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THE INFLUENCE OF PRECEDING PLANT CULTIVATION ON GROWTH AND PHYSIOLOGY OF AN *OCIMUM BASILICUM* L. CULTIVAR

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Abstract

The paper aimed to assess some morphometric and physiological parameters of a purple leaved Ocimum basilicum L. cultivar plants grown in substrates in which other plants (a green leaved Ocimum basilicum L. and Armoracia rusticana Gaertn. Mey. & Scherb.) were grown. The number of lateral stems and leaves and the mass of plants were positively influenced by cultivation after the other plants. Photosynthesis and transpiration rates of the same plants decreased, however the chlorophyll fluorescence parameters (Fv/Fm and ϕPSII) did not reveal a major influence on the photosynthetic apparatus. Chlorophyll and total phenolic contents decreased in plants grown after green basil and increased in plants grown after horseradish. The results show that basil can be grown in the same substrate used by the tested species with positive influences on growth.

***Key words**: biometry, photosynthesis, chlorophyll fluorescence, substrate, horseradish.*

INTRODUCTION

Ocimum basilicum L. known as sweet basil belongs to *Lamiaceae* family, *Ocimum* genus, which includes approximately 60 species. It is a valuable plant species with multiple uses in medicine, cosmetics and gastronomy. Apart from the flavoring properties, the basil has antimicrobial, insecticidal, antioxidant, anti-inflammatory etc. activities (Putievsky and Galambosi, 1999). The properties of this species are mainly owed to the essential oils it synthesizes and to the large infraspecific variability with numerous phenotypes and also chemo-types (Grayer et al., 1996). More than 100 sweet basil cultivars exist that differ in leaf shape, color, height and odor. Originated in subtropical areas, where it can be grown either as an annual or perennial plant, basil is cultivated all over the world as annual plant. Due to its economic importance basil is cultivated on large areas and, thus, the basil crops have to meet the current requirements for agricultural sustainability. Also, as a medicinal and culinary herb, the value of the basil increases when cultivated under organic conditions. Such conditions include crop rotation, intercropping or organic fertilization. The cultivation parameters for basil have been largely investigated, with an emphasis on nitrogen, phosphorus and potassium requirements, time of seeding, distance between plants and rows, soil type, temperature, weeding strategies and pest control (Simon 1996; Meyers, 2003; Arabaci and Bairam, 2004). It is also known that the basil should be rotated once every 4 years and that the most suited preceding crops are cereals and legumes (Pârvu, 2002). However, the influence of the preceding crops on basil physiology and biochemistry has been less studied, rather the species for which basil can be a companion are known, such as tomatoes or marigold (Meyers, 2003; Tringovska et al. 2015).

The effects of preceding crops or intercropping species on other species are mainly due to the substances released in the soil by either leaf litter or roots. Such substances range from high molecular mass compounds such as proteins and polysaccharides, enzymes such as acid phosphatase, nucleases, invertases (Chang and Bandurski, 1964; Chhonkar and Tarafdar, 1981) to low molecular weight phenolic acids (Vaughan et al., 1994). The root exudates, when deposited in soil, a process also known as rhizodeposition, influence the microbiota of the soil and also the growth and development of surrounding plants. The exuded substances may have an effect on proximate plants physiological processes, protein synthesis or enzyme functioning (Bertin et al., 2003; Farooq et al., 2011).

The present paper aimed to test whether basil grown on substrates following other plants is influenced at a physiological and biochemical level in its development. Prior to cultivating a purple leaved basil cultivar, another, green leaved basil cultivar or horseradish (*Armoracia rusticana* Gaertn. Mey. & Scherb.) were grown in the same substrates. *Armoracia rusticana* is a crop specie grown for culinary uses, due to its pungent taste which is given by glucosinolates and their transformation products such as isothiocyanates, but also for medicinal purposes, with proven in vitro anti-inflammatory activity (Yamaguchi, 2012; Marzocco et al., 2015). The specie presents an increasing demand on various markets and is also used in crop rotation for preventing potato and tomato crops pests (Filipović et al., 2015). Horseradish is a specie which is known to exert negative influences on the surrounding plants due to the root exudates it synthesizes (Dias and Moreiro, 1988; Itani et al., 2013). Several parameters were evaluated, to assess the opportunity of cultivating sweet basil in a culture comprising different basil cultivars or other spices which could influence the succeeding crops due to chemicals secreted in soil.

MATERIALS AND METHODS

Plant material

The tested specie was *Ocimum basilicum* L. cv. “Violet de Buzau”, which was grown from seeds obtained from the Agricultural Research and Development Station at Buzau, Romania. The species grown prior to this cultivar in the substrate were *Ocimum basilicum* L. cv. “Aromat de Buzau”, obtained from the same source and grown from seeds and *Armoracia rusticana*, planted as one year old roots obtained from local suppliers.

Experimental conditions

For the growth of plants, 4 L (15 cm height x 18 cm diameter) plastic pots were used. Pots were filled with 3 L of a mixture composed of commercial soil (60% v/v), peat moss (30% v/v) and perlite (10% v/v). For each variant, 3 pots were used.

Three experimental variants were set up: pots with purple basil seeded in substrate mixture alone (control plants), in substrate mixture in which green basil (4 plants per pot) was previously grown and in substrate mixture in which horseradish (4 plants per pot) was previously grown. Both the green basil cultivar and the horseradish plants were grown for approximately 3 months, period which, in the case of basil, coincided with the fruiting stage. After this period, the green basil and the horseradish plants were removed manually from the pots. Each pot was seeded with 10 purple basil seeds. The plants were thinned at 4 individuals per pot after 1 week.

The pots were kept at constant temperature regimes, between 22° C (night) and 25° C (day). Artificial light was supplied by 4800K fluorescent tubes for 14 h each 24 h. The atmospheric humidity was relatively constant, around 50%. Plants were irrigated twice a week with distilled water. The growth of purple basil lasted for 3 months, until the fruiting stage of plants.

Morphometrical assessments

For each variant, 12 individuals were assessed for stem height, number of leaves, number of lateral stems, number of inflorescences and mass of fresh plants.

Physiological measurements

The photosynthetic activity, transpiration rates and stomatal conductance were measured with an ADC Bioscientific LCi apparatus, on 3 leaves (from the lower, middle and upper regions of the plants) from 3 individuals each per treatment, 5 readings per leaf. Measurements were done during the light regime.

Chlorophyll fluorescence was evaluated by measuring F_0, Fm, Fv, Fv/Fm, Fs, Fm’, ϕPSII on 5 leaves per treatment. Fv/Fm was measured following a 20 minutes dark adaptation of leaves using provided clips. ϕPSII was measured at normal light regime.

Biochemical parameters

Assimilatory pigments were extracted from grounded leaves (approx. 0.1 g) using 80% aqueous acetone. The absorbance of extracts were recorded at 470, 646 and 663 nm with a

Shimadzu UV 1240 spectrophotometer in 1 cm light path length glass cuvettes. Pigment contents were calculated using Wellburn (1994) equations.

The total phenolic contents were determined following the method described in Herald et al. (2012). An aliquot of 0.1 ml of 5% (w/v) extracts in 30% ethanol was mixed with Folin reagent, incubated for 5 min, Na_2CO_3 7.5% was added and incubated for further 90 min. Results were calculated using absorbance at 760 nm and expressed as gallic acid equivalents per gram fresh weight.

Total flavonoids were assessed in the same extracts using the $AlCl_3$ method and expressed as quercetin equivalents per gram fresh weight while antioxidant activity of extracts was determined according to the DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) method (Herald et al. 2012).

The statistical analyses conducted were represented by analyses of variance among treatments and the Tukey post hoc test at $p<0.05$, the results being expressed as means and standard errors.

RESULTS AND DISCUSSIONS

Morphometry

From a morphometric point of view, the analyzed parameters of the purple basil plants recorded certain significant variations. An increase in the number of lateral stems (Fig. 1) and leaves (Table 1) were recorded for plants grown after green basil. For the same plants, a significant decrease in height was recorded, thus the plants presented a more compact habitus.

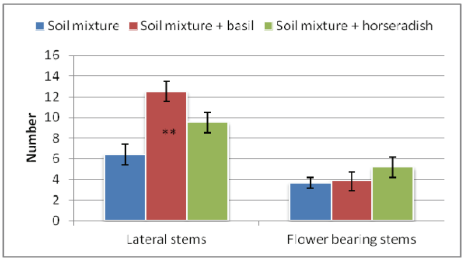

Figure 1. Number of lateral stems and flower bearing stems of purple basil plants

(*-significant differences from control plants at $p < 0.05$; **-significant differences from control plants at $p < 0.01$).

Increases in mass and number of leaves, although not significant, were recorded also for plants grown after horseradish. The number of flower bearing stems was not significantly modified in any of variants.

Table 1. Morphometric indices of purple basil plants

Treatments / Parameters	Height (cm)	No. leaves	Mass (g)
Soil mixture	74.5±3.07	119.33±9.36	22.9±1.48
Soil mixture + basil	53.9±2.08**	188±14.11**	27.3±3.61
Soil mixture + horseradish	71.17±4.3**	133.83±9.19**	29.38±3.4

(*-significant differences from control plants at $p < 0.05$; **-significant differences from control plants at $p < 0.01$).

Physiological analyses

The photosynthetic rate of purple basil plants significantly decreased when grown after the green basil cultivar and it remained within similar values with control plants when grown after horseradish plants (Table 2). Transpiration rates decreased significantly for both purple basil individuals grown after green basil and for those grown after horseradish plants.

Table 2. Physiological parameters of purple basil plants

Treatment/ Parameter	Photosynthesis rate (μmolls $CO_2/m^2/s$)	Transpiration rate (molls $H_2O/m^2/s$)	Stomatal conductance (molls/m²/s)
Soil mixture	2.31±0.1	2.9±0.15	0.25±0.02
Soil mixture + basil	1.45±0.14**	1.67±0.15**	0.1±0.02
Soil mixture + horseradish	2.26±0.15	2.41±0.1*	0.35±0.22

(*-significant differences from control plants at $p < 0.05$; **-significant differences from control plants at $p < 0.01$).

The stomatal conductance (Table 2), although not statistically significant, was lower in basil plants grown after green basil and higher when grown after horseradish plants compared to control plants.

Chlorophyll fluorescence measured in the light adapted state (ϕPSII) increased in a significant manner in both purple basil plants grown after green basil and also grown after horseradish (Table 3).

The higher values of ϕPSII occurred due to significantly lower values in both Fs and Fm' for the two variants compared to the control.

Table 3. Chlorophyll fluorescence parameters in purple basil plants

Treatments /Parameters	Soil mixture	Soil mixture + basil	Soil mixture + horseradish
F_0	22.4±1.87	23.8±5.11	28±2.35
Fm	246.4±10.92	236.6±27.88	255.8±27.86
Fv	224±9.59	212.8±24.74	227.8±25.89
Fv/Fm	0.91±0.01	0.9±0.02	0.89±0.01
Fs	300.5±17.99	113.5±10.2**	146.67±9.08**
Fm'	1461.08±90.93	623.58±40.27 **	790.75±47.75**
PSII	0.79±0	0.82±0.01**	0.81±0*

(*-significant differences from control plants at $p < 0.05$; **-significant differences from control plants at $p < 0.01$).

The Fv/Fm parameter, measured under a dark adapted state, recorded lower values, but not significantly, in treatments compared to the controls. These results are the effect of increased values of the F_0 parameter, although not significantly, in both variants compared to control plants.

Biochemical parameters

Among assimilatory pigments, the contents of chlorophyll *a* and carotenoids increased in purple basil grown after horseradish, while in the other cases, the contents remained similar compared with the controls (Fig. 2)

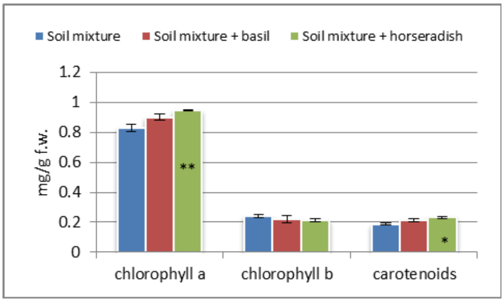

Figure 2. Assimilatory pigments contents in basil plants (*-significant differences from control plants at $p < 0.05$; **-significant differences from control plants at $p < 0.01$).

The total content of phenolic compounds and of flavonoids, determined in ethanolic extracts, increased in plants grown after horseradish compared to controls, with significance in the case of total phenolics. Purple basil plants grown after green basil registered a decrease of these parameters, significantly so in the case of flavonoids (Fig. 3).

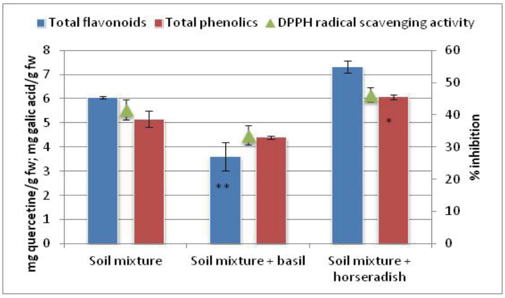

Figure 3. Total phenolics and flavonoid contents and free radical scavenging activity of purple basil plants 2.5% ethanolic extracts
(*-significant differences from control plants at $p < 0.05$; **-significant differences from control plants at $p < 0.01$).

These values were reflected also in the scavenging activity of the extracts, which was higher in plants grown after horseradish and lower in plants grown after green basil compared to control plants.

In crops, as in natural populations, the plants can influence each other through various processes. These include direct competition for light, water or nutrients, release of volatile compounds and soil mediated interactions. The soil represents a medium in which numerous substances are released, either by decomposition of plant material or by root exudates. These processes represent the basis for many agricultural practices that are included in organic farming systems, practices such as crop rotation, intercropping, mulching etc. (Farooq et al., 2011).

In the area around the roots, various substances can be found, originating from root exudates. These substances are represented by ions, oxygen, low molecular weight organic compounds (such as arabinose, glucose, oligosaccharides, amino acids such as arginine, asparagine, cysteine, glutamine etc. organic acids such as acetic, ascorbic, benzoic, ferulic, malic and phenolic compounds), as well as higher-molecular-weight compounds such as flavonoids, enzymes, fatty acids, growth regulators, nucleotides, tannins, carbohydrates, steroids, terpenoids, alkaloids, polyacetylenes, and vitamins. Some of these compounds, especially the phenolics, influence the growth and development of surrounding plants and soil microorganisms (Bertin et al., 2003).

The basil roots are known, under certain conditions, to produce rosmarinic acid (Bais et al., 2002). Horseradish roots were proven to secrete, in the substrate, peroxidase (Willey, 2016), probably together with other compounds, as the horseradish root residues are known to be toxic to certain species such as lettuce (*Lactuca sativa*) (Dias and Moreira, 1986; Itani et al., 2013).

In the present study, the fresh mass of basil plants and the number of flowering stems increased (however not significantly), as did number of lateral stems and leaves (significantly) when cultivated after other plants, suggesting a better availability of nutrients in substrates. This type of effect is known to occur as a result of root exudates and biomolecules interacting with compounds present in soil. Root exudates can enhance the solubility of both anions and cations (the latter by the presence of organic acids), can release Al and Fe from compounds, can form nano precipitates and can alter the adsorption of toxic elements and nutrients on substrate particles (Violante and Caporale, 2015). It is considered that certain properties of root exudates can favor agricultural production by increasing nutrient availability (Gianfreda, 2015) and also that proper crop rotation can lead to a 20% increase in yield (Farooq et al., 2011).

A possible stress might have occurred in the case of plants grown after the green basil cultivar, as these plants had reduced height but the highest number of lateral stems and leaves.

Regarding the photosynthetic rates, the decrease in basil plants cultivated after the green basil cultivar may be attributed to the higher number of leaves, but with smaller area per leaf. This kind of effects can occur under the presence of certain organic compounds in substrates such as organic acids which can also determine reduced stomatal opening (Zhou and Yu, 2006), as was found in the present study. The same parameters were not significantly influenced in the case of basil plants grown after horseradish, suggesting that the composition of exudates does not affect the photosynthetic process. The reduced stomatal conductance values of plants grown after green basil may also explain the decrease in transpiration rates (Chapin, 1991). Although apparently the stomatal conductance increased in plants grown after horseradish, the large standard deviations of the mean value may explain the reduction in transpiration rates in the same plants.

Regarding the chlorophyll fluorescence, in our study, the basil plants recorded similar values among treatments for both Fv/Fm and ɸPSII parameters, although the slight increase in the latter was statistically significant. Fluorescence parameters are generally known to decrease under various types of stresses, including the presence in substrates of allelochemicals, as was recorded for *Cucumis sativus* after application of cinamic acid (Zhou and Yu, 2006) or for *Dactylis glomerata*, *Lolium perenne* and *Rumex acetosa* after application of benzoxazolin-2(3H)-one and cinamic acid (Hussain and Reigosa, 2011a) or *Lactuca sativa* when exposed to cinamic acid (Hussain and Reigosa, 2011b). However, other reports have shown that the effect on chlorophyll fluorescence of *Bidens pillosa* and *Lolium perenne* of applied plant leachates was concentration dependent (Rashid et al., 2010). Such concentration dependence is also known as “saw tooth effect” and is explained by the simultaneous influence of chemicals on various physiological processes and the interactions among these (Reigosa et al., 1999). Also, the effects of chemicals on physiological processes depend on the nature of the chemical and on the tested species. For instance, the same compounds (benzoxazolin-2(3H)-one and p-hydroxybenzoic acid) had no effect on the chlorophyll fluorescence of *Polygonum persicaria*, but significantly decrease the values for *Dactylis glomerata*, while ferulic acid had no influence in the case of both species (Reigosa et al., 2001). We could therefore assume that the treatments did not induce a significant stress in tested plants and the concentrations of potentially toxic compounds exuded were low.

Assimilatory pigment content of tested plants increased, significantly for chlorophyll *a* and carotenoids in plants grown after horseradish.

Chlorophyll content is known to increase with the availability of the nutrients as reviewed in Marschner (2011). This is the case for many species, such as wheat (Bojovic and Sojanovic, 2005), melissa (Sharafzadeh et al., 2016) and basil (Politycka and Golcz, 2004), thus further sustaining the idea of improved nutrient availability in the substrates.

The content of phenolic compounds in plants is subjected to biotic and abiotic factors such as light, nutrient, salt stresses or pathogen interaction (Waśkiewicz et al., 2012). Phenolic contents can increase under fertilization of plants, as was observed in the case of basil (Scagel and Lee, 2012). However, a role as growth regulators was suggested for certain phenolics, with positive correlations observed between the amount of phenolics in tissues and plant growth (Kefeli and Kutacek, 1977). Since the basil plants grown after horseradish registered higher amount of phenolics and also increased plant height, while plants grown after green basil registered lower amounts of phenolics and reduced stem height compared to controls, such a hypothesis should be further investigated. Also, the different contents of phenolics in the plants further suggest the different nature of chemicals present in the substrates and their differential effect on basil plants. As a consequence of the amount of phenolics, the antioxidant activity of basil extracts varied accordingly, with higher scavenging ability for plants grown after horseradish, a correlation between these two parameters being proved for many species, including for basil (Juliani and Simon, 2002).

Overall, the data obtained for the growth of purple basil in the present study, indicate that basil can be successfully grown both after other basil cultivars as well as after horseradish. The positive effects on the growth of basil can be attributed to the presence in the substrate of exuded organic compounds such as organic acids and enzymes. Organic acids can improve nutrient availability (Violante and Caporale, 2015) while enzymes such as peroxidase can use organic toxic compounds as a substrate, degrading them and thus decreasing toxicity in the substrate (Vaughan et al., 1994). Although some exuded substances may influence physiological processes (photosynthesis, transpiration, stomatal conductance), cell division and elongation, membrane fluidity, protein synthesis, enzyme regulation (Farooq et al., 2011), the cultivation of several species simultaneously or in succession can positively contribute to the content of organic matter and nitrogen in the soil, improve water and nutrient availability and suppress weeds (Tringovska et al., 2015). *Armoracia rusticana* is a specie already used in crop rotation systems, which protects other crops from pests (Filipović et al., 2015) and it may exert other types of beneficial effects.

CONCLUSIONS

Ocimum basilicum L. plants can be grown on substrates where other basil or horseradish plants were previously grown with some beneficial effects, especially in the case of the latter. Positive effects are represented by increased morphological parameters values and bioactive compounds content. Further analyses are required to determine the type and the concentration of compounds excreted in substrate by roots and a more complete assessment of agronomic, physiological and biochemical parameters of plants grown in succession.

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