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# Essential oil constituents, antimicrobial and herbicidal assays of lesser calamint (*Calamintha nepeta* (L.) Savi subsp. *nepeta*) from East Mediterranean Region of Turkey

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## ABSTRACT

*Calamintha nepeta* (L.) Savi subsp. *nepeta* (Lamiaceae) has a widespread use in the mint family of the Angiosperms. Uses of the spectacular aromatic feature as well as immense medicinal properties of this species have been dated back to the antiquity in the Mediterranean life. Aerial parts of the *Calamintha nepeta* (L.) Savi subsp. *nepeta* were collected from the wild ecosystems of the Amanos Mountains (East Mediterranean of Turkey). The hydrodistillated essential oil (EO) of the species were predominantly rich in with the following constituents; pulegone (43.02%) and menthone (28.09%). EO of the tested species had also notable antimicrobial activities. Minimum Inhibitory and Bacterio/Fungio-Cidal Concentrations (MIC and MBC/MFC) of *C. nepeta*' EO were 0.39/0.78 µl/ml for *B. subtilis*, 0.19/0.78 µl/ml for *C. albicans* and 0.78/0.78 µl/ml for *C. parapsilosis*. In the herbicidal assay, a 100% inhibition of the seed germination, radicle and plumule growth of *L. sativa* was found at 0.5, 1.0 and 2.0 mg /ml of the EO. The germination and seedling lengths of *P. oleracea* and *L. sativum* in response to 1.0 and 2.0 mg /ml of the EO treatment were the same as those found for *L. sativa*. Essential oil composition, antimicrobial as well as the herbicidal assays of the species from Amanos Mountans (East Mediterranean region of Turkey) could be regarded as the first report.

**Keywords:** *Calamintha nepeta* (L.) Savi subsp. *nepeta*; *Lamiaceae; essential oil; Candida; herbicidal*.

## 1. INTRODUCTION

In the Lamiaceae family, aromatic and taste properties of *C. nepeta* are very popular in the preparations of various Mediterranean food. The leaves of *C. nepeta* have been used as the flavouring herbs for artichoke, egg and/or snail dishes, bread, oil and salads [1]. In the folk cosmetics, it has been also in use as a deodorising agent in the clothes [2]. In the ethnomedicine, the local habitants of the Mediterranean region have widely used the aerial parts of *C. nepeta*. Applications are used for the treatment of various ailments, disorders and diseases such as stomache [1, 3, 4, 5, 6, 7, 8], palpitations [3], cholagogue [1], insomnia [3, 7], spasm, hiccups and emmenagogue [7], toothache and decayed tooth [8], abscess [9], stimulant and cholagogue [1]. Furthermore, other treatments in practical uses are as antiseptic [1], antidiabetic [10], antiparasitic [2, 4, 11], antirepellent [4, 12], antipyretic [5, 7], antisudoral and anticatarrhal [5, 7], antirheumatismal [13, 14], antiarthritic and antiasthenia [7].

Essential oil (EO) composition of *C. nepeta* subsp. *glandulosa* have been reported from the Central Anatolia, Northeast Anatolia and Eastern Mediterrenean part of Turkey [15, 16, 17, 18, 19]. EO of the species of *C. nepeta* (L.) Savi was found to be rich in limonene (7.1, 7.5 and 0.1), menthone (3.0, 11.9 and 1.0), pulegone (0.8, 12 and 1.2), trans-piperitenone oxide (11.69, 1.4 and 6.9), caryophyllene oxide (3.8, 0.8 and 7.8), carvacrol (0.0, 10.0 and 1.2) in the regions of Silifke and Tarsus (İçel) (East Mediterranean Region) and Bartın (Black Sea Region), respectively [19].

EO composition of *C. nepeta* (L.) Savi has been also under focus in some European countries. In Italy, major compounds were pulegone (46.0 and 49.6), menthone (9.82 and 9.4), d-limonene (6.40 and 7.0) in the region of Molina di Quosa (Pisa) [20, 21], piperitenone (16.4), isopulegone (14.1), pulegone (21.4), menthone (19.8) in the region of Castelbuono (Sicily) [22], and limonene (4.8), isomenthol (64.4) and piperitenone (6.4) in the region of Baunei (Sardinia) [23]. In France, the predominant compounds were limonene (5.2, 12.8 and 6.0), menthone 43.4, 9.3 and 20.0), pulegone (18.9, 12.4 and 55.6), piperitone oxide II (8.3, 30.5 and 1.2), piperitenone oxide (0.8, 12.5 and 0.6) in the region of Corsica Island [24]. In Portugal, isomenthone (52), isomenthol (19), 1,8-cineole (11) in the region of Castelo Branco [25] and 1,8-cineole (21.1), isomenthone (35.8), trans-isopulegone (7.8) in the region of Vilarinho de Baixo Coimbra (Portugal) [23].

The secondary metabolites of plant species have differences from each other because of the environmental and genetic factors. The variations are also known at species and subspecies category. Therefore, bioconstituents of each organisms living in different environments deserve to be explored in relation to find out novel bioactive compounds in many fields (pharmaceutical, food and etc.) for the benefits of human life. As of date, there has been no document on the essential oil composition as well as antimicrobial and herbicidal assays of *C. nepeta* subsp. *nepeta* from Amanos Mountains (East Mediterranean Region of Turkey).

## 2. EXPERIMENTAL SECTION

**2.1. Plant and essential oil (EO) hydrodistillation.** *C. nepeta* subsp. *nepeta* at the blooming season were collected from Bağlıca Plateau (Amanos Mountains, Hatay-İskenderun, 36°36'46,43" N, 36°14'12,94"E). EO was recovered after hydrodistillation (3h)

with Clevenger apparatus. In each distillation stage, a total of 100 air dried aerial parts were used. Anhydrous sodium sulphate was used for removing the residual water from EO, which was then kept in a dark vial at +4°C.

**2.2. Analyses of the EO Constituents of *C. nepeta* subsp. *nepeta* with Gas Chromotagraphy/Mass Spectrometry (GC/MS).**

The constituents of EO from *C. nepeta* subsp. *nepeta* were analyzed with GC/MS [26]. GC was the Shimadzu QP 2010 Plus (Shimadzu, Kyoto, Japan).

**Table 1.** *C. nepeta* (L.) Savi subsp. *nepeta* and essential oil constituents.

| Number | Compound | Retention Time | Retention Index | % |
|---|---|---|---|---|
| 1 | α-Pinene | 12.711 | 1097 | 0.41 |
| 2 | 2,5-Diethyltetrahydrofuran | 13.650 | 1128 | 0.05 |
| 3 | Camphene | 14.644 | 1160 | 0.05 |
| 4 | β-Pinene | 16.602 | 1223 | 0.46 |
| 5 | Sabinene | 17.311 | 1246 | 0.16 |
| 6 | β-Myrcene | 19.517 | 1318 | 0.21 |
| 7 | Limonene | 21.196 | 1375 | 2.71 |
| 8 | Eucalyptol | 21.558 | 1387 | 0.11 |
| 9 | para-Cymene | 24.696 | 1497 | 0.10 |
| 10 | 3-Octanol | 30.293 | 1711 | 2.16 |
| 11 | 1-Octen-3-ol | 32.861 | 1819 | 0.13 |
| 12 | trans-Sabinene hydrate | 33.558 | 1848 | 0.08 |
| 13 | Menthone | 33.992 | 1867 | 28.08 |
| 14 | Menthofuran | 34.592 | 1892 | 0.09 |
| 15 | Isomenthone | 34.972 | 1909 | 2.55 |
| 16 | β-Bourbonene | 36.296 | 1969 | 0.13 |
| 17 | Linalool | 36.982 | 2000 | 0.17 |
| 18 | Isopulegone | 38.390 | 2012 | 1.12 |
| 19 | 4-Terpineol | 39.419 | 2020 | 0.54 |
| 20 | Caryophyllene | 39.677 | 2023 | 1.58 |
| 21 | Dihydrocarvone | 39.833 | 2024 | 0.05 |
| 22 | Mentha-2,8-dien-1-ol | 40.546 | 2030 | 0.12 |
| 23 | Pulegone | 41.792 | 2040 | 43.02 |
| 24 | β-Farnesene | 42.089 | 2043 | 0.33 |
| 25 | α-Humulene | 42.460 | 2046 | 0.49 |
| 26 | α-Terpineol | 43.117 | 2051 | 0.62 |
| 27 | α-Terpinyl acetate | 43.325 | 2053 | 2.31 |
| 28 | Germacrene | 43.965 | 2058 | 0.56 |
| 29 | Piperitone | 44.549 | 2063 | 3.12 |
| 30 | L (-)-Carvone | 44.760 | 2065 | 0.09 |
| 31 | Carveol | 48.240 | 2094 | 0.07 |
| 32 | Isopiperitenone | 48.651 | 2097 | 0.08 |
| 33 | Verbenone | 51.643 | 2122 | 3.12 |
| 34 | Piperitone oxide | 52.791 | 2132 | 1.47 |
| 35 | (-)-Caryophyllene oxide | 53.867 | 2141 | 1.96 |
| 36 | Humulene oxide | 55.720 | 2156 | 0.26 |
| 37 | Spathulenol | 58.196 | 2177 | 0.77 |
| 38 | Eugenol | 59.433 | 2187 | 0.04 |
| 39 | Thymol | 59.824 | 2190 | 0.18 |
| 40 | Carvacrol | 60.749 | 2198 | 0.30 |
| 41 | Menthalactone | 63.994 | 2303 | 0.14 |
| | | | Total | 100.00 |

The colon consisted of TRB-Wax (Teknokroma, Barcelona, Spain) fused silica capillary column (60 m × 0.25 mm i.d. and film thickness, 0.25 µm). Different temperature and time (40°C/ 5 min, 3°C/1 min, 240°C/15 min were used during total running time (86 h). AOC-20i/20s autosampler was used as the injector. EO: n-hexane was injected as 1:100 (v/v). The carrier gas was the helium at flow rate of 1 ml per min. The detector was the MS-QP 2010 series mass-selective detector. The split ratio, electron energy, mass spectra and scanning rate were 1/50, 70 eV, 35-450 m/z and 1 scan/s, respectively [26].

Retention Index (Kovats Indices) of individual constituent in the test EO was calculated with the mixture of n-alkane [27]. The comparison of the mass spectra and the retention indices of the compounds and those of the references from NIST (National Institute of Standards and Technology, 2013, Gaithersburg, MD, USA), and Wiley database were carried out during the identification period. The peak space normalization measurement was employed to calculate the quantity of the compounds in the EO. All analytical analyses were repeated triplicate and the average values were indicated in Table 1.

**2.3. Antimicrobial assay.** Six gram positive bacteria (*Bacillus subtilis* ATCC 6633, *Bacillus cereus* EU (food isolate), *Enterococcus faecalis* ATCC 29212, *Enterococcus casseliflavus* ATCC 700327, *Staphylococcus aureus* ATCC 29213, *Staphylococcus aureus* ATCC BAA 977), four gram negative bacteria (*Enterobacter hormaechei* ATCC 700323, *Klebsiella pneumoniae* ATCC 700603, *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922) and two yeast (*Candida parapsilosis* ATCC 22019 and *Candida albicans* ATCC 14053) were used for antimicrobial assays. Fresh culture of each assayed microorganism in appropriate medium was subjected for calibration to obtain required amount of cell in physiological saline solution using MacFarland unit. A 0.1 ml of the adjusted culture ($10^8$ cfu/ml) was distributed into freshly prepared test tubes including at varying concentrations of the EO (200.0 to 0.0485 µl/ml) in appropriate medium plus 0.5% of Tween 80. Mueller Hinton Broth and Sabaroud Dextrose Broth were the used media during experiments. In addition to treatments, controls were included and monitored during assay. After incubation (37°C/24 h), tubes were checked for the growth in order to determine the Minimum Inhibitory Concentration. In the assayed tubes, the first tube and onwards had no indication of microbial growth were plated onto agar medium to find out the cidal effect of the EO on test microorganisms [28]. For bacteria and yeast cultures, streptomycin and fluconazole were used as the standards for comparative purposes, respectively.

**2.4. Herbicidal assay.** In the direct-contact assay, test organisms were *Lactuca sativa* (lettuce), *Lepidium sativum* (cress) and *Portulaca oleracea* (common purslane). Seeds of these species in treated with aqueous dysinfection solution (sodium hypochlorite, 1.5%, v/v), 10 min) were placed into petri dishes including two layered filter membranes [29, 30]. For the treatments, EO of *C. nepeta* changed from 2.0 to 0.062 mg/ml in 0.5% Tween 80. Distilled water was used as the control. After treatments, outer parts of the petri dishes sealed with parafilm were incubated at 24°C for 168 h (12/12 h, 1.500 lux and 80% relative humidity). The counts of the germinated seeds as well as the seedling lengths (mm) were determined. The herbicide "Trifluralin" was used for the comparison.

**2.5. Statistical analyses.** In this study, all assays were repeated three times. SPSS17 was used for assessing the results. Within the programme, analyses of the variance (ANOVA) were used. The differences within the test results were assessed using the Tukey-Multiple Comparative test. The results were evaluated statistically and shown as the mean with standard deviations.

## 3. RESULTS SECTION

**3.1. Essential oil yield and composition.** Hydrodistillation of the EO was followed by analyzing its constituents with GC/MS. Retention time, indices and % percentage of the constituents and major compounds were listed in Table 1 and Figure 1, respectively. The major compounds (%) were pulegone (43.02), menthone (28.09), piperitone (3.12), verbenone (3.12) and limonene (2.71). The yield of the EO was 1% (v/w). In previous studies, EO yield was also reported by different authors e.g. 0.2%, 1.5%, and trace in *C. nepeta* subsp. *nepeta* [19], 0.60% in *C. nepeta* [20], 0.40-1.2% in *C. nepeta* [24] and 1.43% in *C. nepeta* [25]. The oil yield differences as well as the constituents (%) could be attributed to species/subspecies, part of the plant material, vegetation period, geographical properties of the collecting site, distillation method and time and etc.

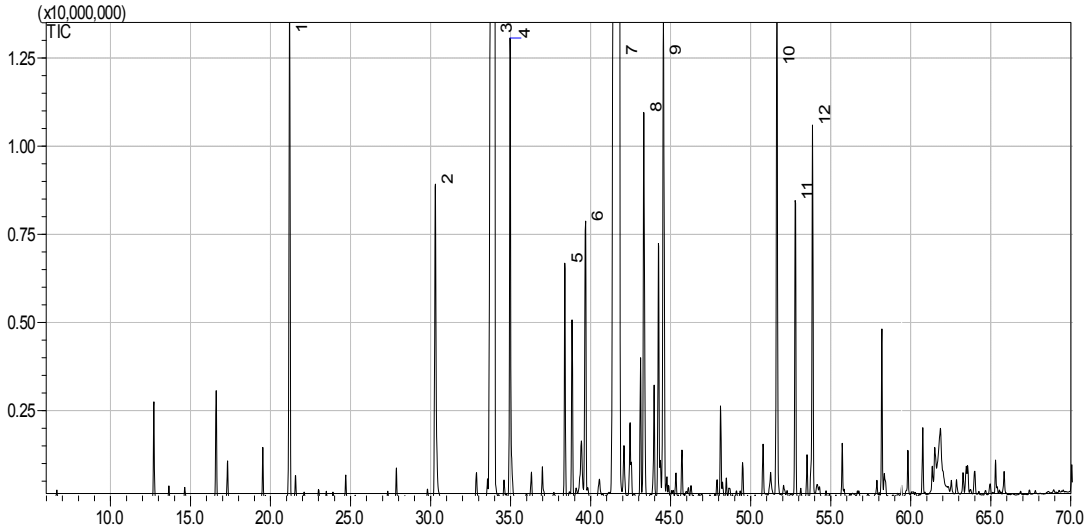


**Figure 1.** GC/MS chromatogram of the aerial parts of *C. nepeta* (L.) Savi subsp. *nepeta*' essential oil. The peaks in the chromatogram were above the main compounds (>1%) in the essential oil constituents:1) Limonene, 2) 3-Octanol, 3) Menthone, 4) Isomenthone, 5) Isopulegone, 6) Caryophyllene, 7) Pulegone, 8) α-Terpinyl acetate, 9) Piperitone, 10) Verbenone, 11) Piperitone oxide, 12) (-)-Caryophyllene oxid.

**3.2. Antimicrobial assays of the EO.** After determining the Minimum Inhibitory Concentration (MIC) of the EO on each test microorganism, cidal action was determined and expressed by the Minimum Bactericidal/Fungicidal Concentration (MBC/MFC). The standard antibiotics (Streptomycin and Fluconazole) were used during the assays.

**Table 2.** Minimum inhibitory and cidal concentrations of the essential oil of *C. nepeta* (L.) Savi subsp. *Nepeta.*

| Microroorganism | | EO (µl/ml) | | Strep (µg/ml) | | Flu (µg/ml) | |
|---|---|---|---|---|---|---|---|
| | | MIC | MBC/MFC | MIC | MBC | MIC | MFC |
| ***B. subtilis*** | ATCC 6633 | 0.39±0.00$^{a}$ | 0.78±0.00$^{a}$ | 31.25±0.00$^{a}$ | 31.25±0.00$^{a}$ | - | - |
| ***B. cereus*** | EU | 1.56±0.00$^{ab}$ | 50.0±0.00$^{f}$ | 5.20±0.00$^{a}$ | 31.25±0.00$^{a}$ | - | - |
| ***E. faecalis*** | ATCC 29212 | 5.20±1.80$^{cd}$ | 12.5±0.00$^{d}$ | 125.0±0.00$^{b}$ | 250.0±0.00$^{ab}$ | - | - |
| ***E. casseliflavus*** | ATCC 700327 | 5.20±1.80$^{cd}$ | 6.25±0.00$^{c}$ | 31.25±0.00$^{a}$ | 500.00±0.00$^{b}$ | - | - |
| ***S. aureus*** | ATCC 29213 | 6.25±0.00$^{d}$ | 6.25±0.00$^{c}$ | 15.62±0.00$^{a}$ | 62.5±0.00$^{a}$ | - | - |
| ***S. aureus*** | ATCC BAA977 | 5.20±1.80$^{cd}$ | 25.0±0.00$^{e}$ | 15.62±0.00$^{a}$ | 62.5±0.00$^{a}$ | - | - |
| ***K. pneumoniae*** | ATCC 700603 | 6.25±0.00$^{d}$ | 25.0±0.00$^{e}$ | 3.90±0.00$^{a}$ | 31.25±0.00$^{a}$ | - | - |
| ***E. hormaechei*** | ATCC 700323 | 3.12±0.00$^{bc}$ | 6.25±0.00$^{c}$ | 15.62±0.00$^{a}$ | 26.04±9.02$^{a}$ | - | - |
| ***P. aeruginosa*** | ATCC 27853 | 6.25±0.00$^{d}$ | 12.5±0.00$^{d}$ | 104.16±36.08$^{b}$ | 500.00±0.0$^{b}$ | - | - |
| ***E. coli*** | ATCC 25922 | 3.12±0.00$^{bc}$ | 3.12±0.00$^{b}$ | 07.81±0.00$^{a}$ | 13.02±4.51$^{a}$ | - | - |
| ***C. parapsilosis*** | ATCC 22019 | 0.78±0.00$^{ab}$ | 0.78±0.00$^{a}$ | - | - | 37.5±0.00$^{a}$ | 75±0.00$^{a}$ |
| ***C. albicans*** | ATCC 14053 | 0.19±0.00$^{a}$ | 0.78±0.00$^{a}$ | - | - | >300.00±0.00$^{b}$ | >300±0.00$^{b}$ |

EO: Essential Oil; MIC: Minimum Inhibitory Concentration; MBC: Minimum Bactericidal Concentration MFC: Minimum Fungicidal Concentration; Strep: Streptomycin; Flu: Fluconazole. The statistical differences in each column were indicated by different letters* ($p<0.05$). –not tested;

As shown in Table 2., *B. subtilis* and *B. cereus* had the lowest MIC values (µl/ml) which indicates the most sensitive bacteria, and these were followed by *E. hormaechei* = *E. coli* > *E. faecalis* = *E. casseliflavus* = *S. aureus* BAA > *S. aureus* 29213 = *K. pneumoniae* = *P. aeruginosa*. *C. albicans* was the most susceptible yeast species when compared to *C. parapsilosis*. After determining the static value of the EO, cidal action was determined with transferring the broth culture onto agar media. The results indicated that the cidal effect varied among the test microorganisms. The highest cidal effect was observed on *B. subtilis*> *E. coli* > *E. hormaechei* = *S. aureus* 29213 = *E. casseliflavus* > *P. aeruginosa* = *E. faecalis* > *S. aureus* BAA = *K. pneumoniae* > *B. cereus*. For Candida species, the cidal actions were the same both for *C. parapsilosis* and *C. albicans*.

In previous studies, EO of *C. nepeta* was determined as MIC/MBC on variety of test microorganisms. MIC/MBC values (µg/ml) were 5/5 for *S. aureus* ATCC 25923, 10/10 for *E. coli* ATCC 25922, 20/20 for *P. aeruginosa* ATCC 14207, 2/2 for *B. subtilis* BGA and 5/5 for *C. albicans* [20]. The essential oil of Portugal and Italian *C. nepeta* was found to be effective with the following MIC/MBC (µl/ml) values for *C. albicans* ATCC 10231 (2.5/2.5 and 1.25/1.25), *C. parapsilosis* ATCC 90018 (2.5/5.0 and 1.25/1.25), respectively [23].

In this study, MIC and MBC values of the EO from *C. nepeta* (L.) Savi against *B. subtilis*, *S. aureus*, *E. coli*, *K. pneumoniae*, *P. aeruginosa* were higher than the findings of Panizzi et al. [20]. In their study, essential oil of *C. nepeta* (L.) Savi was rich in pulegone (46.0; 49.6), menthone (9.82; 9.4) and d-limonene (6.40; 7.0). Differences withe results of Panizzi et al.'study could be either related to the essential oil composition, subspecies of the test plant and/or microbial strain differences. In this study, observed values for Candida species were lower than the findings of Marongiu et al. [23]. The differences than those of Marongiu et al.' findings could be mostly related to essential oil composition of *C. nepeta* (L.) Savi subsp. *nepeta* as the EO was found to be rich in 1,8-cineole (21.1), isomenthone (35.8), trans-isopulegone (7.8) in the region of Vilarinho de Baixo Coimbra (Portugal), and limonene (4.8), isomenthol (64.4) and piperitenone (6.4) in the region of Baunei (Sardinia, Italy) [23].

**3.3. Results of the herbicidal assay of the EO.** As shown in Table 3., EO and the standard herbicide (2.0-0.0625-mg/ml) were assayed on on *L. sativa*, *L. sativum* and *P. oleracea* for herbicidal activities. At all concentrations, the herbicide was not as effective as the EO on the germination, radicle and plumule growth of all test species. The herbicidal action of the EO seemed to be dose dependent effect on tested seeds under assay. A complete inhibition of the seed germination of *L. sativa* was found at 0.5, 1.0 and 2.0 mg /ml of the EO treatment; however, inhibition of the seed germination of *L. sativum* and *P. oleracea* at 0.5 mg /ml of the EO was 73 and 96.67%, respectively. The germination of *P. oleracea* and *L. sativum* in response to 1.0 and 2.0 mg /ml of the EO treatment revealed the same as those found for *L. sativa*.

**Table 3.** The herbicidal effect of the essential oil of *C. nepeta* (L.) Savi subsp. *nepeta.*

| (mg/ml) | SG (%) | RL (mm) (Mean±SD) *L. sativa* | PL (mm) (Mean±SD) | SG (%) | RL (mm) (Mean±SD) *L. sativum* | PL (mm) (Mean±SD) | SG (%) *P. oleracea* | RL (mm) (Mean±SD) | PL (mm) (Mean±SD) |
|---|---|---|---|---|---|---|---|---|---|
| **Control** | 94.43±1.52$^{a}$ | 32.74±1.86$^{a}$ | 24.26±1.64$^{a}$ | 100.0±0,00$^{a}$ | 40.63±3.59$^{a}$ | 24.86±1.87$^{a}$ | 100.0±0.00$^{a}$ | 53.83±3.04$^{a}$ | 42.06±1.61$^{a}$ |
| **EO-0.062** | 93.33±2.46$^{a}$ | 26.16±1.86$^{b}$ | 17.20±1.38$^{b}$ | 100.0±0,00$^{a}$ | 37.13±2.74$^{a}$ | 17.86±2.90$^{b}$ | 100.0±0.00$^{a}$ | 50.56±1.91$^{ab}$ | 30.50±2.05$^{b}$ |
| **EO-0.125** | 57.76±2.51$^{b}$ | 25.82±1.63$^{b}$ | 17.00±1.30$^{b}$ | 100.0±0,00$^{a}$ | 30.63±2.64$^{b}$ | 13.33±1.82$^{c}$ | 100.0±0.00$^{a}$ | 48.16±1.81$^{b}$ | 25.83±1.42$^{c}$ |
| **EO-0.25** | 28.86±0.57$^{c}$ | 25.27±1.73$^{b}$ | 14.18±1.91$^{b}$ | 100.0±0,00$^{a}$ | 12.06±0.38$^{c}$ | 08.50±1.00$^{d}$ | 20.00±2.00$^{b}$ | 28.80±3.25$^{c}$ | 12.43±1.91$^{d}$ |
| **EO-0.50** | 00.00±0.00$^{d}$ | 00.00±0.00$^{c}$ | 00.00±0.00$^{c}$ | 27.00±3,60$^{b}$ | 03.50±0.85$^{d}$ | 01.70±0.67$^{e}$ | 03.33±0.57$^{c}$ | 01.00±0.00$^{d}$ | 00.00±0.00$^{e}$ |
| **EO-1.00** | 00.00±0.00$^{d}$ | 00.00±0.00$^{c}$ | 00.00±0.00$^{c}$ | 00.00±0.00$^{c}$ | 00.00±0.00$^{d}$ | 00.00±0.00$^{e}$ | 00.00±0.00$^{d}$ | 00.00±0.00$^{d}$ | 00.00±0.00$^{e}$ |
| **EO-2.00** | 00.00±0.00$^{d}$ | 00.00±0.00$^{c}$ | 00.00±0.00$^{c}$ | 00.00±0.00$^{c}$ | 00.00±0.00$^{d}$ | 00.00±0.00$^{e}$ | 00.00±0.00$^{d}$ | 00.00±0.00$^{d}$ | 00.00±0.00$^{e}$ |
| **T- 0.062** | 91.10±2.61$^{ab}$ | 12.84±1.84$^{b}$ | 16.65±1.49$^{b}$ | 100.0±0.00$^{a}$ | 04.02±0.71$^{b}$ | 06.83±0.17$^{b}$ | 100.0±0.00$^{a}$ | 10.96±1.54$^{b}$ | 13.50±2.55$^{b}$ |
| **T-0.125** | 90.00±2.35$^{ab}$ | 06.88±1.27$^{c}$ | 12.50±1.53$^{c}$ | 100.0±0.00$^{a}$ | 04.00±0.50$^{b}$ | 05.53±0.62$^{b}$ | 100.0±0.00$^{a}$ | 08.19±1.32$^{bc}$ | 12.45±1.84$^{bc}$ |
| **T-0.25** | 90.00±2.61$^{ab}$ | 05.37±1.75$^{cd}$ | 10.64±1.43$^{c}$ | 100.0±0.00$^{a}$ | 03.31±0.65$^{b}$ | 05.36±0.65$^{b}$ | 100.0±0.00$^{a}$ | 06.86±2.47$^{bc}$ | 10.39±1.70$^{bc}$ |
| **T-0.50** | 86.66±1.00$^{bc}$ | 04.36±0.76$^{cd}$ | 06.03±0.74$^{d}$ | 73.33±2.30$^{b}$ | 03.13±0.63$^{b}$ | 05.00±0.52$^{b}$ | 83.33±1.00$^{b}$ | 05.09±1.44$^{c}$ | 07.89±1.41$^{cd}$ |
| **T-1.00** | 82.20±0.57$^{c}$ | 02.23±0.43$^{d}$ | 05.70±0.53$^{d}$ | 68.86±3.73$^{b}$ | 02.58±0.45$^{b}$ | 02.22±0.44$^{c}$ | 76.66±1.00$^{c}$ | 04.13±0.62$^{c}$ | 03.54±0.80$^{de}$ |
| **T-2.00** | 74.43±1.52$^{d}$ | 01.95±0.47$^{d}$ | 04.45±0.73$^{d}$ | 34.43±2.78$^{c}$ | 01.76±0.59$^{b}$ | 01.00±0.50$^{c}$ | 66.66±1.00$^{d}$ | 03.09±1.47$^{c}$ | 02.71±0.99$^{e}$ |

EO: Essential Oil; T: Trifluralin; SG: Seed Germination; RL: Radicle Length; PL: Plumule Length; SD: Standard Deviation;
* the same letters in each column indicates the statistical differences in the results of the applied doses ($p<0.05$).

EO concentrations lower than that of 0.5 mg/ml revealed less inhibitory effects on the growth of radicle and plumule growth of *L. sativa*. Likewise, the effect was more or similar for *L. sativum* and *P. oleracea*. As in Table 3, it appears that radicle and plumule growth of *L. sativa* were completely inhibited at 0.5 mg/ml and above concentrations. It seemed that upper two concentrations of the EO (1 and 2 mg/ml) completely inhibited the radicle and plumule growth of *L. sativum* and *P. oleracea*.

In a previous study of the herbicidal assay of the EO from *C. nepeta* L. (Savi) grown in Italy, a dose dependent manner (0.125, 0.25 and 0.5 µl/ml) was observed on the germination and radicle growth of *L. sativa*. At 0.125, 0.25 and 0.5 µl/ml of the EO, they found that seed germination of *L. sativa* was reduced by 40%, 100% and 100%, respectively. At the same concentrations, radicle growth of *L. sativa* was reduced by 89%, 83% and 89%, respectively [31]. Differences than those of Araniti's et al.' [31] findings could be related the subspecies of the test species and/or essential oil composition.

## 4. CONCLUSIONS

*C. nepeta* (L.) Savi subsp. *nepeta* from Amanos Mountain include significant sources of phytocompounds acting notable antimicrobial and herbicidal functions on assayed organisms. Further studies are required I) to test the individual and combinatory actions of the compounds to find out their most static and cidal actions on test organisms; II) to determine detrimental effects, practical uses and economics; III) to test active compounds on test plants in agriculture.

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