

Effects of Gamma Irradiated and Non-Irradiated Sewage Sludge on Essential Oil Content and Constituents of *Ocimum basilicum* L.

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Abstract

Background: The increasing amount of sewage sludge (SS) and more legislative regulation of its disposal have stimulated the need for developing new technologies including gamma irradiation to recycle SS efficiently.

Objective: To evaluate the effects of gamma irradiated and non-irradiated SS (SSGI and SSNI, respectively) on essential oil (EO) content and composition of *Ocimum basilicum* L.

Methods: A greenhouse experiment was conducted with 15, 30, and 60 g kg⁻¹ of SS irradiated with absorbed doses of 5, 10, and 20 kGy gamma-ray and soil without SS and irradiation as the control treatment. The essential oil was isolated by hydro-distillation and analyzed by Gas chromatography and Gas chromatography-mass spectrometry techniques.

Results: The applications of SSGI and SSNI at all levels except 15 g kg⁻¹ significantly enhanced EO content compared to the control. The using of 60 g kg⁻¹ SS in each absorbed dose significantly reduced EO yield relative to the control, while it was significantly increased under 15 and 30 g kg⁻¹ SSNI and SSGI. Also, in 60 g SS kg⁻¹, the restriction effects on EO yield were mitigated with increasing the absorbed dose of gamma-ray, but it was insignificant with increasing absorbed dose to 10 and 20 relative to 5 kGy. The methyl chavicol as a major constituent of EO was significantly increased in SSGI and SSNI as compared with the control. However, the increment in the methyl chavicol content was not significant between the treatments ≥ 30 g kg⁻¹ SSGI or SSNI and 15 g kg⁻¹ SS at absorbed dose of ≥ 10 kGy.

Conclusion: To improve essential oil content and composition, application of ≤ 30 g kg⁻¹ sewage sludge with 5 kGy absorbed dose of gamma-ray can be suggested at similar conditions.

Keywords: Basil, Essential oil, Gamma irradiation, Medicinal plant, Sewage sludge

Introduction

The disposal of sewage sludge (SS) has increased dramatically in a human environment due to the rapid growth of industrialization and urbanization [1]. The SS is produced by wastewater treatment processes (WWTPs), where solids and liquids are separated [2]. The sludge produced by WWTPs including primary and secondary sludge is usually processed via aerobic and anaerobic digestion to reduce the water content, stabilize the organic matter in the final product and be disinfected; thus it becomes suitable for final disposal and utilization in agriculture [3]. In general, SS consists organic matter, macro- and micronutrients, and therefore can be utilized in agricultural lands as organic fertilizer to produce better yield especially in the light-textured sandy soils [4]. However, the utilization of SS in agricultural lands could create a few environmental and public health concerns through entering pathogenic bacteria and viruses [5], toxic organic substances and heavy metals (HMs) to the food chain and/or environment [6, 7], due to not completely successful reducing biological and chemical contamination of SS to the desired levels [8]. Ionizing radiation technology especially gamma-ray is regarded as a promising alternative, effective and rapid method of SS treatment [9]. The gamma radiation can cause the following events in SS: (i) inactivation of microbial populations; (ii) oxidation of organic compounds, which can result in the formation of CO₂ and H₂O [10] (iii) degradation of the SS structure, promoting the release of more easily adsorbed free forms of metals derived from the degradation of soluble organic complexes

thereby altering mobility of HMs and micronutrients [6].

On the other hand, SS is mainly used in cultivation of vegetables due to its low cost in Iran. Basil (*Ocimum basilicum* L.), a well-known vegetable and/or non-food crop (medicinal and aromatic plant) belonging to Lamiaceae family [11]. Further, due to the transfer of potential contaminants in SS into the human food chain directly via uptake from agricultural soils, its application to non-food crops seems of great potential [12]. Since the harvested foliage of medicinal and aromatic plants is a source of essential oil (EO) (the marketable revenue-generating products of aromatic), and EO is free from the risk of heavy metals accumulation from initial plant biomass [13, 14]. The previous studies mostly focused on the effects of non-irradiated SS (SSNI) and other organic materials including farmyard manure, poultry manure and vermicompost on EO production and chemical composition of medicinal plants EOs [15-18]. Therefore, there exist very sparse if any reliable and comprehensive data regarding the use of gamma irradiated SS (SSGI) on secondary metabolite biosynthesis of basil plant. Hence, this study aims to evaluate the effects of gamma irradiated and non-irradiated sewage sludge on essential oil production and constituents of basil plant.

Materials and Methods

Materials

All the chemicals (e.g. diethylenetriaminepentaacetic acid (DTPA), sodium bicarbonate, ammonium acetate, hydrochloric acid, nitric acid, sodium

hexametaphosphate, sulfuric acid, ferrous sulfate, sodium hydroxide, potassium antimony tartrate, and potassium dihydrogen phosphate) were purchased from Merck Company, Germany.

Experimental set up

A pot experiment was conducted in a naturally lighted greenhouse at Nuclear Agriculture Research School, Nuclear Science and Technology Research Institute, Karaj, Iran during November 2017. Basil seeds (*O. basilicum* L.) after providing from Pakan Bazr Company, Esfahan, Iran were cultivated in chest and after reaching the three-leaf stage, four uniform seedlings were transplanted in each pot. Plastic pots with diameter and height of 15 and 18 cm, respectively, were used and filled with 3 kg of dried soil. The pots were randomly rotated on a weekly basis in the benches to avoid any potential localized effects on plants. The seedlings were irrigated daily with distilled water to 70-80% field capacity by weighing the pots. The plants were harvested at the fully flowering growth stage (12 weeks after sowing), when the quantity and quality of essential oil (EO) are usually maximum. At harvest, the aboveground plant parts were carefully cut at 2-3 cm above the pot soil level and half of the shoot was shade dried for 14 days at room temperature (20–25 °C) and distilled for EO extraction. The second half of the vegetative tissue were oven-dried (70 °C for 72 h) and subsequently weighed using a digital precision scale (± 0.0001 g). Then the roots were carefully separated from the soil and to prevent losses of hairy roots, it was washed on the sieve with distilled water and dried in a

ventilated oven at 70 °C to a constant weight. Finally, after drying, the samples were ground and sieved by a 0.5 mm sieve and stored in polyethylene bottles at room temperature for further analyses.

Gamma irradiation of sewage sludge and treatment details

Sewage sludge (SS) was collected from Southern Municipal Wastewater Treatment Plant of Tehran, Iran. The wastewater treatment processes of the plant involve gravity and pumping, screening, grit, oil, fat and grease removal, primary sedimentation, aeration tanks and activated sludge, trickling filters, secondary sedimentation and chlorination unit. Also, in order to treat the sludge, Southern Municipal Wastewater Treatment Plant of Tehran has adopted a gravitational condensation of sludge produced from preliminary and secondary treatment, anaerobic digester, storage and stabilization, dewatering with belt filter press and storing dewatered sludge for further drying. The examined SS was collected by a sterilized special recipient immediately after the dewatering treatment.

Gamma irradiation was performed at room temperature from a Gamma Cell ^{60}Co source with a dose rate of 124 Gy min^{-1} and specific activity of 8677 Curie with different absorbed doses of 0, 5, 10 and 20 kGy in the Nuclear Science and Technology Research Institute, Atomic Energy Organization of Iran. Since 10 kGy absorbed dose of gamma radiation has been recommended by United States Environmental Protection Agency (USEPA) as a process to further reduce pathogens in municipal sludge [19]; and considering the

trend of changes in SS properties following gamma irradiation, it was irradiated with half of the recommended dose, the recommended dose and twice the recommended dose of USEPA. The dose of irradiation was determined by the standard Fricke dosimeter.

Both SSGI and SSNI were applied at levels of 0, 15, 30, and 60 g kg⁻¹ soil (approximately equivalent to 0, 30, 60, and 120 tons dw ha⁻¹). Totally, the experiment consisted of 13 treatments which were applied as: soil without SS and irradiation as the control, 15, 30, and 60 g kg⁻¹ of SSNI (abbreviated as SS₁₅NI, SS₃₀NI and SS₆₀NI, respectively), irradiated with 5 kGy (abbreviated as SS₁₅GI₅, SS₃₀GI₅ and SS₆₀GI₅, respectively), 10 kGy (abbreviated as SS₁₅GI₁₀, SS₃₀GI₁₀ and SS₆₀GI₁₀, respectively) and 20 kGy (abbreviated as SS₁₅GI₂₀, SS₃₀GI₂₀ and SS₆₀GI₂₀, respectively).

Essential oil extraction and analysis

Essential oil extraction

The EO of *O. basilicum* plants (one half of the air dried shoot) was isolated by hydro-distillation for 3 h using a Clevenger-type apparatus according to the method recommended by British Pharmacopoeia [20]. The isolated oil was dried over anhydrous sodium sulfate and then stored in tightly closed dark vials at 4 °C in the refrigerator until analysis. The essential oil yield was computed by multiplying the dry shoot yield by the oil content.

Gas chromatography analysis

Gas chromatography (GC) analysis was performed using a Thermoquest-Finnigan gas chromatograph, Model 2000, USA, with a flame ionization detector (FID). The analysis

was carried out on fused silica capillary DB-5 column (30 m (length) × 0.25 mm (internal diameter), and film thickness of 0.25 µm). The injector and detector temperatures were kept at 250 °C and 300 °C, respectively. Nitrogen was used as the carrier gas at a flow rate of 1.1 mL min⁻¹; oven temperature program was 60–250 °C at the rate of 4 °C min⁻¹ and finally held isothermally for 10 min, and split ratio was 1: 50.

Gas chromatography- mass spectrometry analysis

The chemical characterization of the essential oil was performed by a Thermoquest-Finnigan gas chromatography, Model 2000, USA, coupled with Trace mass spectrometry, UK. The temperature program conditions and the column characteristics were the same as those employed and described for GC. Temperature of the transfer line was kept to 250 °C. The carrier gas (helium) was used at a constant and linear flow rate of 1.1 mL min⁻¹. Also, FID split ratio was equal to 1:50. Mass spectra were obtained over the range of 35–465 a.m.u. (atomic mass units) in the electron ionization mode with a voltage of 70 eV.

Identification of essential oil constituents

The constituents of the EO were identified by calculating their retention indices under temperature-programmed conditions for n-alkanes (C₆-C₂₄) and the oil on a DB-5 column under the same chromatographic conditions. Identification of individual compounds was made by comparing their mass spectra with those of the internal reference mass spectra library (Adams and Wiley 7.0) or with authentic compounds and confirmed by comparing their retention indices with authentic compounds or

with those of reported in the literature [21]. For quantification purpose, relative area percentages obtained by FID were used without the use of correction factors.

Chemical analysis of soil and sewage sludge

The soil sample was taken from the surface down to 25 cm depth at the Research Farm of Nuclear Agriculture Research School, located in the Ramandeh village in Savojbolagh (35° 49' 26 " N, 50° 44' 38" E), Alborz Province, Iran. After air drying, crushing and passing through a 2 mm sieve, some physicochemical properties of the soil as well as SSGI and SSNI were measured (Tables 1 and 2, respectively). The chemical characteristics of SSGI and SSNI such as total concentrations of some nutrients and HMs including phosphorus (P) (measured by vanado-molybdate yellow method), potassium (K), sodium (Na), calcium (Ca), magnesium (Mg), copper (Cu), zinc (Zn), manganese (Mn), lead (Pb), cadmium (Cd), iron (Fe) and nickel (Ni) were determined by dry ashing the SS and the ash treatment with diacid mixture [hydrochloric acid (HCl) + nitric acid (HNO₃) in 3:1 ratio] according to Westerman [22]. Also, the concentrations of

Cu, Zn, Mn, Ni, Fe, Pb and Cd in plant shoot and root were measured as mentioned above method for SS. Additional chemical properties of the SSGI and SSNI including electrical conductivity (EC) and pH (1: 5 sludge to water ratio) and total Kjeldahl nitrogen (TKN) were analyzed using standard methods [23].

Also, some physical and chemical properties of the soil sample such as available-P were measured by Olsen method, total nitrogen by Kjeldahl method, available-K by 1 N acetate ammonium, available-Cu, Zn, Fe, and Mn by DTPA-TEA, EC and pH in saturated extract, cation exchange capacity (CEC) by Bower method, soil texture by hydrometric method and organic carbon (OC) by the wet oxidation method were measured [25, 26]. Overall, the concentrations of Ca, Mg, Cu, Zn, Pb, Cd, Fe, Mn, and Ni in extracts were analyzed using atomic absorption spectrophotometer (Shimadzu model AA-6300, Japan), Na and K by flame photometer (Corning-410, UK), pH by pH meter (Mettler Toledo Instrument Co. Ltd., MA235, USA), EC by JENWAY EC meter (model 4320, UK), and P by SU6100 spectrophotometer (Philler Scientific, USA).

Table 1- General physicochemical properties of the studied soil

Characteristic	Quantity	Characteristic	Quantity
Soil texture	Sandy clay loam	CEC (cmol _c kg ⁻¹)	11.7
Soil taxonomy	Typic Haploxerepts	Total N (mg kg ⁻¹)	350
Clay (%)	28.3	Available-P (mg kg ⁻¹)	3.4
Silt (%)	18	Available-K (mg kg ⁻¹)	261
Sand (%)	53.7	Available-Fe (mg kg ⁻¹)	12.3
pH _e	7.18	Available-Mn (mg kg ⁻¹)	2.8
EC _e (dS m ⁻¹)	0.98	Available-Cu (mg kg ⁻¹)	0.8
CaCO ₃ (%)	6.53	Available-Zn (mg kg ⁻¹)	0.45
OC (%)	0.37	Available-Cd (mg kg ⁻¹)	0.22
FC (%)	18.1		

Table 2-Changes in chemical characteristics of the sewage sludge following gamma irradiation

Characteristics	Absorbed dose (kGy)				limit value*
	0	5	10	20	
Ni (mg kg ⁻¹)	36.8 ± 0.75a	36.35 ± 0.2a	35 ± 1.1a	34.8 ± 1.6a	420
Zn (mg kg ⁻¹)	1495 ± 3.8a	1491 ± 7.9a	1483 ± 4.4a	1472 ± 13.1a	7500
Cu (mg kg ⁻¹)	164.2 ± 1a	161.8 ± 1.1ab	157.3 ± 1.95bc	154.7 ± 2.7c	4300
Pb (mg kg ⁻¹)	117.3 ± 1.3a	115.4 ± 1.8a	111.3 ± 3.3a	108.9 ± 6.3a	840
Cd (mg kg ⁻¹)	3.03 ± 0.18a	2.76 ± 0.11a	2.68 ± 0.13a	2.62 ± 0.12a	85
Fe (mg kg ⁻¹)	5750 ± 14.4a	5725 ± 28.9a	5683 ± 74.1a	5583 ± 58.3a	
Mn (mg kg ⁻¹)	149 ± 2.1a	149.6 ± 0.16a	145.2 ± 2.85a	145.8 ± 2.2a	
Mg (mg g ⁻¹)	4.5 ± 0.12a	4.6 ± 0.13a	4.6 ± 0.26a	4.4 ± 0.22a	
Ca (mg g ⁻¹)	33.9 ± 0.8a	34.2 ± 2.2a	37.8 ± 1.6a	37.2 ± 1.4a	
Na (mg g ⁻¹)	1.13 ± 0.08a	1.03 ± 0.06a	1.10 ± 0.05a	1.16 ± 0.06a	
K (mg g ⁻¹)	2.03 ± 0.06a	2.07 ± 0.08a	1.90 ± 0.11a	1.90 ± 0.11a	
P (mg g ⁻¹)	2.42 ± 0.01a	2.53 ± 0.04a	2.45 ± 0.02a	2.51 ± 0.05a	
N (mg g ⁻¹)	26.7 ± 0.98b	28.7 ± 0.49ab	30.6 ± 1.65ab	31 ± 1.59a	
pH (1:5)	6.91 ± 0.02a	6.75 ± 0.06ab	6.68 ± 0.08ab	6.61 ± 0.12b	
EC (1:5) (dS m ⁻¹)	2.97 ± 0.04a	2.75 ± 0.12ab	2.7 ± 0.08ab	2.62 ± 0.12b	

Different letters in each row show significant difference at $p \leq 0.05$ by Duncan multiple range test.

*USEPA standard for biosolid applied to land [24]

Statistical analysis

All data were processed based on a completely randomized design with three replications. Statistical analyses including analysis of variance and means comparison by Duncan's multiple range test ($p < 0.05$) were performed by SAS software (computer SAS software version 9.1; CoHort Software). Values were expressed as mean $\pm$ standard error (SE). The Excel software (Excel software 2013, Microsoft Inc., WA, USA) was used to draw the figures.

Results

Effects of SSGI and SSNI applications on essential oil content and yield

The data pertaining to essential oil (EO) yield and content of sweet basil were presented in Figures 1a and b. The EO content under all SSGI and SSNI applications treatments except SS₁₅NI, SS₁₅GI₅, SS₁₅GI₁₀ and SS₁₅GI₂₀ was

significantly increased as compared to the control ($p < 0.05$), albeit even no significant differences were found between the other treatments (SS₃₀NI, SS₃₀GI₅, SS₃₀GI₁₀, SS₃₀GI₂₀, SS₆₀NI, SS₆₀GI₅, SS₆₀GI₁₀, and SS₆₀GI₂₀) ($p > 0.05$). The EO yield was significantly promoted and/or restricted under the treatments applications compared to the control. So that, application of 60 g SS per kg soil in each absorbed dose of gamma-ray (SS₆₀NI, SS₆₀GI₅, SS₆₀GI₁₀, and SS₆₀GI₂₀ treatments) significantly reduced EO yield relative to the control ($p < 0.05$), while it was significantly increased in the other treatments ($p < 0.05$), although no significant differences were found between SS levels with different absorbed doses except SS₃₀NI as compared with SS₃₀GI₅, and SS₃₀GI₁₀ as well as SS₃₀GI₂₀ and SS₆₀NI as compared with SS₆₀GI₅, SS₆₀GI₁₀, and SS₆₀GI₂₀ treatments. However, the

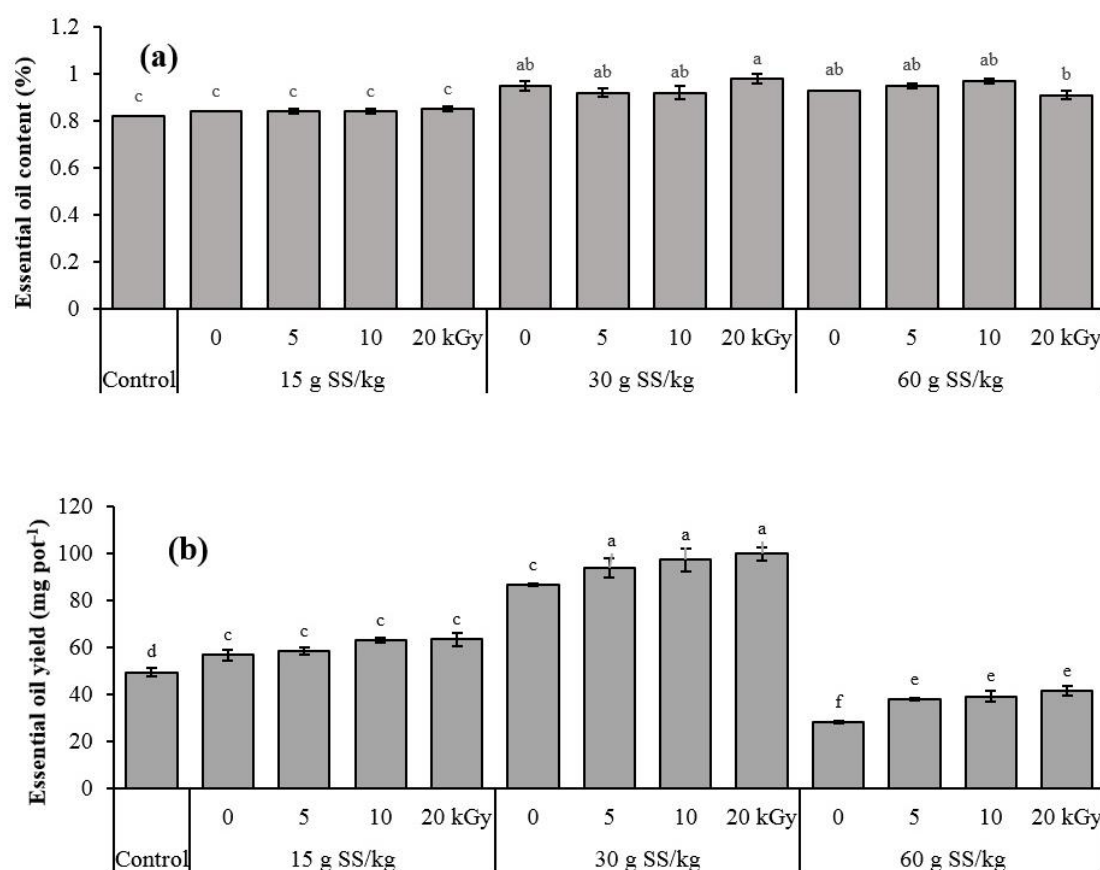


Fig. 1- Essential oil content (a) and yield (b) of *Ocimum basilicum* L. under different levels of gamma irradiated and non-irradiated sewage sludge

maximum EO content and yield were observed in SS₃₀GI₂₀, which had 21 and 102% increase over the respective control, respectively.

Effects of SSGI and SSNI applications on essential oil constituents

The extracted EO was submitted to GC–MS analysis (Fig. 2). Also, sample chromatograms of GC-FID of EO isolated from aerial parts of *O. basilicum* in some treatments were presented in the Fig. 3. The chemical composition of EO of sweet basil under different treatments was listed in the Table 3. Twenty-one constituents according to 98.35-99.89% of the total oil, with the methyl chavicol as the major component were identified for EO of all treatments.

However, methyl chavicol as dominant components ranging from 92.54 to 96.68% for all 13 treatments tested. Methyl chavicol content for all treatments was significantly higher than the control treatment ($p < 0.05$), and the lowest and the highest of its contents occurred in the control and SS₆₀GI₂₀ treatments. Although, no significant differences existed between SS₁₅GI₁₀, SS₁₅GI₂₀, SS₃₀NI, SS₃₀GI₅, SS₃₀GI₁₀, SS₃₀GI₂₀, SS₆₀NI, SS₆₀GI₅, SS₆₀GI₁₀, SS₆₀GI₂₀ ($p > 0.05$). Therefore, SS₆₀GI₂₀ showed the best performance in terms of the increase in Methyl chavicol content, which was higher than that of other treatments and was 4.47% greater than that in the control treatment. On the other hand, as indicated in the Table 3,

the other components were negligible and were less than 7% of total in different treatments. So, due to the negligible portion of other components, further discussion was done about the results of methyl chavicol in the following. Classification of the identified compounds based on functional groups was summarized in the Table 3. As seen, a small fraction of essential oils was devoted to various groups of terpenes including oxygenate monoterpenes, hydrocarbonate monoterpenes, oxygenate sesquiterpenes and hydrocarbonate

sesquiterpenes (1.88 to 5.59%). So that, the maximum amounts of terpenes compounds were recorded in the control treatment and their amounts were decreased with the application of gamma irradiated and non-irradiated sewage sludge. Therefore, the major part of essential oils related to phenylpropanoids was categorized as "other" group. The application of gamma irradiated and non-irradiated sewage sludge increased the amount of phenylpropanoids compounds.

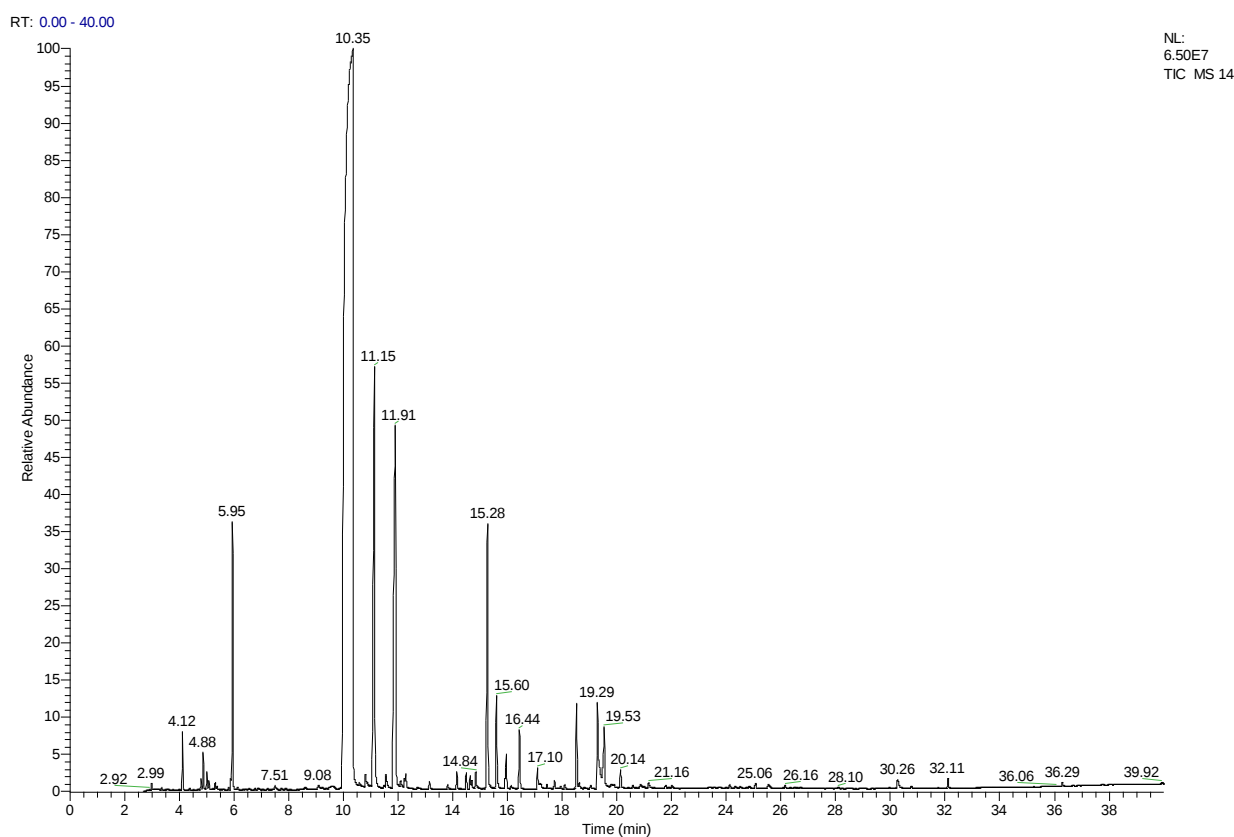
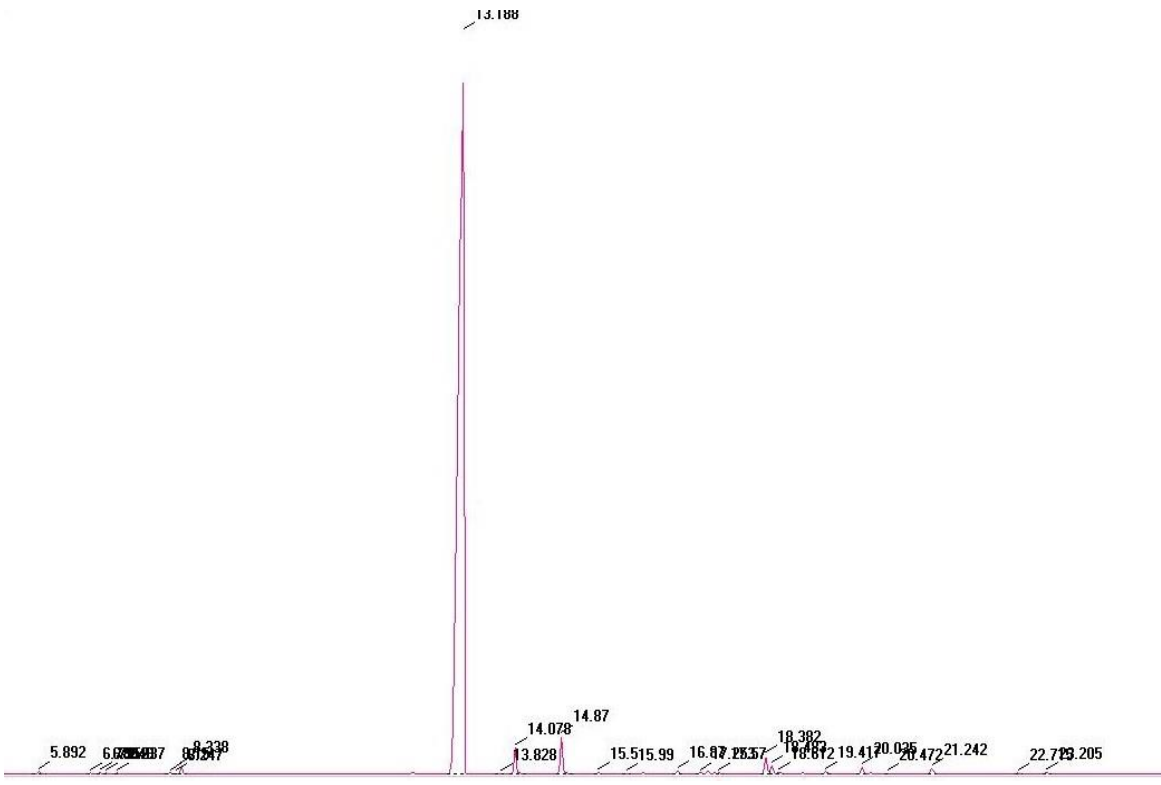
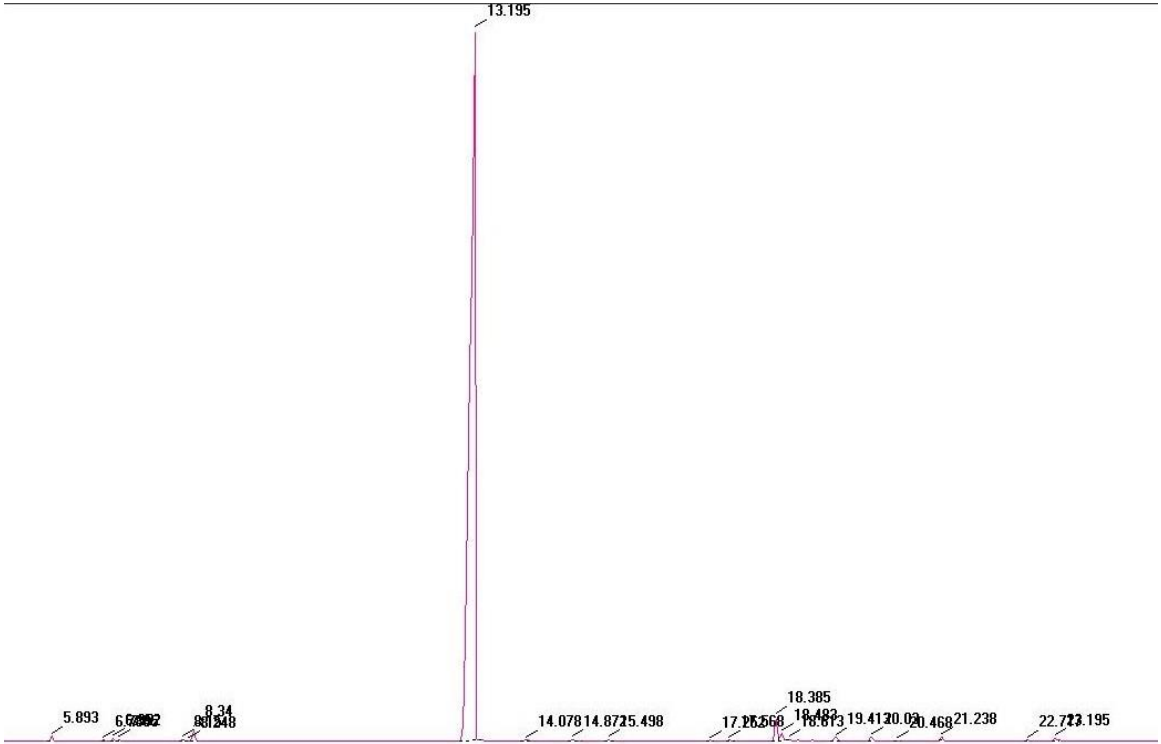


Fig. 2- The GC–MS chromatogram of the chemical composition of the essential oil from *Ocimum basilicum* L. aerial parts

(A)



(B)



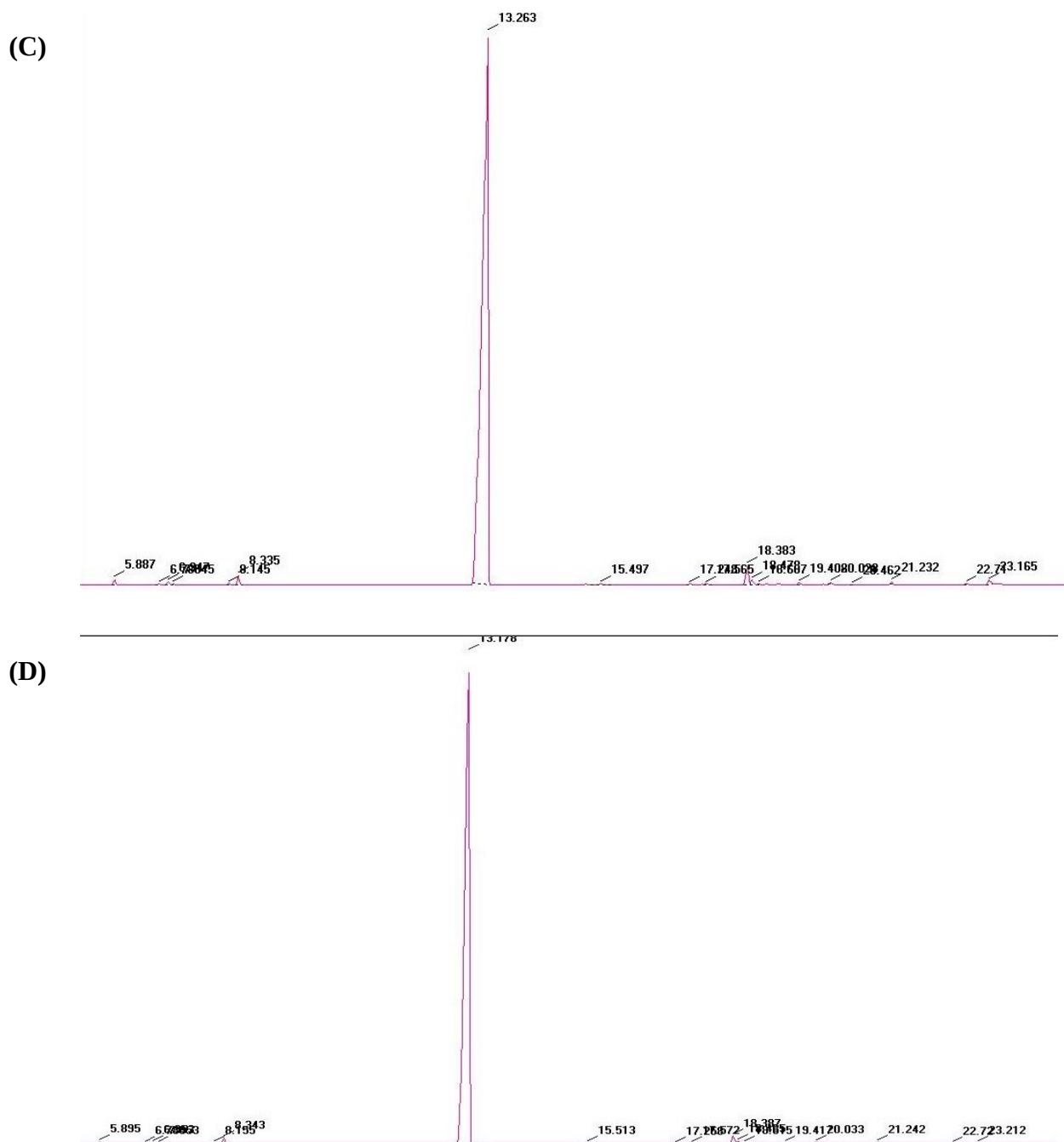


Fig. 3- Sample chromatograms of GC-FID of essential oil isolated from *Ocimum basilicum* L. aerial parts in treatments of (A): Control as soil without sewage sludge and irradiation; (B): 15 g kg⁻¹ of sewage sludge irradiated with 20 kGy; (C): 30 g kg⁻¹ of sewage sludge irradiated with 20 kGy; (D): 60 g kg⁻¹ of sewage sludge irradiated with 20 kGy

Table 3- Variations of essential oil constituents of *Ocimum basilicum* L. under gamma irradiated and non-irradiated sewage sludge

No.	Compound	Control	SS ₁ NI	SS ₁ GI ₁	SS ₁ GI ₁₀	SS ₁ GI ₂₀	SS ₃₀ NI	SS ₃₀ GI ₁
1	α -Pinene	0.15 $\pm$ 0.00cd	0.08 $\pm$ 0.00e	0.17 $\pm$ 0.00cd	0.17 $\pm$ 0.00cd	0.27 $\pm$ 0.00a	0.17 $\pm$ 0.00cd	0.24 $\pm$ 0.00ab
2	β -Pinene	0.12 $\pm$ 0.00cd	0.07 $\pm$ 0.00e	0.13 $\pm$ 0.00bc	0.12 $\pm$ 0.00cd	0.15 $\pm$ 0.00a	0.11 $\pm$ 0.00ab	0.14 $\pm$ 0.00ab
3	6-Methyl-5-heptene-2-one	tr*	tr	0.05 $\pm$ 0.00 a	tr	0.06 $\pm$ 0.00a	tr	tr
4	β -Myrcene	0.05 $\pm$ 0.00a	tr	tr	tr	tr	tr	tr
5	<i>p</i> -Cymene	tr	tr	0.06 $\pm$ 0.01a-c	0.07 $\pm$ 0.00a-c	0.08 $\pm$ 0.00a	0.06 $\pm$ 0.00bc	0.07 $\pm$ 0.00ab
6	Limone	tr	tr	tr	tr	tr	0.02 $\pm$ 0.00a	tr
7	1,8-Cineole	0.74 $\pm$ 0.04a	0.38 $\pm$ 0.06c	0.63b $\pm$ 0.03ab	0.57 $\pm$ 0.11abc	0.75 $\pm$ 0.05a	0.68 $\pm$ 0.1ab	0.55 $\pm$ 0.05abc
8	Methylchavicol	92.54 $\pm$ 0.77d	94.25 $\pm$ 0.40c	94.52 $\pm$ 0.45bc	95.23 $\pm$ 0.35abc	95.24 $\pm$ 0.53abc	95.91 $\pm$ 0.16ab	95.90 $\pm$ 0.64ab
9	Neral	1.19 $\pm$ 0.11a	1.22 $\pm$ 0.18a	0.46 $\pm$ 0.03b	0.28 $\pm$ 0.23b	tr	tr	tr
10	Geranial	1.71 $\pm$ 0.35a	0.46 $\pm$ 0.24b	0.51 $\pm$ 0.09b	0.08 $\pm$ 0.01c	tr	tr	tr
11	E-anethole	tr	0.06 $\pm$ 0.00b	0.06 $\pm$ 0.00b	tr	0.08 $\pm$ 0.00a	0.06 $\pm$ 0.00b	0.09 $\pm$ 0.00a
12	Neryl acetate	0.05 $\pm$ 0.00b	0.14 $\pm$ 0.02a	0.05 $\pm$ 0.00b	tr	tr	tr	tr
13	α -Copaene	0.05 $\pm$ 0.00a	0.06 $\pm$ 0.00a	tr	tr	tr	tr	tr
14	Germaerene D	0.1 $\pm$ 0.02e	0.38 $\pm$ 0.03b	0.19 $\pm$ 0.02cde	1.48 $\pm$ 0.10a	0.29 $\pm$ 0.01bc	0.28 $\pm$ 0.02bc	0.15 $\pm$ 0.00de
15	Methyl Eugenol	1.24 $\pm$ 0.06bc	1.17 $\pm$ 0.09b-d	1.42 $\pm$ 0.12ab	0.51 $\pm$ 0.05f	1.30 $\pm$ 0.09b	1.42 $\pm$ 0.05ab	0.94 $\pm$ 0.04de
16	trans-Caryophyllene	0.34 $\pm$ 0.04cd	0.50 $\pm$ 0.03bc	0.48 $\pm$ 0.03bc	0.06 $\pm$ 0.00b	0.32 $\pm$ 0.06b	0.38 $\pm$ 0.02bc	0.30 $\pm$ 0.02bc
17	trans- α -Bergamotene	0.07 $\pm$ 0.00b	0.07 $\pm$ 0.00b	0.07 $\pm$ 0.01b	0.21 $\pm$ 0.00a	0.08 $\pm$ 0.00b	0.07 $\pm$ 0.01b	0.07 $\pm$ 0.00b
18	α -Humulene	0.12 $\pm$ 0.00abc	0.16 $\pm$ 0.00ab	0.13 $\pm$ 0.00abc	0.18 $\pm$ 0.00a	0.14 $\pm$ 0.00abc	0.12 $\pm$ 0.00abc	0.09 $\pm$ 0.00c
19	trans- α -Bisabolene	0.24 $\pm$ 0.04bc	0.35 $\pm$ 0.01a	0.19 $\pm$ 0.01de	0.17 $\pm$ 0.00def	0.25 $\pm$ 0.00bc	0.26 $\pm$ 0.00b	0.16 $\pm$ 0.00ef
20	(E)- <i>p</i> -methoxy Cinnamaldehyde	0.12 $\pm$ 0.01a	tr	tr	tr	tr	tr	tr
21	Caryophyllene oxide	0.21 $\pm$ 0.00cd	0.08 $\pm$ 0.00f	0.23 $\pm$ 0.00bc	0.16 $\pm$ 0.00e	0.05 $\pm$ 0.00g	0.36	0.19 $\pm$ 0.00d
	Hydrocarbonate monoterpenes	0.87	0.15	0.36	0.36	0.50	0.36	0.45
	Oxygenate monoterpenes	3.94	2.26	1.75	1.65	0.83	0.74	0.83
	Hydrocarbonate sesquiterpenes	0.97	1.52	1.06	2.1	1.08	1.11	0.78
	Oxygenate sesquiterpenes	0.21	0.08	0.23	0.16	0.05	-	0.19
	Other	93.78	95.42	95.94	95.74	96.54	97.33	96.84
	Total identified	99.77	99.43	99.34	100.00	99.00	99.50	98.10

Table 3 - Continue

No.	Compound	SS ₀ GI ₁₀	SS ₀ GI ₂₀	SS ₀ NI	SS ₀ GI ₅	SS ₀ GI ₁₀	SS ₀ GI ₂₀
1	α -Pinene	0.24 $\pm$ 0.01ab	0.14 $\pm$ 0.00d	0.17 $\pm$ 0.00cd	0.26 $\pm$ 0.00ab	0.23 $\pm$ 0.00b	0.18 $\pm$ 0.00c
2	β -Pinene	0.14 $\pm$ 0.00ab	0.08 $\pm$ 0.00e	0.10 $\pm$ 0.00d	0.13 $\pm$ 0.00bc	0.13 $\pm$ 0.00bc	0.13 $\pm$ 0.00bc
3	6-Methyl-5-heptene-2-one	tr	tr	tr	tr	tr	0.06 $\pm$ 0.00a
4	β -Myrcene	tr	tr	tr	tr	tr	tr
5	<i>p</i> -Cymene	0.06 $\pm$ 0.00bc	tr	0.06 $\pm$ 0.00bc	0.05 $\pm$ 0.00c	0.05 $\pm$ 0.00bc	0.06 $\pm$ 0.00babc
6	Limonene	tr	tr	tr	0.01 $\pm$ 0.01a	tr	tr
7	1,8-Cineole	0.63 $\pm$ 0.06ab	0.65 $\pm$ 0.09ab	0.51 $\pm$ 0.01bc	0.69 $\pm$ 0.04ab	0.51 $\pm$ 0.02bc	0.69 $\pm$ 0.03ab
8	Methylchavicol	95.27 $\pm$ 0.55a	96.65 $\pm$ 0.29a	96.08 $\pm$ 0.16a	96.18 $\pm$ 0.41a	96.11 $\pm$ 0.31a	96.68 $\pm$ 0.22a
9	Neral	tr	tr	tr	tr	tr	tr
10	Geranial	tr	tr	0.06 $\pm$ 0.00c	tr	tr	tr
11	E-anethole	0.08 $\pm$ 0.00a	0.08 $\pm$ 0.00ab	0.06 $\pm$ 0.00b	tr	0.08 $\pm$ 0.00ab	0.08 $\pm$ 0.00ab
12	Neryl acetate	tr	tr	tr	tr	tr	tr
13	α -Copaene	tr	tr	tr	tr	tr	tr
14	Germacrene D	0.23 $\pm$ 0.01cd	0.16 $\pm$ 0.00de	0.16 $\pm$ 0.00de	0.11 $\pm$ 0.00e	0.10 $\pm$ 0.00e	0.18 $\pm$ 0.00cde
15	Methyleugenol	0.89 $\pm$ 0.03e	0.85 $\pm$ 0.02e	1.57 $\pm$ 0.03a	1.33 $\pm$ 0.13ab	1.20 $\pm$ 0.04bc	1.02 $\pm$ 0.10cde
16	trans-Caryophyllene	0.41 $\pm$ 0.02bc	0.31 $\pm$ 0.02bc	0.30 $\pm$ 0.00bc	0.30 $\pm$ 0.01bc	0.29 $\pm$ 0.01bc	0.32 $\pm$ 0.01bc
17	trans- α -Bergamotene	0.08 $\pm$ 0.00b	0.08 $\pm$ 0.01b	0.07 $\pm$ 0.00b	tr	tr	0.07 $\pm$ 0.00b
18	α -Humulene	0.11 $\pm$ 0.06bc	0.09 $\pm$ 0.01bc	0.09 $\pm$ 0.00bc	0.12 $\pm$ 0.01bc	0.11 $\pm$ 0.00bc	0.08 $\pm$ 0.00c
19	trans- α -Bisabolene	0.21 $\pm$ 0.00cd	0.17 $\pm$ 0.00def	0.16 $\pm$ 0.00ef	0.16 $\pm$ 0.00ef	0.13 $\pm$ 0.00f	0.14 $\pm$ 0.00f
20	(E)- <i>p</i> -methoxy Cinnamaldehyde	tr	tr	tr	tr	tr	tr
21	Caryophyllene oxide	tr	0.14 $\pm$ 0.00e	0.24 $\pm$ 0.01b	0.21 $\pm$ 0.00cd	0.27 $\pm$ 0.01a	0.05 $\pm$ 0.00g
	Hydrocarbonate monoterpenes	0.44	0.22	0.33	0.45	0.41	0.54
	Oxygenate monoterpenes	0.71	0.73	0.65	0.69	0.59	0.83
	Hydrocarbonate sesquiterpenes	0.73	0.81	0.78	0.69	0.63	0.79
	Oxygenate sesquiterpenes	-	0.14	0.24	0.21	0.27	0.05
	Other	96.16	97.50	97.51	97.51	98.00	97.7
	Total identified	98.00	99.4	99.60	99.60	99.90	99.9

SS: Sewage sludge (g kg⁻¹ soil); GI: Gamma irradiation absorbed dose (kGy); NI: Non-irradiation; Control: soil without SS and irradiation Different letters in each row show significant difference at P $\leq$ 0.05 by Duncan multiple range test.
*Trace

Discussion

It can be concluded that essential oil (EO) of basil was mainly composed of terpenes/terpenoids constituents and originated from two distinct biosynthetic pathways including shikmic and mevalonic pathways. The shikmic pathway is mediated by the activity of phenylalanine ammonia-lyase (PAL) and leads to accumulation of phenolic compounds while mevalonic pathway is connected with photosynthesis, CO₂ fixation, glucose production, as well as terpenoid biosynthesis [27]. In other words, the biosynthetic EO requires adenosine triphosphate (ATP) and nicotinamide adenine dinucleotide phosphate (NADPH) [18]. Thus, organic material containing macro- and micronutrients and due to created more favorable conditions for better plant growth [17], nutrient uptake [7], and consequently supplying source of carbon utilized in terpene, biosynthesis pathway may positively effect on EO quantity [14]. The results have been supported by various researchers such as Bajeli *et al.* [17]. They reported that the increase in EO content can be attributed to the effect of organic materials including farmyard manure, poultry manure and vermicompost on the dilution of EO concentration in the leaves. Hussein *et al.* [28] reported that organic manures such as compost increase the EO content due to the effect of compost on accelerating metabolism reactions as well as stimulating enzymes. On the other hand, it has been reported that accumulation of secondary metabolites could be occurred in medicinal and aromatic plants in response to different types of stresses [13, 29, 30]. In other words, the stress of heavy metals

(HMs) induced biosynthesis pathway (elicitation) and caused production of secondary metabolites through stimulation of immune responses in medicinal plant [13]. Thus, the sewage sludge (SS) contains coexisting of nutrients and excessive amounts of HMs (Table 2), which consequently causes elicitation of EO content as mentioned above. Also, the increase in EO yield in treatment mentioned above could be due to the effects of gamma irradiated and non-irradiated sewage sludge (SSGI and SSNI) on biomass production and/or oil content. Other researchers previously reported the positive role of organic matter on the improvement of EO yield of medicinal plants [15, 16, 18, 28, 31]. Najafi *et al.* [32], Mahmoudi *et al.* [33] and Chu *et al.* [7] reported that several physicochemical and biological soil properties such as pH, bulk density, porosity, water-holding capacity, and supplying nutrients showed improvement because of the SS addition and its positive influence on soil properties generally resulted in a positive effect on the growth and nutrient uptake by plants. Similarly, Mohamed *et al.* [34] suggested that improved shoot yield of Moroccan sunflower may be attributed to potential source of nitrogen and phosphorus for plants in SS treatments. Furthermore, excess amounts of SS reported to have adverse effects on different parameters such as biomass production [7, 354], and balance of macro- and micronutrients levels [36, 37]. Overall, the positive effects of SSGI and SSNI at lower and/or moderate levels (15 and 30 g kg⁻¹) and the negative effect at high levels of SS (60 g kg⁻¹) on EO yield is possibly a consequence of the positive and negative effects of SS on plant growth and/or essential



oil content in different levels of SS.

In the case of the effects of SSGI and SSNI applications on essential oil constituents, it could be concluded that applications of SSGI and SSNI did not alter the trend of chemotype as compared to the control. Many reports on the chemical composition and chemotype of EO of sweet basil plants have been published previously [11, 38-40]. However, our findings were in agreement with those of Kasali et al. [41], Ghanati et al. [27], and Kumar et al. [42] who noted that methyl chavicol was the dominate constituent. On the other hand, it is well known that there are various classifications for chemotypes of basil oils according to their major compounds, including (1) methyl chavicol-rich, (2) linalool-rich, (3) methyleugenol-rich, (4) methyl cinnamate-rich, and also numerous subtypes [39]. For this reason, the sample of basil plants in all treatments showed high content of methyl chavicol which could be grouped into methyl chavicol chemotype.

It is well known that elicitation of secondary metabolites often occurs in plants subjected to different types of stresses and is regulated by the several factors including genetic and evolution, growing conditions, physiological variations, climate, photoperiod, temperature, light, agronomic factors such as soil amendments, mineral elements (such as N, P, K, Ca, Cu, Zn, and Fe), and trace elements (including Pb, Cd, Cr, and Ni) [13, 14, 43, 44]. However, little information is available about the effect of SSGI on quality of secondary metabolites biosynthesis in medicinal plants especially in basil. It has been reported that application of SS irradiated with 6 kGy

absorbed dose of gamma-ray in a sandy soil caused an increase in Fenchone content as one of the major constituents of fennel plant EO, while t-anethole was relatively higher in plants grown under SSNI [4]. Patel and Patra [31] reported that the major constituents of *Tagetes minuta* oil such as limonene, Cis-ocimene, Z-tagetone and E-tagetone were found to be maximum in 50:50 combinations of both studied soils with tannery sludge. So that, even 100:0 combination of tannery sludge and soil (full sludge and no soil) had no adverse effect on amount of mentioned constituent. Nutritional treatment (Cu and Zn) had a significant effect on linalool and methyl chavicol as major EO constituent of basil in comparison to control plants [11]. On the contrary, Bolechowski et al. [45] reported that mixing organic material (different composts such as citrus juice waste, tomato soup waste and cattle manure in combining with exhausted grape marc) with perlite and peat at different levels decreased major constituent of *Thymus vulgaris* including *p*-cymene and thymol. Totally, relative increase of major compounds of basil EO in plants cultivated on SSGI and/or SSNI treated soil suggested that treatment of medicinal plants such as basil with SSGI and/or SSNI can be introduced as a tool in order to produce more desired medicinal compounds.

On the other hand, one positive point in medicinal and aromatic plants is that in the process of EO extraction by distillation, there is very less probability of heavy metals being present in the oil and metal-free extracted oils could be safely marketed [13]. Several authors have reported effects of HMs on changes of chemical composition of EO in medicinal

plants [14, 29, 40]. Even though, some studies have been done on sole application of organic matter including compost on altering chemical composition of sweet basil. For example, Zheljazkov and Warman [46] reported that additions of municipal solid waste compost with concentrations of Cu 311 mg kg⁻¹, Pb 223 mg kg⁻¹, Mo 17 mg kg⁻¹, and Zn 767 mg kg⁻¹ to soil altered the chemical composition of basil oil, but the basil oil obtained from crops grown on enriched medium would be easily marketable. But, the literature review showed that no study has been carried out on the comparison of SSGI and SSNI effects on chemical composition of volatile oil of sweet basil. However, the observed modification in the synthesis of some EO constituents in treated plants considered as a defense response to metal stresses of SSNI and SSGI. So that, methyl chavicol as dominant constituent of EO of basil have known antioxidant properties [47], which may play a role in the defense mechanisms of sweet basil against heavy metal contents of SSGI and SSNI as antioxidant compounds.

The changes in classification of the identified compounds with SSGI and SSNI applications could be mentioned that essential oils are originated from two distinct biosynthetic pathways: shikmic pathway and mevalonic pathway. Shikmic pathway is mediated by the activity of phenylalanine ammonia-lyase (PAL) enzyme and leads to production of phenolic compounds while mevalonic pathway is connected with photosynthesis, assimilation, and growth and results in production of terpenoids. They are although produced by the Methylerythritol phosphate [27]. The expression and activity of

PAL enzyme has a direct correlation with the amount of phenylpropanoids, and by decreasing its activity, the amount of phenylpropanoids is also reduced [48]. The factors affecting the expression of PAL enzyme and subsequently the production of phenylpropanoids compounds in plants including plant age, concentration of growth regulators, various abiotic stresses such as herbicides, temperature, macro- and micronutrients and heavy metals contents, and microbial contaminations such as viral, bacterial and fungal [49]. Wen et al. [50] reported that increasing the expression of PAL enzyme and consequently increasing the content of phenolic compounds caused induction of the plant's resistance to stress. In other words, it can be stated that stimulating the production of these compounds may have been achieved by inducing the systematic resistance of the plant to external agents. Systematic resistance stimulation increases plant cell wall resistance, changes plant physiologic and metabolic responses, and increases the production of defense chemicals in abiotic-stress conditions [51]. However, the altering essential oil composition biosynthesis of basil such as phenylpropanoids and terpenes probably could be due to the bioenergetic changes in the plant cells in response to the treatments.

Conclusion

Sewage sludge improved essential oil content and yield of basil as well as chemical constituent of essential oil. When, soil received gamma irradiated and non-irradiated sewage sludge in the rate of 60 g kg⁻¹, essential oil yield

significantly reduced relative to other treatments. Applications of gamma irradiated and non-irradiated sewage sludge even at the highest level (60 g kg⁻¹ soil), not only had no negative effect on basil essential oil content, but also increased it as compared to the control. Thus, the decrease in essential oil yield is possibly a consequence of the negative effect of sewage sludge on plant growth because of increasing soil solution salinity. In total, 21 constituents were identified that representing about 98.35-99.89% of the essential oil compositions. The methyl chavicol as the major component of *O. basilicum* essential oil was significantly improved by the applications of gamma irradiated and non-irradiated sewage

sludge as compared with the control. The best performance in terms of methyl chavicol content was obtained by the application of 60 g gamma irradiated sewage sludge per kg soil. However, the enhanced methyl chavicol content was not significant between the treatments of ≥ 30 g kg⁻¹ gamma irradiated or non-irradiated sewage sludge and 15 g kg⁻¹ sewage sludge at absorbed dose of ≥ 10 kGy. With considering a significant improvement of essential oil content and composition and cost-effective application of gamma-ray, it may be concluded that the application of ≤ 30 g sewage sludge per kg soil with 5 kGy absorbed dose of gamma-ray can be suggested as practical recommendation under similar conditions.

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