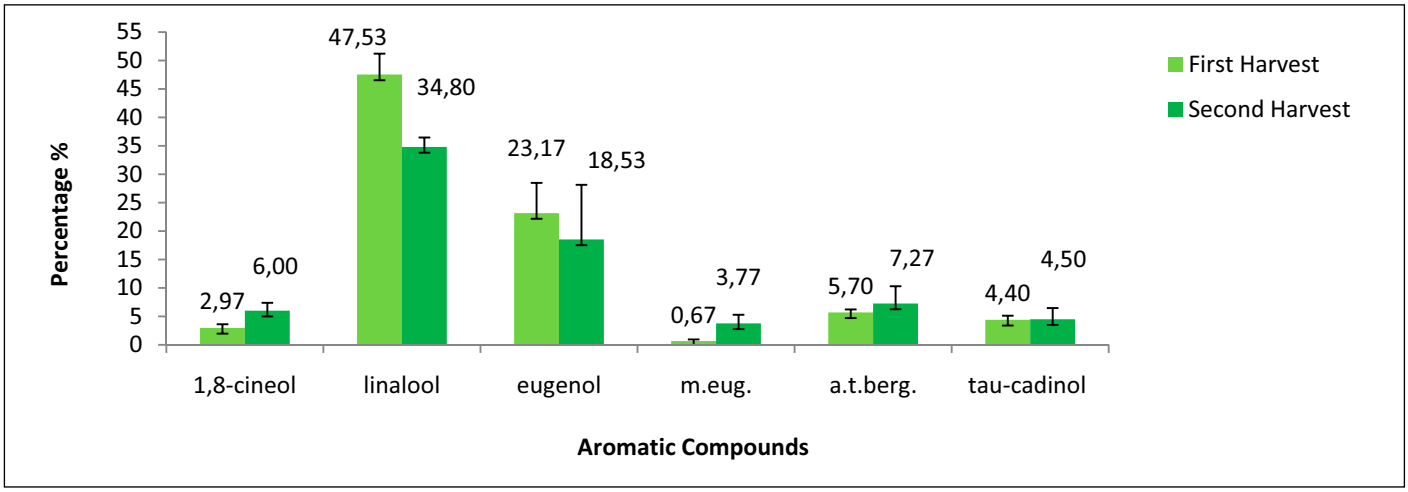

Fig. 5 - Percentages (relative to total in essential oil= 100%) of the main aromatic compounds of basil essential oil for green genotype at first and second harvest. Mean values ± standard error (Methyl eugenol= m.eug, Alpha-trans-bergamotene= a.t.berg.).

Robison and Barr, 2006). Methyl- eugenol is in the group of the alikobenzenes together with iso-eugenol, eugenol, estragol and safrol, which are considered carcinogenic following the tested effects on rats and mice after intake of high doses. The effect in human beings generates certain concern, however the doses at which humans are exposed through diet (mainly via intake) are very low (Robison and Barr, 2006).

Methyl- eugenol content is related to vegetal tissue age. Greater enzyme activity in young leaves was reported (Lewinsohn *et al.,* 2000). This supports other authors who state that enzyme activity is significantly greater in young and developmental leaves than in mature leaves, because as the leaf develops it produces glandular trichomes with high levels of this enzyme (Gang *et al.,* 2002). In young leaves there are more trichomes per unit area before cell expansion (Gang *et al.,* 2001) but when glands reach maturity, enzyme levels decrease. In totally mature plants of *O. basilicum* var. Genovese methyl- eugenol was not found (Marotti *et al.,* 1996). In mature hydroponic plants in greenhouse a percentage similar to this study was found for *O. basilicum* var. Genovese (0, 6% methyl-eugenol in leaves) (Hassangpouraghdam *et al.,* 2010).

In the present investigation, agronomic techniques (pruning) and environmental conditions may have influenced the content of this compound. Pruning after the first harvest decreased sinks, caused regrowth and the development of abundant young tissue, which might have influenced the rise in glandular trichome density, and as a consequence, the higher amount and activity of the eugenol-O-methyl-transferase

At the sixth sample date (second harvest), methyl-eugenol content increased in the essential oil of both genotypes. The increase was remarkably higher in purple basil (33.80%). This result could be due to the slower regrowth of this variety, with a lower foliar apparition rate throughout the cycle (at sixth sample date foliar apparition rate was 1.01 leaves.day^{-1} in purple basil and 1.52 leaves.day^{-1} in green basil) which led to a greater amount of young and developmental leaves with elevated density of trichomes and eugenol-O-methyl-transferase enzyme activity.

4. Conclusions

The agronomic performance of green basil was greater throughout the growing cycle. This genotype showed greater fresh and dry weight (aerial and root), dry matter percentage, number of leaves, plant height and total biomass yield than purple basil. The environment could have influenced agronomic performance and volatile oils composition. Hence, it can be concluded that variations in aromatic compounds and the productive behavior were affected by temperature and radiation.

Differences in volatile oils between dates of harvest and genotypes was clear. At the fifth sample date (first harvest) linalool prevailed in green and purple basil, but in green basil it was significantly higher. At the sixth sample date (second harvest) methyl- eugenol content increased in both genotypes but the increase was markedly higher in purple basil. Pruning after the first harvest might have promoted sprouts, but in purple basil it also might have decreased yield because regrowth after the cut is more difficult for this variety. This difficulty was also enhanced by the environmental conditions (sub-optimal radiation and temperatures). It is also concluded that this agronomic technique could have altered the methyl- eugenol content.

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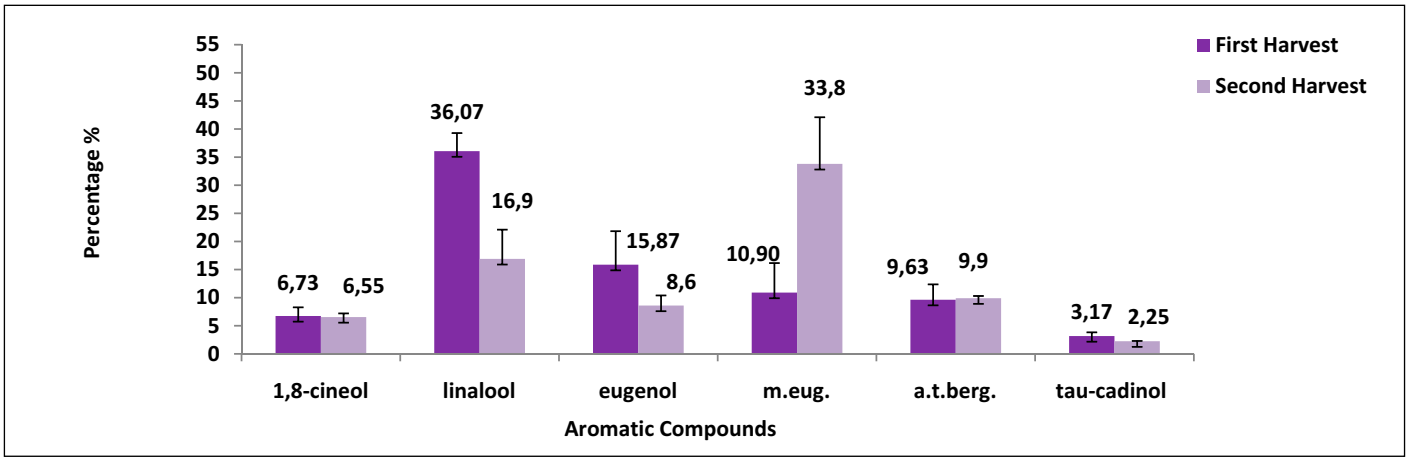

Fig. 6 - Percentages (relative to total in essential oil= 100%) of the main aromatic compounds of basil essential oil for purple genotype at first and second harvest. Mean values ± standard error (Methyl eugenol= m.eug, Alpha-trans-bergamotene= a.t.berg.).

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