Yield and Composition of *Ocimum basilicum* L. and *Ocimum sanctum* L. Grown at Four Locations

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Abstract. Sweet basil (*Ocimum basilicum* L.) and holy basil (*Ocimum sanctum* L.) are the most widely grown basil species in the world either for the fresh market or for essential oil production. Both species are considered to be promising essential oil crops in the southeastern United States; however, research on oil production and composition of these species in Mississippi and the southeastern United States is lacking. The objective of this study was to evaluate biomass productivity, oil content, and oil composition of sweet basil (*Ocimum basilicum* L.) cv. German and Mesten and holy basil (*Ocimum sanctum* L.) cv. Local grown at four locations in Mississippi. Overall, the three basil cultivars grew well; the fresh herbage and essential oil yields at three of the locations were high and comparable to basil yields reported in the literature. Essential oil content in air-dry herbage and the essential oil yields were as follows: 0.07% to 0.50% and 0.7 to 11.0 kg ha−1 in sweet basil cv. Mesten, 0.2% to 0.5% and 1.4 to 13.0 kg ha−1 in sweet basil cv. German, and 0.08% to 0.40% and 0.6 to 5.3 kg ha−1 in holy basil cv. Local, respectively. The major constituent of sweet basil cultivars was (−)-linalool with other constituents being (−)-camphor, α-humulene, eucalyptol, eugenol, (−)-bornyl acetate, methyl chavicol, (−)-trans-caryophyllene, α-trans-bergamotene, and cadinol. The major constituents of holy basil were methyl chavicol, eugenol, and eucalyptol with other constituents being α-humulene, humulene-epoxide II, (−)-trans-caryophyllene, α-trans-bergamotene, and γ-cadinene. Our results suggest sweet and holy basils have a potential as new essential oil crops for Mississippi and possibly the southeastern United States and can provide oil yields and composition typical for the respective species.

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Materials and Methods

Plant materials and growing conditions. A replicated field experiment (a randomized complete block design with four replications) was conducted in the 2006 cropping season at four locations in Mississippi: Beaumont, Crystal Springs, Stoneville, and Verona, located in the four main geographic areas of Mississippi (Fig. 1). The test material was cv. German and cv. Mesten of sweet basil (*O. basilicum* L.) and cv. Local of holy basil (*O. sanctum* L.). All seedling production was initiated in March in one greenhouse at Verona using certified seeds from the Research Institute for Roses and Medicinal Plants in Kazanluk, Bulgaria. Basil seedlings were initiated in 48-cell plastic trays filled with Metro-mix 300 growth medium (The Scotts Co., Marysville, OH) sown by hand and thinned after emergence to one plant per cell. Basil seedlings grew for 6 weeks under natural light with a day temperature of 22 to 25 °C and night temperature of 18 °C. Plants were irrigated once every 24 h and fertilized weekly with 1.8 g of 20-20-20 N-P2O5-K2O dissolved in 300 mL of water during the greenhouse seedling production period. All basil plants were transplanted into the field in Mississippi (Fig. 1). The test material was cv. German and cv. Mesten of sweet basil (*O. basilicum* L.) and cv. Local of holy basil (*O. sanctum* L.). All seedling production was initiated in March in one greenhouse at Verona using certified seeds from the Research Institute for Roses and Medicinal Plants in Kazanluk, Bulgaria. Basil seedlings were initiated in 48-cell plastic trays filled with Metro-mix 300 growth medium (The Scotts Co., Marysville, OH) sown by hand and thinned after emergence to one plant per cell. Basil seedlings grew for 6 weeks under natural light with a day temperature of 22 to 25 °C and night temperature of 18 °C. Plants were irrigated once every 24 h and fertilized weekly with 1.8 g of 20-20-20 N-P2O5-K2O dissolved in 300 mL of water during the greenhouse seedling production period. All basil plants were transplanted into the field in

There are more than 150 basil species belonging to the genus *Ocimum* (Javanmardi et al., 2002). The two most widely grown species for essential oil production are holy basil (*Ocimum sanctum* L.) and sweet basil (*Ocimum basilicum* L.). Basil oil is used for flavor and fragrance in the food, pharmaceutical, cosmetic, and aromatherapy industries. The essential oil possesses antimicrobial activity (Bixin et al., 2006; Elgayar et al., 2001; Kristinsson et al., 2005; Suppakul et al., 2003) and insecticidal (Aslan et al., 2004; Bowers and Nishida, 1980) activity. In addition, basil extract and essential oil have been shown to possess antioxidant activity (Gulcin et al., 2007; Junkachote and Berghofer, 2005; Kelm et al., 2000; Polito et al., 2007; Trevisan et al., 2006). Basil essential oil has been traditionally, extracted from whole aboveground herbage (stems, leaves, and flowers) using steam distillation (Topolov, 1962; Trevisan et al., 2006). The optimal harvesting stage for essential oil production is at flowering, when the oil content and preferred composition are the highest (Topolov, 1962; Zheljazkov, 1998). Various basil species and cultivars provide essential oil with different compositions and aroma. The chemotaxonomical range of sweet basil is very wide. For example, in a study on 270 sweet basil accessions, the major constituents were found to be (−)-Linalool (up to 71%), methyl chavicol, or citral and 1,8-cineole, (−)-camphor, thymol, methyl cinnamate, eugenol, methyl eugenol, methyl isoeugenol, and elemicin (Kruger et al., 2002). According to Marotti et al. (1996), the European basil type has (−)-linalool and methyl chavicol as the major oil constituents. The Reunion basils, another chemotype, have methyl chavicol as a major constituent, whereas tropical chemotypes of basil have methyl cinnamate as the major constituent. Another basil chemotype grown in North Africa, Russia, Eastern Europe, and parts of Asia has eugenol as the major constituent (Marotti et al., 1996).

Crop producers are always looking for high-value specialty crops, and some producers expressed interest in basil as an essential oil crop in Mississippi. However, research on basil productivity, the essential oil content and composition and production of these species in Mississippi and the southeastern United States is lacking. The objective of this study was to evaluate productivity, oil content, and composition of sweet and holy basils grown at four locations in Mississippi.

May 2006 in previously prepared black plastic-covered raised beds.

Before land preparation, soil samples were taken and were extracted for phyto-available nutrients using the Lancaster soil test method (Cox, 2001) (Table 1). Land preparation in early spring included disking several times and the formation of raised beds using a press-pan-type bed shaper. The bed shaper machine also covered the beds with black plastic mulch and placed a drip-tape irrigation tube under the plastic. The system of black plastic mulch combined with drip irrigation was selected to provide uniform weed control, improved water relations, metered fertility, and cleaner harvested crop than a bare ground system would have. Also, the black plastic reduced cultivation adjacent to plants. The resulting beds were 15 cm high and 75 cm wide across the top. Basil seedlings were transplanted in two rows on each bed, in an offset pattern, with 30 cm in-row and between-row spacing. The individual plots (replication) had 40 plants and were 6 m long. Irrigation was done through the subsurface drip irrigation tape on a weekly basis; fertilizers were supplied through the drip tape weekly to supply a total of 120, 80, and 100 kg·ha⁻¹ of N, P₂O₅, and K₂O over the growing season. There are only two common weeds in the area that can grow through the plastic—yellow nutsedge (Cyperus esculentus) and purple nutsedge (C. rotundus)—and these were removed several times by hand-weeding. In general, sweet basil reached beginning of flowering 2 to 3 d earlier than holy basil. Both species set up many flowers and have a much extended flowering stage, which allowed for a harvest at the same time. Also, although the shape of inflorescences and individual leaves are different between the species, both species have similar overall plant shape.

All basil plants were harvested by hand in full bloom (7 weeks after transplanting) by cutting at 10 cm aboveground, the fresh and air-dry weights were recorded, and the plants were dried at a temperature of 40 °C to preserve the essential oil content and composition (Topalov, 1962). Basil could provide two to three harvests; however, in that case, it must be cut relatively high so some leaves and secondary branches remain intact. Basil cutting at 10 cm above soil surface (which is typical for field production systems where basil is cut with machinery and distilled for essential oil) does not allow for regrowth. Hence, the data in this study reflect a single harvest. The essential oil from all air-dried basil samples was extracted using a modified Cleveeng collector apparatus (Furnis et al., 1989). A sample size of 150 g of air-dry aboveground material (stems, leaves, and flowers from six plants from the middle of every plot) and a distillation time of 120 min were used.

**Essential oil sample preparation and compositional gas chromatography–mass spectrometry analysis.** The essential oil from each treatment was weighed, and the oil content was calculated as the weight (g) of oil per weight (g) of dried basil herbage. Essential oil samples were prepared using a microcentrifuge by transferring 100 μL of oil from each sample into a 10-mL volumetric flask and brought to volume with CHCl₃. Furthermore, a 1-mL aliquot of each oil sample was transferred into a gas chromatograph vial for analysis. Gas chromatograph grade chemical standards and the basil oils from the field experiment were analyzed by gas chromatography–mass spectrometry (GC-MS) on a Varian CP-3800 (Palo Alto, CA) GC attached to a Varian Saturn 2000 MS/MS. The GC was fitted with a DB-5 fused silica capillary column (30 m × 0.25 mm, with film thickness of 0.25 μm, splitless injection) operated under the following program: injector temperature, 240 °C; column temperature, 60 to 240 °C at 3 °C/min then held at 240 °C for 5 min; carrier gas, He; injection volume, 1 μL (splitless); MS mass range from 40 to 650 m/z; filament delay of 3 min; target total ion chromatogram (TIC) of 20,000; a prescan ionization time of 100 μsec; an ion trap temperature of 150 °C; manifold temperature of 60 °C; and a transferline temperature of 170 °C.

**Quantitative analysis.** Commercial standards of eugenol, methyl chavicol, and (-)-trans-caryophyllene were purchased from Aldrich (St. Louis, MO), whereas (-)-linalool, (-)-camphor, α-humulene, eucalyptol,
and (−)-bornyl acetate were purchased from Fluka (Buchs, Switzerland). A previously
isolated and characterized compound, humulene epoxide II, was used as a standard as well
(Cantrell et al., 2005). With five concentration points, an external standard least squares
regression for quantification was used. Separate calibration curves were formulated for
each of the nine analytes. Linearity was imposed by using response factors and regression
coefficients independently. The response factors were calculated using the equation
\[ RF = \frac{DR}{C} \]
where DR is the detector response in peak area (PA) and C is the analyte concen-
tration. The range of analysis was from 1.0 mg mL\(^{-1}\) to 0.0001 mg mL\(^{-1}\) and \(R^2\) values
were 0.994 or higher for all analytes.

The resulting oil chromatograms from each sample from the field experiments were
compared with the standard injections, and the target peaks were confirmed by both retention
time and mass spectra. The confirmed integrated peaks were used to determine the percentage of each chemical
constituent in the essential oil from each sample. The “percent in oil” for each sample
was determined using the RF of the target chemical constituent and using the equation:
\[ \left( \frac{PA}{RF/C} \right) \times 100 = \% \]
(\(PA = \) peak area of analyte from sample injection; \(RF = \) response
factor of analyte; \(C = \) concentration of sample) in oil. Percent is therefore a wt/wt
percentage of analyte in oil. Injection volume was 1 \(\mu\)L for samples and standards.

There were three commercially unavailable analytes (α-trans-bergamotene, γ-cadinene,
and cadinol) that were major constituents in some of the basil samples (Fig. 2). Identification
of these components was obtained using retention times, Kovats indices, and
mass spectra. Kovats indices were calculated using the equation
\[ K_I(x) = 100 \left( \frac{\log RT(x) - \log P_2}{\log RT(P_{x+1}) - \log RT(P_x)} \right) \]
where: \(RT(P_x) \leq RT(x) \leq RT(P_{x+1})\) and \(P_4 \ldots P_{25}\)
are n-paraffins.

All data analyses of oil content, air-dry herbage, and essential oil yields were done
using one-way analysis of variance in Quatro Pro (Corel, Ottawa, Ont., Canada).

**Results and Discussion**

The four locations had dissimilar soil characteristics; soil pH was lower in Bea-
umont and Crystal Springs and higher in Stoneville and Verona, and the soil at Bea-
umont was lower in organic matter relative to the other locations (Table 1). The phyto-
available nutrients (extracted with the Lancaster soil test method) had different con-
centrations at the four locations; the soil in Verona had relatively lower concentra-
tions of P and K compared with the other locations. Stoneville had the highest values for nearly
every soil parameter measured. Although basil is notorious for diseases, especially in
warm and humid climates (Dudai et al., 2002; Holcomb and Cox, 1998), we did not de-
tect any disease infestation on sweet or holy basils at the four experimental sites in
Mississippi.

### Herbage yields

The three basil cultivars yielded differently at the four locations (Table 2). Yields of cv. Mesten were the
highest in Stoneville, lower in Crystal
Springs, and the lowest in Verona and Bea-
umont. Air-dry matter yields of cv. German of
sweet basil were the highest in Crystal
Springs, lower in Stoneville and Verona, and the lowest in Beaumont. Yields of holy
basil cv. Local were the highest in Verona, lower in Crystal
Springs and Stoneville, and the lowest in Beaumont. In Beaumont and Stoneville, cv. Mesten of sweet basil had higher yields than the other two cultivars. In
Crystal Springs, cv. German of sweet basil provided the highest yields, whereas in Verona, holy basil cv. Local had higher yields than the other two cultivars (Table 2). Overall, the lowest basil herbage yield across the three cultivars was in Beaumont, the southernmost location, most probably the result of differences in soil type and climate. Beaumont has sandier soil and higher temperatures relative to the other locations (Table 1). Yields of all three cultivars at the other three locations (Crystal Springs, Stoneville, and Verona) were relatively high and comparable to literature reports from other countries (Bowes and Zheljazkov, 2004; Topalov, 1962; Zheljazkov et al., 1998).

**Essential oil content.** Oil content of cv. Mesten was also different at the four locations, with higher oil content in Crystal Springs, Stoneville, and Verona and lower in Beaumont (Table 2). Oil content of sweet basil cv. German was also lower in Beaumont relative to the other locations, although it was the most southern location with higher temperatures, which was supposed to increase oil content as indicated by previous research (Topalov, 1962). Oil content of holy basil cv. Local was the highest in Crystal Springs, lower in Verona, and the lowest in Beaumont and Stoneville (Table 2). At Beaumont and Crystal Springs, sweet basil cv. German provided higher oil yields than the other cultivars. At Stoneville, sweet basil cv. Mesten provided higher oil yields, whereas at Verona, all three cultivars had similar oil yields (Table 2).

Oil content of sweet and holy basil in this study was similar to literature reports (Anwar et al., 2005; Bowes and Zheljazkov, 2004; Marotti et al., 1996; Topalov, 1962). For example, previous reports found basil essential oil content variation between 0.07% and 0.07% (Anwar et al., 2005; Pino et al., 1994; Wetzell et al., 2002). Simon et al. (1999) reported basil oil content ranging from 0.04% to 0.70% in a study of a number of basil accessions in the United States. In a large study on 270 sweet basil accessions in Germany, oil content in air-dry leaves (leaves only) varied from traces to 2.65% (Kruger et al., 2002). The same authors reported a great variation in chemical composition among the 270 accessions. Except for the oil yields at Beaumont, oil yields of the cultivars in this study were within the range for sweet and holy basil reported in the literature (Bowes and Zheljazkov, 2004; Marotti et al., 1996; Topalov, 1962; Zheljazkov, 1998).

**Essential oil composition.** Overall, the oil composition was representative for sweet and holy basil (Table 3, Fig. 2) (Bowes and Zheljazkov, 2004; Zheljazkov et al., 2008). The major constituents of sweet basil cultivars were (-)-linalool and eucalyptol, whereas the major constituents of holy basil oil were eucalyptol, eugenol, and methyl chavicol. Sweet basil cultivars did not contain detectable amounts of humulene epoxide II or (-)-camphor or (--) bornyl acetate (Table 3). All three cultivars contained a significant concentration of α-trans-bergamotene (Fig. 2), which could not be properly quantified as a result of the unavailability of a commercial standard. Also, sweet basil cultivars contained cadinol, whereas cv. German and cv. Local had γ-cadinene (Fig. 2), which again could not be quantified as a result of the unavailability of commercial standards. As expected, oil composition of the three cultivars was slightly different at the four locations, confirming literature reports (Bowes and Zheljazkov, 2004; Topalov, 1962). For example, holy basil oil accumulated (--)linalool only at Stoneville, sweet basil cv. Mesten did not have (--)camphor at Beaumont, whereas cv. German did not have eugenol at Crystal Springs or Beaumont (Table 3). Our results suggest sweet basil cvs. German and Mesten could fit into the (-)linalool chemotype or to the European basil according to the classification of Marotti et al. (1996).

In another study, Zheljazkov et al. (2008) analyzed commercially available essential oils from sweet basil produced in Bulgaria, Italy, India, the Seychelles, and the United States. They identified 23 compounds (Table 3). Differences in oil composition among the 270 accessions were found, with compounds that were identified in a few accessions only. The content of (-)linalool, the major constituent of basil oil, varied from traces to 2.65%. The content of eucalyptol, eugenol, and methyl chavicol, the major components of holy basil oil, varied from traces to 2.65% (Kruger et al., 2002). The content of α-trans-bergamotene, the major component of sweet basil oil, varied from traces to 2.65% (Kruger et al., 2002).
States. In general, the essential oil composition of basil cultivars from this study was similar in composition to the analyzed commercial basil. Hence, basil essential oils from Mississippi and possibly the southeastern United States could be marketed the same way as basil oils already on the world market. In addition, the oil composition of the sweet and holy basil cultivars in our study was within the typical variation of the oil composition of commercial basil oils from India, France, Australia, and the Seychelles as reported earlier by Lachowicz et al. (1996).

The essential oil composition of the sweet and holy basins grown in Mississippi was comparable to another study on cv. Menesten of Ocimum basilicum and cv. Local of Ocimum sanctum conducted in Atlantic Canada by Bowes and Zheljazkov (2004). Interestingly, the (-)-linalool concentration in both studies was similar; in the study in Atlantic Canada, the (-)-linalool concentration in cv. Menesten oil varied between 38% and 65%, whereas in this study, the (-)-linalool concentration varied between 30% and 65%, indicating a relatively stable range of variation for this trait. However, eugenol accumulation in cv. Menesten in Atlantic Canada was lower than eugenol in cv. Menesten in this study. Regarding cv. Local of holy basil, methyl chavicol and eucalyptol in all locations plus eugenol in Crystal Springs and Verona were the major oil constituents in our study in Mississippi, whereas the major constituents of this cultivar grown in Atlantic Canada were carene, methyl chavicol, elemene, or (E)-humulene (Bowes and Zheljazkov, 2004). These compositional differences between the same cultivars grown in Mississippi and Atlantic Canada could be the result of differences in climatic conditions, soil characteristics, or production systems between the two locations and, apparently, the relatively less conservative nature of the synthesis or accumulation of these constituents at harvest. Our results suggest that even within the state of Mississippi, basil oil composition could vary considerably. This study suggests growing conditions could alter herbage yields, essential oil content, yield, and composition of sweet and holy basil. However, the exact nature of the modifying factors is not clear. There are many factors that might have influenced the chemical profile of basil (e.g., soil type, pH, extractable nutrients, and temperature) (Table 1). This supports previous reports on the significant effect of environmental conditions on basil productivity, oil content, and composition (Javanmardi et al., 2002; Topalov, 1962).

Conclusions

The results from this study suggest that sweet basil cv. Menesten and German and holy basil cv. Local have potential as new high-value essential oil crops for Mississippi. Except in the seasonal microclimatic conditions around Beaumont during this study, the three cultivars of sweet and holy basil could develop well under Mississippi climatic and soil characteristics and provide essential oil with typical composition for the respective species, which would facilitate marketability.

Literature Cited


