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Fertilization modifies the essential oil and physiology of basil varieties

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ABSTRACT

Medicinal and aromatic crops are economically important because they are sources for secondary metabolites such as essential oils (EOs) and polyphenolic compounds. High biomass yields are obtained by conventional chemical fertilizers, whereas the production of EOs is more influenced by elicitors that act on secondary metabolic pathways. The objective of the field trials was to evaluate the effects of biologic and conventional fertilizers (biosolids: 40 and 20 t ha^{-1}, organic: 600 kg ha^{-1}, microorganisms (arbuscular mycorrhizal fungi, AMF): 60 kg ha^{-1}, chemical NPK 20-20-20: 300 kg ha^{-1}) on plant growth, EO chemical profile and yield, and physiology of sweet basil (*Ocimum basilicum* L.). The two sweet basil cultivars (varieties) used were the green-leafed 'Aromat de Buzau' and the purple-leafed 'Violet de Buzau'. The greatest increases in fresh yields compared with unfertilized plants, 116% and 68% increases for the 'Aromat de Buzau' and 'Violet de Buzau, respectively, were obtained from the conventionally (chemical) fertilized plants. The 40 t ha^{-1} biosolids treatment increased the EO content of the 'Aromat de Buzau' by 30%, while the AMF and organic treatments increased the EO content of the 'Violet de Buzau' by 21%. The major constituents of 'Aromat de Buzau', methyl chavicol and β-linalool, were increased by 4 and 17% with the AMF and organic treatments, respectively. Eucalyptol and δ-guaiene concentrations in the EO were higher in the 40 t ha^{-1} biosolids treatment, while τ-cadinol and γ-cadinene were higher in the 20 t ha^{-1} biosolids treatment. For 'Violet de Buzau', the AMF treatment led to an increase of β-linalool, limonene, and (+)-camphor, and decreased the concentration of methyl chavicol. The organic treatment increased the concentrations of limonene, (+)-camphor, terpinene-4-ol, and β-caryophyllene, and decreased the concentration of methyl chavicol. The highest concentration of methyl chavicol was observed in the chemical fertilizer treatment. The 20 and 40 t ha^{-1} biosolids treatments led to highest yields of eucalyptol, (+)-camphor, terpinene-4-ol, β-caryophyllene, germacrene D, and τ-cadinol. The results demonstrated that conventional fertilizers can increase fresh yield while biological fertilizers positively alter the EO composition, leading to increased crop quality.

1. Introduction

Basil (*Ocimum basilicum* L.) is an economically important herb cultivated worldwide for commercial production of its essential oil (EO), as a medicinal and ornamental plant, and also as a fresh-market herb. Since ancient times, basil has been used as an ethnobotanical plant or as an ingredient in various dishes and beverages, particularly in Mediterranean cuisine. Basil is a rich source of natural plant chemicals, including monoterpenes, sesquiterpenes, and phenylpropanoids that possess antioxidant activities and proven human health benefits (Makri and Kintzios, 2008; Zamfirache et al., 2011; Szymanowska et al., 2015; Onofrei et al., 2017). Furthermore, basil extracts have antimicrobial and insecticidal activities, and for that reason have extensive applications not only in the food, but also in pharmaceutical, perfumery, cosmetic, and aromatherapy industries (Stefan et al., 2013).

Basil cultivars currently available on the international market display significant variation in EO composition and phenotype (Grayer et al., 1996; Marotti et al., 1996). The European chemotype is known for its high linalool and methyl chavicol concentration in the oil. The Reunion chemotype is characterized by a high concentration of methyl

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chavicol. Other tropical basil chemotypes are characterized by the high concentration of methyl cinnamate in their EOs. The chemotype containing eugenol as a major constituent is cultivated in Eastern Europe, Russia, and many parts of Asia and North Africa (Zheljazkov et al., 2008a,b).

The synthesis of secondary metabolites in plants that produce EOs and phenolic compounds is related to specific phenophases (such as flowering) or to environmental stressors. Such compounds are responsible for pigmentation or attraction of pollinators, and offer protection against stresses including UV light and pests (Kliebenstein 2004; Mazid et al., 2011). In order to obtain high herb yields and increased amounts of bioactive compounds, basil plants need well-drained soil rich in nitrogen (N), and other macro and micronutrients. Because of increasing demands for natural products with potential health benefits and for industrial purposes, efforts are underway to develop sweet basil as a high-value EO crop in the European Union (EU).

Selecting an appropriate fertilizer may lead to improved crop yields and quality. Fertilization with organic soil amendments is central in organic farming (Stoleru et al., 2014); organic fertilizers are utilized in conventional systems as well. Organic and sustainable agriculture practices need to be further developed and fine-tuned to obtain not only high yields, but also yield stability in order to fulfill the need for greater food and environmental security (Stoleru et al., 2014; Rouphael et al., 2015). The role of organic soil amendments, such as biosolids, arbuscular mycorrhizal fungi (AMF), or manure, on production and quality of crop plants has been an objective of previous studies and reports (Aguilera-Gomez et al., 1999; Smith and Read, 2008).

Biosolids represent sanitized sewage sludge resulting from wastewater treatment processes that can produce approximately 10 million t dry solids (d.s.) in the EU and 7 million t d.s. in the USA every year (Kelessidis and Stasinakis, 2012). There is great potential to use biosolids in agriculture in Eastern European countries such as Romania and Bulgaria (Burducea et al., 2016). Therefore, the Sewage Sludge Directive 86/278/EEC was developed to encourage the use of biosolids in agriculture because they contain an array of macro- and micronutrients, and organic matter of up to 50–60% d.s. Biosolids may improve crop yield because of their nutrient content (Vaca et al., 2011; Özyazici, 2013; Chrysargyris and Tzortzakis, 2015). Biosolids can improve the physico-chemical characteristics of soil, such as water holding capacity, aeration, and soil bulk density (Holz et al., 2000; Ros et al., 2003; Gu et al., 2013; Mihalache et al., 2014). However, the potential contamination of soils, and, implicitly, plants, with heavy metals or other pollutants has led to resistance to the agricultural utilization of biosolids. Previous research indicated that basil can be grown on biosolids-amended substrates, with increased biomass and contaminant-free EOs (Zheljazkov and Warman, 2004).

Arbuscular mycorrhizal fungi (AMF) are known to improve plant nutrition through enhanced plant nutrient scavenging and uptake (Smith and Read, 2008). They are extensively utilized in horticulture, particularly *Rhizophagus* (formerly known as *Glomus*) *intraradices* and *Funneliformis* (formerly known as *Glomus*) *mosseae* (Krüger et al., 2012). Positive effects from the use of AMF on horticultural crops were demonstrated both under field and greenhouse conditions (Rouphael et al., 2015; Nadeem et al., 2014). Colonization with *Glomus mosseae* positively affected plant aboveground shoots, roots, and EO yields of basil 'Genovese' (Copetta et al., 2006).

With goals of high crop quality, environmental protection, and reduced use of chemical fertilizers, the first objective in this study was to evaluate the effects of biological and conventional fertilizers on growth indicators and EO production of two basil cultivars. The second objective was to assess the chemical profile of the two basil cultivars and to quantify possible physiological and biochemical influences on basil plants grown under field conditions.

2. Materials and methods

2.1. Experimental design

The field study was conducted at the research farm "Vasile Adamachi" of the University of Agricultural Sciences and Veterinary Medicine in Iaşi, Romania (http://www.uaiasi.ro/, N = 47°11′76″ E = 27°33′71″) during two cropping seasons, in 2016 and in 2017.

Basil seeds of the green-leafed 'Aromat de Buzau' and purple-leafed 'Violet de Buzau', were sown in a glasshouse with automatically controlled environmental conditions: 25/20 °C day/night temperature, 60% relative humidity, and natural light. Plants were watered daily in the first 2 weeks after sowing, and after that period once a week. The containers used were black plastic trial cells (30/60 cm), while the growth medium was a peat based substrate. Seedlings were transplanted into the field site after 5 weeks.

A complete randomized block design with six treatments in triplicates (n = 3) was established. The crop was set as follows: the in-row space was 25 cm with 50 cm distance between the rows, resulting in a density of 8 plants m^{-2}. Each replicate consisted of 18 plants. The field site soil was an anthropic chambic chernozem. The soil physico-chemical properties were: clay – 32%, potential of hydrogen (pH) – 7.20; electrical conductivity (EC) – 478 μS/cm, calcium carbonate ($CaCO_3$) – 0.41%, organic matter (OM) – 28.56%, carbon/nitrogen (C/N) – 5.91, nitrogen (N) − 0.175%, phosphorus (P_2O_5) – 0.0073%. The local meteorological conditions of the two years and cropping seasons represented by the total precipitation and the average temperature, are presented in Figs. 1 and 2.

2.2. Fertilization regimes

The fertilizing treatments were:
(1) 40 t ha^{-1} Biosolids.
(2) 20 t ha^{-1} Biosolids.

The biosolids were obtained from the municipal waste water treatment plant (Iasi, Romania); they were platform dried for 2 years. Physico-chemical composition of the biosolids were: pH – 6.96; EC – 6280 μS/cm; OM – 26.6; N – 4.83%; P_2O_5 – 2.43%; K_2O – 0.51%, while the heavy metal content did not exceed the admissible concentration of the respective elements for agricultural use.

(3) Orgevit® is an organic fertilizer based on granulated poultry manure. The physico-chemical composition of this organic fertilizer was: pH 7, OM – 65%, N total – 4%, P_2O_5 – 3%, K_2O- 2.5%, MgO- 1%, and traces of Fe, Mn, B, Mo, Zn, Cu (dose 600 kg ha^{-1}).

(4) Micoseed® is a fertilizer based on microorganisms, that consists of an inoculum containing spores of arbuscular mycorrhizal fungi (AMF), and is based on *Glomus* spp. and enriched with *Beauveria* sp., *Metarhizium* sp., and *Trichoderma* sp. (dose 60 kg ha^{-1}).

(5) Nutrispore® is a solid chemical fertilizer. The composition of this fertilizer was: NPK 20-20-20, (N – 20% of which nitric N 5.6%,

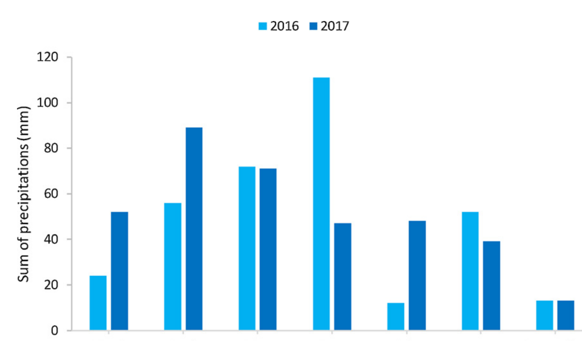

Fig. 1. Monthly variation of precipitations in Iasi, during the vegetation period of basil crop, for the 2 years of the experiment.