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Eirini Sarrou
Hellenic Agricultural Organization "Demeter", Institute of Breeding and Plant Genetic Resources, Department of Medicinal and Aromatic Plants, Thermi 57001, Thessaloniki, Greece

Paschalina Chatzopoulou
Hellenic Agricultural Organization "Demeter", Institute of Breeding and Plant Genetic Resources, Department of Medicinal and Aromatic Plants, Thermi 57001, Thessaloniki, Greece

Theodoros Koutsos V
Hellenic Agricultural Organization "Demeter", Institute of Breeding and Plant Genetic Resources, Department of Medicinal and Aromatic Plants, Thermi 57001, Thessaloniki, Greece

Stavros Katsiotis
Aristotle University of Thessaloniki, School of Pharmacy, Department of Pharmaceutics, Thessaloniki, Greece, 54124

Correspondence
Eirini Sarrou
Hellenic Agricultural Organization "Demeter", Institute of Breeding and Plant Genetic Resources, Department of Medicinal and Aromatic Plants, Thermi 57001, Thessaloniki, Greece, esarroy@gmail.com

Herbage yield and essential oil composition of sweet basil (*Ocimum basilicum* L.) under the influence of different mulching materials and fertilizers

Eirini Sarrou, Paschalina Chatzopoulou, Theodoros Koutsos V, Stavros Katsiotis

Abstract
Field experiment was conducted in order to evaluate the effect of synthetic and organic mulching on weed control, biomass production and essential oil yield of sweet basil (*Ocinum basilicum* L.). Moreover, the effect of fertilizers containing different forms of nitrogen on sweet basil essential oils and herbage yield was also examined. The applied fertilization (one inorganic and four organic), affected significantly the herbage yield compared to the control. Namely, the treatments of organic Agrobiosol (O-A) and Neem-cake (O-NK) increased the herbage yield by 33 and 47.75%, respectively. The essential oil content ranged between 1.06-1.27% and 0.88-1.36% under mulching and fertilizing treatments, respectively, with main constituents: linalool, 1.8 cineol, 4 terpineol, α-bergamotene and t-cadinene. Moreover significant quantitative differences in basil volatile constituents were observed under the influence of different types of fertilizers. The components 1, 8 cineol, α-bergamotene and t-cadinol were mostly affected by organic fertilization (O-L).

Keywords: sweet basil, fertilization, mulching, essential oil, linalool

Introduction
The genus *Ocimum* of the Lamiaceae family comprises about 30 species, known as basil, occurring mainly in tropical and subtropical regions (Paton, 1992). It is an annual crop cultivated extensively in the United States, France, Egypt, Hungary, Israel, Italy, Morocco etc. It is cultivated as a culinary herb, ornamental, spice or condiment and its essential oil is used for flavoring foods, in perfumery as well as in pharmacy (Simon, 1999) [41]. Basil essential oil has antioxidant (Lee *et al*., 2005) [19], antimicrobial (Koba *et al*., 2008) [17], antifungal and insect repelling properties (Dube *et al*., 1989) [9]. Different basil varieties, cultivars and chemotypes are globally traded, and they are mostly known by their origin. Depending on the chemotype, the essential oil is characterized by high concentration of linalool, methylchavicol, methylcinnamate or eugenol respectively (Grayer *et al*., 1996; Vieira and Simon, 2006) [12, 46]. The essential oil accumulation and composition is affected by different factors such as genetic, environmental conditions, agronomic techniques (irrigation, fertilization, time of harvesting) etc. (Marotti *et al*., 1996) [22].
Nowadays, there is an increasing consumers' demand for organic MAPs products (Craker, 1997) [8]. Organic cultivation of MAPs adds to their quality, which is associated to their essential oil content or other secondary metabolites. Weed management is of major importance for MAPs crops, especially those under organic farming, where the use of synthetic herbicides is prohibited (Carruba *et al*., 2007) [7]. Several practices, alternative to chemicals, have been utilized for weed control, in many crops, but they have been limited applied in MAPs organic cultivation. Mulching with synthetic or organic materials is a common technique for increasing profitability of many crops (Palada *et al*., 2008; Najafabadi *et al*., 2012) [25]. Mulching effectiveness is attributed to its ability to increase soil moisture, temperature, insect repellence, efficient utilization of soil nutrients, reduce weed growth and stimulate higher crop yield (Ricotta and Masiounas, 1991; Budnik *et al*., 1999; Kashi *et al*., 2004) [36, 16]. Moreover, a reduced competition for light due to weed absence has resulted in low density of trips, aphides and flies that causes damage in cultivations (Ricotta and Masiounas, 1991) [36].
Different materials may be used for mulching, including crop residues and organic mulches, various plastic materials, paper mulches, biodegradable films etc. (Haapala *et al*., 2014) [13]. The efficiency of mulching is dependent on the crop species, the cultivation practices etc. Genetics and other factors such as climate, harvest time and the use of fertilizer affect the essential oil content and composition in medicinal and aromatic plants (Dudai, 1992; Nurzyńska-Wierdak, 2013) [10, 26].

Plant nutrition enhances plant production. Particularly, nitrogen may affect essential oil accumulation according to the applied form (organic/ inorganic), the rate-concentration, the tissue is applied to (roots/ leaves), the way of application (one dose in the beginning of the harvest period or more) and the interaction with other minerals such as potassium, phosphorus etc. (Nurzyńska-Wierdak, 2013) [26]. It has been documented that nitrogen applications may increase the essential oil production in MAPs, by enhancing herbage yield and photosynthetic rates (Ram *et al*., 1995; Meneghini *et al*., 1998; Rao, 2001; Sangwan *et al*., 2001) [32, 23, 35, 37].

NO_3^- and NH_4^+ are the main inorganic forms of N, presented naturally in the soil solution and absorbed by plants, while NH_4^+ is the most applied form of N fertilizers that is rapidly nitrified to NO_3^- in regards to soil texture and its pH (Haynes, 1986; Adler *et al*., 1989; Omer *et al*., 2008) [14, 2, 28]. Previous reports stated that both N forms didn't alter the concentration of monoterpenes, while NH_4^+ favored the sesquiterpenes' accumulation in *Ocimum basilicum* essential oil (Adler *et al*., 1989) [2]. Moreover, Kandeel *et al*. (2002) [15] observed significantly higher herbage and oil yield under the influence of organic and inorganic N (in combination).

Due to the great industrial importance of basil, and aiming at the evaluation of some organic cultivation practices on Medicinal and Aromatic Plants production, we investigated the effect of different mulching materials and nitrogen fertilizers (organic and inorganic) on weed control, biomass yield and the essential oil of a Greek local variety of sweet basil (*Ocimum basilicum* L.).

Materials and methods

Cultivation and Experimental design

Two separate field experiments a) mulching and b) fertilizing, were conducted at the experimental field of Medicinal and Aromatic Plants Department (Thessaloniki, North Greece), from mid-March to the end of July (2011).

Seedling production: The plants were grown from seeds of a Greek local variety of sweet basil (*Ocimum basilicum* L.), in pots filled with peat, and kept in nursery for one month. The seedlings were transplanted in the field, in rows, at distances 1 m between rows and at 33 cm within the rows.

The experimental layout in both trials was Randomized Complete Block Design (RCBD), with 4 replications for each treatment (each plot contained 30 plants in 10 m^2).

The soil properties at the experimental site were as follows: Soil type: Red loam, pH 7.73%, clay: 39.0, Organic matter: 1.43%, P_2O_5 (ppm): 45, K_2O (ppm): 520. All plots were equally and on a regular basis drip irrigated.

In mulching experiment 3 different mulching materials in comparison with 2 control treatments (non weeded and normal weeded) were applied:

a) M-GT (geo textile, permeable to water)
b) M-BP (black thick plastic)
c) M -WS (wheat straw)
d) N-WD (Non-weeded) and
e) C-HW (control: hand weeded 2 times during the cultivation period.

In fertilizing experiment, 5 types of fertilizers were applied in comparison with a control treatment:

a) O-L containing 8% total N (of which 6% Urea), 2% P_2O_5, 2% K_2O and trace elements Mg, Mn, Fe, Zn, Ball in chileic form, Ca and Al, (*Lipanfyt*, GEOVET-HELLAS S.A, Veria Imathias, Greece) in rate 6 lt/acre,
b) O-A containing 6-8% total N, 0.5% P_2O_5, 0.5% K_2O, (*Agrobiosol,* INTRACHEM, Athens, Greece) in rate 100 kg/acre,
c) O-NK containing 2% total N, 0.7% P_2O_5, 2.3% K_2O, (*Neem-cake*, GEOVET-HELLAS S.A, Veria Imathias, Greece) in rate 60 kg/acre,
d) O-CM, cattle manure, in rate 2.000 kg/acre,
e) I-NN, NH_4NO_3, 33.5% N, (of which 50% NH_4 and 50% NO_3) in rate 25 kg/acre and
f) C- Control: no additional fertilization.

The fertilization treatments were estimated on the same N rate for each plot. All the fertilizers were incorporated into the soil at the beginning of the experiments. The herbage yield and the essential oil content were estimated at the end of both experiments, after 120 days from transplanting.

Essential oil

The essential oil content (ml 100 g $^{-1}$ on wet basis) was determined using a Clevenger-type apparatus. The fresh leaves of basil were subjected to hydrodistillation for 1.5 hour, with a distillation rate 3 - 3.5 mL min $^{-1}$. The essential oil was dried over anhydrous sodium sulphate and stored at 4-6ºC, until analyzed.

The essential oils were analyzed by GC/MS on a fused silica DB-5 column, using a Gas Chromatograph 17A Ver. 3 interfaced with a Mass Spectrometer Shimadzu QP-5050A supported by the Class 5000 software. Injection temperature: 260 ºC, interface heating: 300 ºC, ion source heating: 200 °C, EI mode: 70 eV, scan range: 41 – 450 amu, and scan time 0.50 seconds. The oven temperature programs were as follows: a) 55 – 120 °C (3 °C/ min), 120 – 200 °C (4 °C/min), 200 – 220 °C (6 °C/min) and 220 °C for 5 min; and b) 60 – 240 °C at 3 °C/min, carrier gas He, 54.8 kPa, split ratio 1:30. The percentage composition of the oils was computed after 3 GC runs of each sample from the peak areas without correction factors.

The identification of the constituents was based on comparison of their retention indices (RI) relative to n-alkanes, with corresponding literature data, and by matching their spectra with those of the MS libraries (NIST 98, Wiley) (Adams, 1995) [1].

Statistical analysis

The data were analysed with Analysis of Variance (ANOVA), using the statistical package SPSS 11 17.0 (SPSS Inc. Chicago, Illinois, USA). For mean comparison, the Duncan's multiple range test and standard error (S.E) were used at $P \leq 0.05$ to establish significant differences.

Results and Discussion

Herbage yield

The effect of different mulches and fertilization on basil potential yield and essential oil was investigated, under the environmental conditions of N. Greece. Basil is an annual crop and growth and yield data may be obtained during the annual growing season. Under the different mulch treatments, the fresh herbage basil yield was significantly lower in the straw mulch (10.75 kg/10 m^2), compared to the other treatments, and to the control as well. Moreover, both geo textile and black plastic increased significantly the herbage yield (19.89 and 19.93 kg/10 m^2, respectively), compared to unweeded plants, but they did not differ statistically from the control (16.05 kg/10 m^2) (Figure 1A). Additionally, all mulch treatments were effective for the weed management in basil cultivation. Similarly, Duppong *et al*. (2004) [11] reported no significant effect of flax straw and wool mat treatment on catnip and St.

John's wort yield, in the second year of cultivation. On the other hand, Palada *et al*. (2008) reported an increase of sweet basil biomass, under the effect of organic mulch comparing to synthetic ones.

As previously suggested, the effectiveness of various mulches could be attributed to an elevated water use efficiency, a moderated soil temperature, an increase of CO_2 level around the plants, a more efficient utilization of soil nutrients, a reduced soil compaction, which can increase root development of medicinal plants under synthetic mulch (geo textile and black plastic) (Kim *et al*., 1998; Tarara, 2000; Kashi *et al*., 2004; Palada *et al*., 2008) [45, 16].

Previous studies have shown an increase in soil moisture, and a significant decrease of daily average and maximum temperature under mulches, in particular under straw (Duppong *et al*. 2004) [11]. In our experiment, the increased soil moisture due to mulch treatment might have a positive effect on basil plants growth. Oppositively, decrease of soil temperature, especially during the first stage of plant development, occurring in spring and under the climatic conditions of N. Greece, possibly affected negatively the plant growth and yield, since basil is well developing at higher temperature (25 °C). These opposite effects and the particular environmental conditions at the site of cultivation, could probably explain the decrease under straw and the non-significant effect of black plastic and geo textile, on basil herbage yield.

The applied fertilization (one inorganic and four organic), affected significantly the herbage yield of basil, compared to the control (Figure 1B). More specific, the treatments O-A and O-NK increased the basil biomass by 33 and 47.75%, respectively. Our data are in agreement with previous studies, which reported an enhancement of fresh or dried yield in *O. americanum*, *O. canum* and *O. basilicum,* under the influence of ammonium forms of fertilizers, while these reactions were sometimes dose-depended (Omer *et al*., 1998; Youssef *et al*., 1998; Mousa-Fatma, 2000; Omer *et al*., 2008) [27, 47, 24, 28]. Moreover, the effect of cultivar on N use efficiency for biomass or oil production has previously suggested (Rao, 2001; Ram *et al*., 2003; Sifola and Barbieri, 2006) [35, 33, 39].

Essential Oil

The essential oil yield was higher in the unweeded and mulch treatments compared to the control (1.22, 1.16, 1. 22, 1.27 and 1.06%, respectively), though this difference was not statistically significant. (Figure 2A). The no significant difference of essential oil yield in unweeded plants could be attributed to an elevated secondary metabolism of basil plants, in order to compete /survive from the coexistence with weeds, thereby reinforcing the hypothesis of the allelopathetic action of basil essential oils. Moreover, the essential oil content did not differ among the mulches. Similarly, Singh (2013) [43] reported that *Rosmarinus* essential oil was not influenced by the application of organic mulch and N fertilization. Our findings are probably correlated to the almost equal effect of mulches on basil herbage yield, considering however, that other processes, viz. photosynthesis, boost the accumulation of plant volatiles.

The fertilization treatments O-NK and I-NN promoted the essential oil accumulation, while, the O-L treatment decreased the essential oil content almost 35% compared to the above treatments (Figure 2B). Previous report indicated different reactions of *O. americanum* biomass and oil production in relation to nitrogen form (Omer *et al*., 2008) [28]. These authors reported an enhancement of the essential oil content under the influence of urea form, compared to ammonium nitrate, while in our data stated the opposite. Singh and Wasnik (2013) [43] showed in their study that the content and quality of Rosemary essential oil was not affected by the organic fertilization. Other studies, reported a decrease or increase of essential oil content in *O. basilicum* under the effect of ammonium nitrate (Omer *et al*., 1998; Youssef *et al*., 1998) [27, 47]. With regard to the effect of the different cultivar in our experiment, O-L treatment, containing N in the urea form, may be the reason for the decreased essential oil content. According to previous reports, nitrogen fertilization has affected the composition of essential oil in medicinal plants (Ozgüven *et al*., 2001; Asraf *et al*., 2006; Sekeroglou and Ozgüven 2006) [29, 3, 38] through their effect on biomass yield, leaf area and photosynthetic rate (Ram *et al*., 1995; Ram and Kumar, 1997; Alder *et al*., 1998; Kandeel *et al*., 2004) [32, 31]. Hence, higher rates of photosynthesis were recorded in crops when the relative investment of leaf N to chlorophyll (Chl/N) had been increased by N fertilization (Mae, 1997; Ranjith and Meinzer, 1997) [21, 34].

The qualitative composition of basil essential oil obtained from different mulches and fertilization, was similar. Twenty compounds were detected, accounting for the 92.58% and 92.34% of the total oil, under the different mulches and fertilizing respectively. The most dominant compound was linalool, following by 1, 8 cineol, terpinen -4 ol, α-bergamotene and t-cadinol. The Greek sweet basil cultivar can be classified as 'linalool-rich' chemotype, constituted from a relatively high amount of linalool. Moreover, the essential oil is characterized by the absence of eugenol and methyl-chavicol, which are main compounds of other basil varieties, as previously reported in the literature (Grayer *et al*., 1996; Marotti *et al*., 1996; Vieira and Simon 2006) [12, 22, 46].

According to the results presented in Table 1, the highest yield of linalool (63.88%) and consequently of the oxygenated monoterpenes (75.05%), was achieved in the weeded treatment, followed by the straw and the geo textile treatment. Nevertheless, these differences were not statistically significant. Totally, it seems that sesquiterpenes were obtained at higher amounts under the different mulching materials, comparing to the control. The highest content of α-bergamotene (3.13%), was detected in the black plastic treatment, while those of t-cadinol in the straw treatment. Singh (2004) [42] reported that the main compounds of rosemary essential oil, a pinene, 1, 8 cineol, camphor and verbenone were not affected by the use of organic mulching and nitrogen fertilization.

Significant quantitative differences in basil volatile constituents were observed under the influence of different types of fertilizers (Table 2). 1, 8 cineol, α-bergamotene and t-cadinol were mostly affected by fertilization. Organic fertilization (O-L) exerted a positive effect on these compounds; however it didn't seem to affect statistically significant the linalool content. This variation may be ascribed to the activity of some enzymes involved in the biosynthesis of essential oils and the possible improvement of metabolism, as a result of fertilizer application, as suggested in previous study on peppermint (Burbott and Loomis, 1969) [6].

Furthermore, it has been observed that N favored the synthesis of many organic compounds such as proteins, enzymes, amino acids etc. The last two substances have a key role in the biosynthesis of many constituents of essential oils (Koeduka *et al*., 2006) [18].

Monoterpenes are secondary metabolites formed in the chloroplasts (Bohlmann *et al*., 1998) [4]. Their concentration may depend on CO_2 levels and metabolic intermediates, such as carbohydrates, formed during the process of photosynthesis (Loreto *et al*., 1996) [20] and the production of monoterpenes is directly dependent on amount of non-structural carbohydrates available for use as substrate (Charlwood and Panthrope, 1978; Croteau, 1984). At least, such changes observed in our study are difficult to explain because the biochemical pathways of the synthesis of some volatile compounds have not been yet fully-explained. Moreover, in addition to the direct effect of N on plant metabolism, the interaction between N and other minerals contained in the fertilizers might be more important than its individual action, as previously suggested (Nurzyńska-Wierdak, 2013) [26].

The results of our study showed that the different synthetic mulches did not have significant differences among them regarding their effect on basil biomass production and the essential oil yield and composition. From the practical point of view and taking into account the lifetime and the cost of each material, our experiment indicates the use of geo-textile mulch as the best choice for basil weed control. On the other hand, the applied organic and inorganic fertilization affected significantly the basil herbage yield. Moreover, organic fertilization (O-NK) promoted the essential oil content, as well as O-L favored the accumulation of 1, 8 cineol, α-bergamotene and t-cadinol.

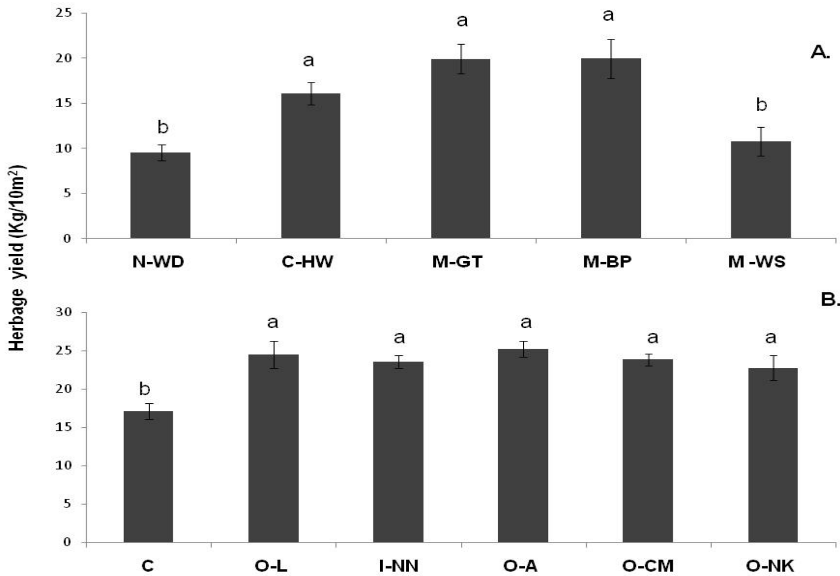

Fig 1: Effect of mulching (A.) and fertilizing (B.) on herbage yield (kg/10 m^2) of *Ocimum basilicum* L. N-WD: Non-weeded, C-HW: control hand weeded, M-GT: geo textile, M-BP: black thick plastic, M-WS: wheat straw, C: control, O-L: Lipanfyt, I-NN: NH_4NO_3, O-A: Agrobiosol, O-CM: cattle manure, O-NK: Neem-cake. Values are mean (n = 4) ± SE. Vertical bars represent ± SE. Different letters indicate a significant difference at $P \leq 0.05$ according to ANOVA and Duncan's multiple range test.

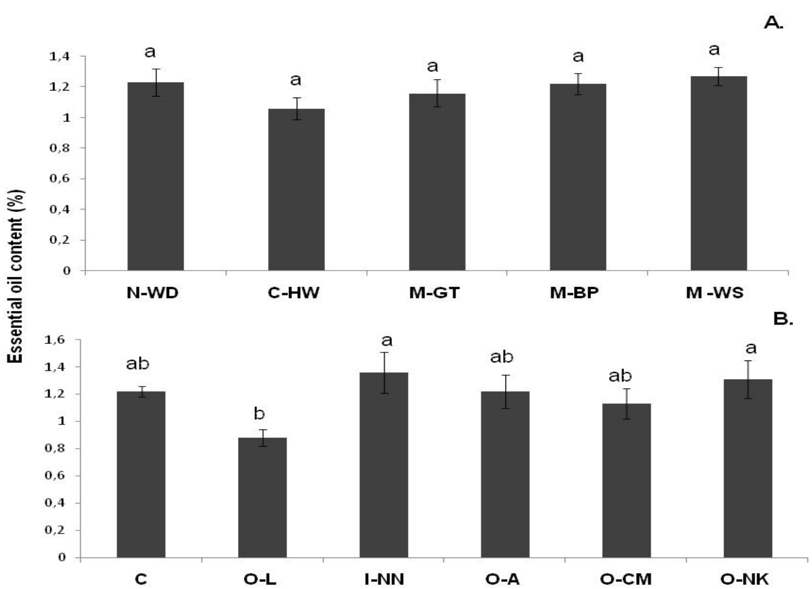

Fig 2: Effect of mulching (A.) and fertilizing (B.) on essential oil content (%) of *Ocimum basilicum* L. N-WD: Non-weeded, C-HW: control hand weeded, M-GT: geo textile, M-BP: black thick plastic , M-WS: wheat straw, C: control, O-L: Lipanfyt, I-NN: NH_4NO_3, O-A: Agrobiosol, O-CM: cattle manure, O-NK: Neem-cake. Values are mean (n = 4) ± SE. Vertical bars represent ± SE. Different letters indicate a significant difference at $P \leq 0.05$ according to ANOVA and Duncan's multiple range test.

Table 1: Percentage composition of basil essential oil under the influence of organic and inorganic fertilizers

		Percentage composition [d,e] %					
No.	**Compounds [a]**	**C**	**O-L**	**I-NN**	**O-A**	**O-CM**	**O-NK**
1	myrcene	1.33 ± 0.07	2.11 ± 0.45	1.49 ± 0.08	1.43 ± 0.19	1.85 ± 0.12	1.28 ± 0.07
2	p-cymene	0.48 ± 0.01	1.18 ± 0.29	0.53 ± 0.03	0.47 ± 0.01	0.48 ± 0.01	0.48 ± 0.03
3	1,8 cineol	6.41 ± 0.30[bc]	7.35 ± 0.36[a]	7.12 ± 0.39[ab]	6.46 ± 0.09[bc]	5.72 ± 0.10[c]	6.27 ± 0.24[bc]
4	cis β-ocimene	0.37 ± 0.02	0.57 ± 0.14	0.43 ± 0.02	0.41 ± 0.08	0.47 ± 0.09	0.37 ± 0.03
5	trans β-ocimene	0.66 ± 0.03	1.00 ± 0.25	0.78 ± 0.03	0.75 ± 0.11	0.81 ± 0.14	0.66 ± 0.06
6	γ-terpinene	0.44 ± 0.00	0.50 ± 0.03	0.58 ± 0.02	0.54 ± 0.00	0.46 ± 0.00	0.46 ± 0.02
7	linalool	58.86 ± 0.01	56.06 ± 0.31	55.31 ± 0.05	58.00 ± 0.13	55.07 ± 2.81	58.45 ± 0.32
8	camphor	0.80 ± 0.01	0.91 ± 0.01	1.02 ± 0.03	0.30 ± 0.05	0.85 ± 0.03	0.85 ± 0.01
9	4 terpineol	3.41 ± 0.00	4.00 ± 0.01	4.13 ± 0.05	4.17 ± 0.03	3.74 ± 0.06	3.77 ± 0.01
10	α-terpineol	0.70 ± 0.02	0.80 ± 0.01	0.78 ± 0.01	0.74 ± 0.01	0.69 ± 0.01	0.71 ± 0.10
11	β-elemene	1.37 ± 0.15	1.12 ± 0.01	1.54 ± 0.10	1.47 ± 0.11	1.28 ± 0.13	1.59 ± 0.03
12	α-bergamotene	4.18 ± 0.10[a]	4.31 ± 0.27[a]	3.38 ± 0.17[b]	3.45 ± 0.03[b]	3.72 ± 0.14[b]	3.59 ± 0.04[b]
13	α-humulene	0.52 ± 0.02	0.54 ± 0.04	0.70 ± 0.03	0.59 ± 0.17	0.52 ± 0.01	0.61 ± 0.03
14	β-copaene	0.57 ± 0.01	0.60 ± 0.03	0.59 ± 0.01	0.51 ± 0.01	0.55 ± 0.01	0.54 ± 0.11
15	Germacrene D	1.33 ± 0.17	1.19 ± 0.17	1.81 ± 0.15	1.61 ± 0.14	1.37 ± 0.14	1.73 ± 0.07
16	γ-elemene	0.58 ± 0.07	0.44 ± 002	0.83 ± 0.07	0.77 ± 0.05	0.63 ± 0.05	0.83 ± 0.06
17	α-bulnesene	0.92 ± 0.07	0.77 ± 0.02	0.89 ± 0.03	0.90 ± 0.04	0.86 ± 0.05	0.97 ± 0.03
18	γ-cadinene	2.09 ± 0.04	2.38 ± 0.16	1.92 ± 0.07	1.89 ± 0.03	1.96 ± 0.08	1.93 ± 0.01
19	spathulenol	0.96 ± 0.05	1.02 ± 0.02	0.76 ± 0.06	0.80 ± 0.01	0.84 ± 0.04	0.80 ± 0.03
20	t-cadinol	4.97 ± 0.06[b]	5.49 ± 0.23[a]	4.86 ± 0.10[b]	4.97 ± 0.05[b]	4.87 ± 0.17[b]	4.83 ± 0.04[b]
Monoterpene hydrocarbons		3.28	5.36	3.81	3.60	4.07	3.25
Oxygenated monoterpenes		70.18	69.12	68.36	69.67	66.07	70.05
Sesquiterpenes		17.49	17.86	17.28	16.96	16.60	17.42
Main constituents		90.95	92.34	89.42	89.23	86.74	90.72

C: control, O-L: Lipanfyt, I-NN: NH_4NO_3, O-A: Agrobiosol, O-CM: cattle manure, O-NK: Neem-cake.
[a] order of elution on DB-5 column; [d] Percentage of the total peak area. Components with percentage ≥ 0.5% are presented; [e]: ± SE. Different letters indicate a significant difference at $P \leq 0.05$ according to ANOVA and Duncan's multiple range test.

Table 2: Percentage composition of basil essential oil under the influence of different mulches

		Percentage composition [d,e] %			
No.	**Compound [a]**	**C-HW**	**M-GT**	**M-BP**	**M-WS**
1	myrcene	1.77 ± 0.05	1.77 ± 0.10	1.61 ± 0.07	1.59 ± 0.15
2	p-cymene	0.37 ± 0.04	0.43 ± 0.03	0.39 ± 0.06	0.35 ± 0.03
3	1.8 cineol	6.12 ± 0.49	6.14 ± 0.37	5.31 ± 0.52	5.19 ± 0.19
4	cis β-ocimene	0.58 ± 0.03	0.56 ± 0.03	0.50 ± 0.03	0.50 ± 0.06
5	trans β-ocimene	1.00 ± 0.03	1.00 ± 0.06	0.93 ± 0.03	0.90 ± 0.09
6	γ-terpinene	0.46 ± 0.03	0.49 ± 0.01	0.50 ± 0.06	0.50 ± 0.04
7	linalool	63.88 ± 1.74	62.51 ± 0.99	61.75 ± 0.83	62.61 ± 0.78
8	camphor	0.89 ± 0.05	0.86 ± 0.06	0.88 ± 0.08	0.95 ± 0.05
9	terpinen 4 ol	3.48 ± 0.12	3.33 ± 0.22	3.51 ± 0.05	3.59 ± 0.13
10	α-terpineol	0.68 ± 0.04	0.63 ± 0.05	0.63 ± 0.01	0.62 ± 0.01
11	β-elemene	1.16 ± 0.16	1.38 ± 0.15	1.53 ± 0.11	1.55 ± 0.09
12	α-bergamotene	2.93 ± 0.42	3.10 ± 0.21	3.13 ± 0.23	2.91 ± 0.09
13	α-humulene	0.43 ± 0.05	0.46 ± 0.03	0.59 ± 0.04	0.51 ± 0.03
14	β-copaene	0.53 ± 0.07	0.52 ± 0.06	0.53 ± 0.04	0.54 ± 0.01
15	Germacrene D	1.13 ± 0.14	1.32 ± 0.20	1.50 ± 0.16	1.57 ± 0.01
16	γ-elemene	0.58 ± 0.08	0.67 ± 0.12	0.78 ± 0.01	0.74 ± 0.06
17	α-bulnesene	0.72 ± 0.11	0.83 ± 0.07	0.89 ± 0.10	0.89 ± 0.04
18	γ-cadinene	1.46 ± 0.22	1.46 ± 0.14	1.54 ± 0.08	1.57 ± 0.05
19	spathulenol	0.65 ± 0.07	0.69 ± 0.05	0.69 ± 0.02	0.66 ± 0.02
20	t-cadinol	3.76 ± 0.48	3.84 ± 0.09	4.24 ± 0.06	4.53 ± 0.10
Monoterpene hydrocarbons		4.18	4.25	3.93	3.84
Oxygenated monoterpenes		75.05	73.47	72.08	72.96
Sesquiterpenes		13.35	14.28	15.42	15.47
Main constituents		92.58	91.99	91.43	92.27

C-HW: control hand weeded, M-GT: geo textile, M-BP: black thick plastic, M-WS: wheat straw
[a] order of elution on DB-5 column; [d] Percentage of the total peak area. Components with percentage ≥ 0.5% are presented; [e]: ± SE.

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