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# Phytotoxic Effects of *Nepeta meyeri* Benth. Extracts and Essential Oil on Seed Germinations and Seedling Growths of Four Weed Species

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Abstract: Essential oil isolated from the aerial parts of Nepeta meyeri Benth. by hydrodistilation was analysed by GC and GC-MS methods. A total 18 components were identified in the oil representing 100.0% of the oil. Main components were  $4\alpha\alpha,7\alpha,7\alpha\beta$ -nepetalactone (80.3%),  $4\alpha\alpha,7\alpha,7\alpha\alpha$ -nepetalactone (10.3%), *trans*-pulegol (3.1%), 1, 8-cineole (3.0%) and  $\beta$ -bourbonene (2.0%). In addition, *n*-hexane extract of *N*. meyeri was analysed by using GC and GC-MS methods and 18 components were identified. Likewise, nepetalactones,  $4a\alpha$ ,  $7\alpha$ ,  $7a\beta$ -nepetalactone (83.7%),  $4a\alpha$ ,  $7\alpha$ ,  $7a\alpha$ nepetalactone (3.6%), 1, 8-cineole (1.9%) and  $\alpha$ -terpinene (1.5%) were the predominat compounds in the hexane extract. Three concentrations (0.5, 1.0 and 2.0 mg/mL) of the essential oil and n-hexane, chloroform, acetone and methanol extracts isolated from the aerial parts and roots were tested for the herbicidal effects on the germination of the seeds of four weed species including Amaranthus retroflexus L., Chenopodium album L., Cirsium arvense L. and Sinapsis arvensis L. The essential oil of N. meyeri completely inhibited the germination of all weed seeds whereas the extracts showed various inhibition effects on the germination of the weed species. Herbicidal effect was increased with the increasing application concentrations of the extracts. In general, the acetone extract was found to be more effective as compared to the other extracts. All extracts also exhibited various inhibition effects on the seedling growths of the weed species. All extracts also tested for their phytotoxic effects on the weeds at greenhouse condition and the results showed that the oil and extracts caused mortality with 22.00-66.00% 48h after the treatments. These findings suggest that the essential oil and the extracts of *N. meyeri* have potentials for use as herbicides against those weed species.

Keywords: Nepeta meyeri; Herbicidal effect; Essential oil; Extract. © 2015 ACG Publications. All rights reserved.

# **1. Introduction**

In recent years, scientists have focused on the increase of food production needed for the fast expansion of world population. Unfortunately, substantial yield losses occur due to insects and plant diseases caused by fungi, bacteria and viruses [1]. Weeds are another major problem in world agriculture because they cause losses in crops. Therefore, farmers have tended to use more herbicide. However, intensive use of synthetic herbicides can result in soil and groundwater contamination, and development of weed resistance [2]. Herbicides at high concentrations can also increase the risk of toxic residues in agricultural products.

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Therefore, researchers have focused on new potential bio-herbicides, having different and selective herbicidal mechanisms in comparison to their synthetic herbicides [2-7].

The genus *Nepeta*, with almost 280 species, is widespread in Europe, Asia, and a few parts of Africa [8]. It comprises 75 species in Iran and is the largest genus of the Lamiaceae family found in the area. Furthermore, the area is one of two throughout the entire world for which the genus is endemic [9]. Many *Nepeta* species have been reported to be biologically active and are widely used in folk medicine because of their antispasmodic, expectorant, diuretic, antiseptic, antitussive, antiasthmatic, and febrifuge activities [10-14]. Previous research on *Nepeta* sp. indicates that it is represented by 44 taxa, indicating that half of them are endemic to Turkey [15,16]. The endemic and non-endemic Turkish species are widespred in East Anatolia and the Taurus Mountains [16]. According to our numerous field observations (Figure 1) and observations, *Nepeta meyeri* does not permit the germination of other wild plant species within its natural environment and this observation was also conformed [17,18]. Such inhibition zones around a dominant plant species can provide an important ecological context for studies of allelopathy [19]. Therefore, this study was carried out to determine herbicidal properties of the essential oil and extracts isolated from *N. meyeri* by different organic solvents.

#### 2. Materials and methods

#### 2.1. Plant materials and isolation of essential oils

*Nepeta meyeri* Benth. was collected at the flowering stage from Erzurum region in 2007. Collected plant material were dried in shadow and ground in a grinder (about 0.100–0.400 mm). The plant material was identified Dr. Meryem Şengül, Ataturk University, Faculty of Science, Department of Biology, Erzurum (Turkey) and a voucher specimen (No:ATA9865) has been deposited in the herbarium of Ataturk University, Erzurum. The dried plant samples (500g) were subjected to hydrodistillation using a Clevenger-type apparatus for 4 h. Hydrodistillation of *N. meyeri* yielded 0.11% (w/w) essential oil. The yield was based on dry materials of plant sample.

The aerial parts and stems of the plant sampled were dried in shadow at room temperature and powdered using a blender. Then, 200g of each sample was individually extracted with *n*-hexane, chloroform, acetone and methanol (750 mL×4) at room temperature. The extracts were filtered using Whatman filter paper (No. 1) and then concentrated under reduced pressure and temperature using a rotary evaporator. The extracts were stored in a freezer at 4°C until further tests. The yields were shown in Table 1.

		Extracts (g/100	g plant sample)	
Plant parts	<i>n</i> -Hexane	Chloroform	Acetone	Methanol
Aerial parts	14.3	13.7	12.7	13.5
Roots	14.1	14.5	12.0	12.6

Table 1. Yields (%) of the extracts isolated from the aerial parts and stems of Nepeta meyeri.

# 2.2. GC analysis

The analyses of the essential oil and *n*-hexane extract were performed using a Thermofinnigan Trace GC/A1300 (E.I.) equipped with a SGE/BPX5 MS capillary column (30 m × 0.25 mm i.d., 0.25  $\mu$ m). Diluted samples (1/100, v/v, in methylene chloride) of 1.0  $\mu$ L were injected in the splitless mode. Helium was the carrier gas, at a flow rate of 1 mL/min. Injector temperature was set at 220 °C. The programme used was 50–150 °C at a rate of 3 °C/min, held isothermal for 10 min and finally raised to 250 °C at 10 °C/min. Quantitative data of the oil and hexane extract was obtained from FID area percentage (Table 2).

RI <sup>a</sup>	Components	The oil	The extract	Identification methods
930	α-Thujene	tr	0.2	GC, MS, RI
994	Myrcene	tr	0.4	GC, MS, RI
1034	<i>p</i> -Cymene	tr	1.0	GC, MS, RI
1042	1,8-Cineole	3.0	1.9	GC, MS, RI
1067	α–Terpinene	tr	1.5	GC, MS, RI
1106	Linalool	tr	tr	GC, MS, RI
1153	Camphor	tr	0.1	GC, MS, RI
1170	δ-Terpineol	0.3	0.4	GC, MS, RI
1178	Terpinen-4-ol	0.2	0.2	GC, MS, RI
1190	α–Terpineol	0.5	0.5	GC, MS, RI
1184	Napthalene	tr	0.2	GC, MS, RI
1210	trans-Pulegol	3.1	0.4	GC, MS, RI
1373	$4a\alpha$ , $7\alpha$ , $7a\alpha$ –Nepetalactone	10.3	3.6	GC, MS, RI
1383	β-Bourbonene	2.0	0.8	GC, MS, RI
1409	4aα,7α,7aβ-Nepetalactone	80.3	83.7	GC, MS, RI
1433	β-Gurjunene	tr	0.3	GC, MS, RI
1486	Germacrene D	0.3	0.4	GC, MS, RI
1513	α–Cadinene	tr	0.2	GC, MS, RI
Group	ed components (%)			
Monot	terpene hydrocarbons	tr	3.1	
Oxyge	enated monoterpenes	97.7	90.8	
Sesqui	terpene hydrocarbons	2.3	1.7	
Oxyge	enated monoterpenes	-	-	
Others	5	tr	0.2	
Total		100.0	95.8	

Table 2. Chemical composition of <i>n</i> -hexane extract and essential oil of aerial p	parts of <i>N. meyeri</i> .
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GC:co-injection with standards; MS; tentatively identified based on computer matching of the mass spectra of peaks with Wiley 7N and TRLIB libraries and published data (Adams, 2007); RI: identification based on comparison of retention index with those of published data (Adams, 2007); tr: traces (less than 0.1%). <sup>a</sup>: Retention index relative to *n*-alkanes ( $C_8$ - $C_{28}$ ) on SGE-BPX5 capillary column

# 2.3. GC–MS analysis

The analyses of the essential oil and *n*-hexane extract were performed with a Thermofinnigan Trace GC/Trace DSQ/A1300 (E.I. Quadrapole) equipped with a SGE-BPX5 MS fused silica capillary column (30 m  $\times$  0.25 mm i.d., film thickness 0.25 µm). For GC–MS detection, an electron impact ionization system with ionization energy of 70 eV was used. Carrier gas was helium at a flow rate of 1 mL/min. Diluted samples (1/100, v/v, in methylene chloride) of 1.0 µL were injected in the splitless mode. Injector and MS transfer line temperatures were set at 220 °C and 290 °C, respectively. The oven temperature was programmed from 50 °C to 150 °C at 3 °C/min, then held isothermal for 10 min and finally raised to 250 °C at 10 °C/min.

The components were identified based on the comparison of their relative retention time and mass spectra with those of standards, Wiley7N, TRLIB library data of the GC-MS system and literature data [20]. The results were also confirmed by the comparison of the compounds elution order with their relative retention indices on non-polar phases reported in the literature [20].

# 2.4. Seed germination and seedling growth experiments in vivo and in vitro

The seeds of *Amaranthus retroflexus* L., *Chenopodium album* L., *Cirsium arvense* L. and *Sinapsis arvensis* L. were collected in Erzurum region (Turkey) in October 2010. Empty and undeveloped seeds were discarded by floating in tap water. To avoid possible inhibition caused by toxins from fungi or bacteria, the seed were surface-sterilized with 15% sodium hypochlorite for 20 min [5] and then rinsed with abundant distilled water.

To determine the contact herbicidal effects of the oil and extracts, the oil and extracts were dissolved in DMSO–water solution (10%, v/v) being 0.5, 1.0 and 2 mg/mL final concentrations. The emulsions were transferred to Petri dish (9 cm diameter) placed on the bottom two layers of filter paper (10 mL/Petri dishes). Afterwards, 50 seeds of *A. retroflexus, C. album, C. arvense* and *S. arvensis* were placed on the filter paper [4-7]. Petri dishes were closed with an adhesive tape to prevent escaping of volatile compounds and were kept at  $23 \pm 2$  °C on a growth chamber supply with 12 h of fluorescent light and humidity of 80% [3,21]. After 10 days, the number of germinated seeds was determined and seedling lengths were measured. Germination was measured as the percentage of seeds from which a radicle emerges. In addition, trifluralin (0.5,1.0 and 2mg/mL) was used as positive control. Petri dishes containing 10 mL DMSO–water solution (10%, v/v), without the oil and hexane extract solutions were used as negative control. The treatments were arranged in a completely randomized design with three replications including controls.

Twenty mg/pot dosage of essential oil and extracts obtained from the aerial parts of *N. meyeri* were tested against the weeds which have 3–4 leaf stage where groving in the greenhouse. In this method, Pots (10 x 10 cm) were filled with 550 g soil (organic material ratio: 2.02%; cation change capacity: 43.34 me/100 g; pH=7.5) sterilized in autoclave. Then, 50 seeds of the weeds were sown into the pots and kept under photoperiod conditions ( $23 \pm 2$  °C, 12 h light and 12 h dark photoperiod) and relative humidity ( $80 \pm 5$ ) in a growth chamber to allow germination and growth of the plant samples. The pots were irrigated with tap water when necessary. The number of germinated seeds of the respective the weed samples in each pot was counted. Afterwards, the oil and extracts were emulsified in 10 mL of DMSO-water solution (10% v/v). The final concentration of the treatments was 20 mg/ml. These emulsions were sprayed uniformly with a glass atomizer on the surface of whole plants in each pot in the stage of 2-4 real leaves. The plants in each pot, sprayed uniformly with 10 mL of DMSO-water solution (1%), were used as negative control groups. The plants sprayed with trifluralin (20 mg for each pot) were used as positive control. Killed plants were counted and recorded at 24th and 48th hour after sample applications. The treatments were arranged in a completely randomized design with three replications including controls. The phytotoxicity of the treatments was expressed as percent mean of killed plants [6].

### 2.5. Soil samples

Soil samples were taken in June-July from the field where plant samples are collected in the flowering time of *N. meyeri*. The surface of the site in which to take soil samples was cleared of grasses, stems and stones, and then the samples were taken from8-10 points, depending on the site. V-shaped holes as deep as a spade were opened with a spade, and the soil from them was put aside. A 3-4-cm-thick soil samples were taken from the smooth part of the hole. In this way, the samples from 0-25 cm [22] were put on a clean nylon and mixed well and carefully. The sample of 1-2 kg taken was put into a sack and taken to laboratory. The soil samples were taken both from where *N. meyeri* is grown and from where *N. meyeri* does not exist though it as the same climate and soil structure.

#### 2.6. Statistical analysis

In order to determine whether there is a statistically significant difference among the obtained results for antifungal and herbicidal activity assays, variance analyses were carried out using SPSS 10.0 software package. Differences between means were tested by Duncan and LSD tests and values with p < 0.05 were considered significantly different.

#### **3. Results and Discussion**

#### 3.1. Chemical composition of the oil and the hexane extract

In the current study, the essential oil and hexane extract of *Nepeta meyeri* were analysed by GC and GC-MS (Table 1). Analyses results showed that 18 components were identified in the oil representing 100.0 % of the oil.  $4a\alpha,7\alpha,7a\beta$ -Nepetalactone (80.3%),  $4a\alpha,7\alpha,7a\alpha$ -nepetalactone (10.3%), *trans*-pulegol (3.1%), 1, 8-cineole (3.0%) and  $\beta$ -bourbonene (2.0%) are major constituents. The oil contains mainly oxygenated monoterpenes representing 97.7% of total oil. Furthermore, GC and GC-MS analyses allowed the identification of 18 components representing 95.8% of the total hexane extract. Likewise, the hexane extract contains  $4a\alpha,7\alpha,7a\beta$ -nepetalactone (83.7%),  $4a\alpha,7\alpha,7a\alpha$ -nepetalactone (3.6%), 1,8-cineole (1.9%),  $\alpha$ -terpinene (1.5%) and *p*-cymene (1.0%) as predominant components and high content of oxygenated monoterpenes (90.8%). Nepetalactones are the principal components of the essential oil of many *Nepeta* 

species at different propertions [23-28]. It has been reported the similar chemical compositions of the essential oils isolated from *N. meyeri* growing different regions of the world [17,18, 28–33]. The essential oils isolated from Turkish *N. meyeri* collected from Eastern Anatolia contain  $4a\alpha$ , $7\alpha$ , $7a\beta$ -nepetalactone (83.4%),  $4a\alpha$ , $7\alpha$ , $7a\alpha$ -nepetalactone (8.83%), (*Z*)-sabinene hydrate acetate (3.26%) and germacrene D (0.98%) as predominant components [17,18,32]. However, in the current study, (*Z*)-sabinene hydrate acetate was not foundt in the essential oil and hexane extract of *N. meyeri*. Likewise, nepetalactones and 1, 8-cineole are the major components of the essential oils of Iranian *N. meyeri* [28,29].

It was documented that nepetalactones were the predominant components responsible for the feline attractant properties of many Nepeta species such as N.meyeri, N. persica, N. pogonosperma, N.racemosa, N.cataria, N. sibirica, N. nuda, N. grandifolia, N. clarkei, N. parnassica, N. camphorata, N. argolica, N. *crassifolia* [9,17,18,24-26,28-47]. For instance, 4aα,7α,7aβ-nepetalactone (57.6%) and 1,8-cineole (26.4%) were reported the major components of Nepeta pogonosperma essential oil [25]. Likewise, it was found that the essential oil of *N. racemosa* contain mainly  $4a\alpha$ ,  $7\alpha$ ,  $7a\beta$ -nepetalactone (33.6%),  $4a\alpha$ ,  $7\alpha$ ,  $7a\alpha$ -nepetalactone (25.6%) and  $4a\alpha$ ,  $7a\beta$ -nepetalactone (24.4%) [26]. It has been also found that N. sibirica oil contained only one compound,  $4a\alpha$ ,  $7\alpha$ ,  $7a\alpha$ -nepetalactone [38]. Birkett et al. reported the essential oil of N. cataria mainly consisted of isomers of nepetaloctones representing about 87.0% of the oil [39]. Likewise,  $4\alpha\alpha$ ,  $7\alpha$ ,  $7\alpha\beta$ nepetalactone (75.7%),  $4\alpha$ ,  $7\alpha$ , (16.3%) were the major components of N. nuda L. ssp. nuda from Greece [40]. Nepetalactones was also found to be principal components in the essential oils of some Nepeta species growing in Turkish flora [17,18,32,34–36]. It has been documented that N. cataria growing in Eastern Anatolia of Turkey contained mainly  $4\alpha_{\alpha},7\alpha_{\gamma},7\alpha_{\beta}$ -nepetalactone (70.4%),  $4\alpha_{\alpha},7\alpha_{\gamma},7\alpha_{\gamma}$ -nepetalactone (8.5%) and thymol (2.3%) [35]. Furhermore, the most abundant components of Turkish N. nuda were 4aa,7β,7aa-nepetalactone (18.1%), germacrene D (15.7%) and elemol (14.4%). On the other hand some reports indicated that the essential oils of some Nepeta species growing in different regions of the world have different chemical compositions and they do not contain nepetalactones or low and/or trace amounts of nepetalactones [44,48–53]. In the reports published by Handlidou et. al. (2012), the essentials oils of two Greek endemics Nepeta species, N. argolica ssp. malacotrichos and M. argolica ssp. vourinensis, contained mainly 1, 8-cineole (30.95 and 55.6%, respectively [50]. It has been declined that no nepetalactones were found as oil consituents in the essential oil of N. betonicifolia growing in Turkish flora and it consisted of mainly linalool (40.5%), 1,8-cineole (20.8%), and caryophyllene oxide (9.2%) [52]. The similar results have been published for the essential oil of Iranian N. prostrata. Spathulenol (36.5%), terpinen-4-ol (13.3%), 1, 8-cineole (8.8%), myrtenal (6.1%), cissabinene hydrate (5.9%), cis-sabinol (5.5%) and  $\gamma$ -terpinene (4.5%) were detected as predominent components in the oil of N. prostrata, whereas nepetalactones were not found in this oil [51]. Furthermore, the volatile oil isolated from Turkish N. nuda L. ssp. nuda did not contain nepetalactones and the main components of the oil were  $\beta$ -caryophyllene oxide (21.8%), spathulenol (13.8%), *allo*-aromadendrene (9.0%) and  $\beta$ -caryophyllene (5.4%) [53].

#### 3.2. Herbicidal effects of the oil and extracts

In the present study, the essential oil and *n*-hexane, chloroform, acetone and methanol extracts isolated from roots and aerial parts of Turkish N. meyeri were tested on seed germinations and seedling growths of A. retroflexus, C. album, C. arvense and S. arvensis, important weeds in cultivated areas in agriculture. Different degrees of the inhibition of germinations and seedling growths of the weeds were observed when compared with control groups. The results showed that, in particular, the oil has potent inhibitory effect on the seed germinations and seedling growths of all weeds tested (Tables 3 and 4). The current results also showed that the *n*-hexane, chloroform, acetone and methanol extracts have low herbicidal effect against the weeds tested as compared with those of the essential oil (Tables 3 and 4). In general, toxic effects of the extracts isolated from the aerial parts on the germinations and seedling growths of the weeds increased with increase of the application concentrations of the extracts. On the other hand, the extracts isolated from the roots was more effective than the extracts isolated from the aerial parts of N. meyeri on the germinations and seedling growths of the weed seeds (Tables 5 and 6). In particular, the acetone extract of the roots was the most effective as compared with other extracts. On the other hand, the radicle growths as well as root growths of A. retroflexus and C. album were promoted by the some applications of the extracts, whereas germination of these weeds were inhibited by all applications of the extracts (Tables 3 and 4). As can be seen from Table 3, 0.5 and 2.0 mg/mL concentrations of the hexane extract isolated from the aerial parts strongly

increased the radicle growth of *A. retroflexus* with the ratio of 183.79% and 86.85, respectively. The increasing effect on the radicle growth of *A. retroflexus* was also found for 0.5 mg/mL concentrations of chloroform and ecetone extracts, all concentrations of methanol extract isolated from the aerial parts of N. meyeri. In particular, all concentrations of the methanol extract strongly promoted the radicle growth of *A. retroflexus* (166.67–85.32%) (Table 3). The similar results were found for the radicle growth of *C. album* and the radicle growth of this weed was promated by some extracts applications,0.5 and 2.0 mg/mL concentrations of hexane, acetone and methanol extracts and 0.5 mg/mL concentration of chloroform extract isolated from the aerial parts of the plant sample, *N. meyeri*. Furthermore, as shown in Table 5, the radicle growths of both *A. retroflexus* and *C. album* were also promoted by some applications of the extracts isolated from the roots of *N. meyeri*. The promated growths of the weeds can be attributed the low germination percentage in the medium.

Herbicidal effects of the essential oil and extracts isolated from the aerial parts and roots of N. meyeri were evaluated for assessing their contact phase effects on the germination of A. retroflexus, C. album, C. arvense and S. arvensis which are important weeds in cultivated area. In our study, their herbicidal effects were compared with a commercial herbicide, trifluralin (Maga-Tref 48 EC) (Tables 3-6). The results obtained from contact phase effect assays showed that the oil and some applications of the extracts completely inhibited the germination and seedling growth of the tested weeds (Tables 3–6). As can be seen from these tables, sometimes, their suppressing effects on the germinations and seedling growths of the weeds were also higher than that of commercial herbicide, trifluralin (Maga-Tref 48 EC) (Tables 3-6). As shown in Table 2, the oil of *N. meyeri* contained the high content of oxygenated monoterpenes (representing 97.7% of the total oil) and nepetalactones,  $4\alpha$ ,  $7\alpha$ ,  $7\alpha$ ,  $7\alpha$ -nepetalactone (80.3%) and  $4\alpha$ ,  $7\alpha$ ,  $7\alpha$ -nepetalactone (10.3%) are the major components. Therefore, the herbicidal effects of the oil can be attributed to its major components, nepetalactones. However, as can be seen from Tables 3 and 4, inhibition effects of the hexane extract isolated from the aerial parts of N. meyeri on the germinations and seedling growths of the weeds were found to be low as compared to inhibition effects of the oil. As similar to essential oil,  $4\alpha\alpha_3,7\alpha_3,7\alpha_3$ nepetalactone (83.7%),  $4a\alpha,7\alpha,7\alpha$ -nepetalactone (3.6%) and 1.8-cineole (1.9%) are predominant components of hexane extract of the aerial parts of N. meyeri (Table 2). On the other hand, Table 1 also shows that the hexane extract contained relatively low content of oxygenated monoterpenes (90.8%) as compared to that of the essential oil. Therefore, low herbicidal properties of the hexane extract of N. meyeri can be attributed to low content of the oxygenated monoterpenes.

The current results show that the essential oil isolated from the aerial parts of N. meyeri possesses potent herbicidal effect against the tested weeds (Tables 3 and 4). In accordance with our findings, it has been documented that the essential oils isolated from N. meyeri growing in different regions of the world, contain mainly nepetalactones, 1,8-cineole,  $\beta$ -bourbonene,  $\alpha$ -terpineol and *trans*-pulegol have bioherbicidal effects against various weed species [5,17,18,42,54]. Previously, it was reported that the essential oil of N. meyeri was completely inhibited seed germination and seedling growth of ten weeds (Agropyron cristatum L., A. retroflexus L., Bromus danthoniae, Bromus tectorum L., Bromus intermedius, C. album L., Convolvulus arvensis L., Cynodan dactylon L., L. serriola L., Portulaca olerace L) [18]. Furthermore, it has been found that the aqueous extracts preperad from the roots and leaves of N. meyeri had phytotoxic effect on the seed germination and seedling growth of economically important crops, barley, wheat, canola, safflower and sunflower [17]. In the previous reports, phytotoxic effects of the essential oils isolated from diffrent Nepeta species against various weeds has also been investigated [55,56]. Mancini et. al. (2009) tested the essential oils of N. curviflora and N. nuda L. subsp albiflora from Lebanon on thegerminations and radical elongations of Raphanus sativus L. and Lepidium sativum L. and the essentail oils had significant inhibitory effects on the germinations and radicle growths of the tested weeds [55]. In this reports, it has also been reported that the essential oil of N. curviflora contained high amounts of  $\beta$ caryophyllene (41.6%), caryophyllene oxide (9.5%), (E)- $\beta$ -farnesene (6.2%) and (Z)- $\beta$ -farnesene (4.8%), whereas N. nuda oil consisted of mainly  $\beta$ -bisabolene (11.8%), pulegone (10.8%), (E,Z)-nepetalactone (8.0%), (E)- $\beta$ -farnesene (7.1%) and caryophyllene oxide (6.9%) [55]. Likewise, 0.3 and 1.0 mg/mL concentartions of the essential oil isolated from N. pannonica, contain 1,8-cineole (28.9%) and  $4\alpha$ ,  $7\beta$ ,  $7\alpha$ nepetalactone as predominant components was more phytotoxic against to lettuce seeds with 100% growth inhibition [56]. However, in the current study, the mechanism of herbicidal activities of the oil, the extracts isolated from the aerial parts and roots of N. meyeri and their constituents was not investigated. On the other hand, it is well known that monoterpenes in the essential oils have phytotoxic effects that may cause anatomical and physiological changes in plant seedlings leading to accumulation of lipid globules in the cytoplasm, reduction in some organelles such as mitochondria, possibly due to inhibition of DNA synthesis or disruption of membranes surrounding mitochondria and nuclei [4,5,32, 57–59]. It has also reported that the essential oil of N. meyeri exhibited the herbicidal effects inducing oxidative stress in early seedlings of some weed species such as A. retroflexus, Bromus danthoniae, Bromus intermedius, Chenopodium album, Cynodon dactylon L., Lactuca serriola and Portulaca oleracea L. [54]. Therefore, the herbicidal activity of the oil in the present study can be attributed to one or more of these factors. In accordance with our results, it has been indicated that the essential oil of N. meyeri have genotoxic effect on the weeds, Bromus danthoniae and Lactuca serriola and two crop plants, Brassica napus and Zea mays [32]. Hence, phytotoxic effect of the oil can be due to its genotoxical potential. The oil, extracts isolated from the aerial parts of N. meyeri and commercial herbicide, trifluralin (Maga-Tref 48 EC) also tested for their phytotoxic effects on the weeds at greenhouse condition and the results showed that the oil and extracts caused mortality with 22.00-66.00% 48h after the treatment as compared with control groups (Table 7). Phytotoxic effects of the oil and extracts were influenced by the exposure time. In contrast to petri experiments, the oil and extracts exhibited similar phytotoxic effects in greenhouse conditions against A. retroflexus, C. album, C. arvense and S. arvensis (Table 7). As can be seen from Table 7, in many times, the oil and extracts applications were effective as commercial herbicide, trifluralin. Among the applications of N. meyeri, the most phytotoxic effect with 66.00% mean death was found for methanol extract against A. retroflexus. Highest photoxic effects with 59.33% and 67.33% mortality rates against S. arvensis were observed for the hexane extract and trifluralin, commercial herbicide as compared with the other applications. On the other hand, chloroform extract of N. meyeri showed the lowest phytotoxic effect with 22.00% mortality against S. arvensis.

#### 3.3. Soil and Plant Analysis

According to our numerous field observations (Figure 1) and litarature survey [17,18,54], Nepeta *meyeri* does not permit the germination of other wild plant species within its natural environment. Therefore, soil samples obtained from the N. meyeri growing region and non-growing region were analysed and the results are shown in Table 8. The results show that the pH value of the soil of N. meyeri growing area was higher than that of non-growing region. As can be seen from this table, N. meyeri increased the amounts of CaCO<sub>3</sub> during growing period as compared with non-growing region. Likewise, Ca amount was measured to be high in the plant growing region. As expected, there is a significant decrease in terms of organic substances and the minerals in soil samples obtained from the plant growing area. However, there were not big problems on both soil to prevent plant development and growth even more the results were blow from normal level. On the other hand, as seen on Table 8, the soil where N. meyeri growing area had higher pH (7.31 vs. 6.81), CaCO<sub>3</sub> (6.0 vs. 2.1%), Ca (19.35 vs. 14.25 me/100g), Mg (7.32 vs. 6.40 me/100g) than non growing area's soil. However, non growing area's soil had higher organic matter (2.58 vs. 1.18%), N (3.16 vs. 1.72 me/100g), P (5.72 vs. 3.48 me/100g) and K (3.12 vs. 2.48 me/100g) values compared to natural growing region. In the view of these results, it can be concluded that N. meyeri does not permit the germination of other wild plant species within its natural environment by increasing the rate of soil lime and by reducing the amounts of basic minerals.

Treatments	Conc. (mg/mL)	Germination (%)	Inh. (%)	Root length (mm)	Inh. (%)	Radicle length (mm)	Inh. (%)
A. retroflexus	5						
Essential Oil	0.5 1.0 2.0	0.00±0.00 a 0.00±0.00 a 0.00±0.00 a	100.00* 100.00* 100.00*	0.00±0.00 a 0.00±0.00 a 0.00±0.00 a	100.00* 100.00* 100.00*	0.00±0.00 a 0.00±0.00 a 0.00±0.00 a	100.00* 100.00* 100.00*
Extracts Hexane	0.5 1.0 2.0	34.00±4.16 f 18.67±3.53e	1.93 46.15*	37.45±1.57 f 18.36±2.14 cde	-12.13 45.03* 28.14*	27.84±1.25 f 7.43±0.64 bc	-183,79* 24.26
	2.0	14.00±2.00 cde	59.62*	20.57±2.73 de	38.14*	18.33±1.26 de	-86.85*
Chloroform	0.5 1.0 2.0	40.67±4.81 f 19.33±3.71 e 7.33±4.06 abcd	-17.30 44.25* 78.86*	36.33±1.19 f 11.83±1.71 bcd 6.50±0.88* ab	-8.77 64.58* 80.54*	25.54±1.29 f 6.17±0.62 abc 9.83±1.21 bc	-160.35* 37.10* -0.21
Acetone	0.5 1.0 2.0	33.33±1.76 f 15.33±1.76 de 5.33±3.53 abc	3.87 55.78* 84.63*	27.30±1.45 ef 11.48±1.26 bcd 9.44±2.12 abc	18.26 65.63* 71.74*	26.30±1.32 f 7.57±0.69 bc 12.22±2.09 cd	-168.09* 22.83 -24.57
Methanol	0.5 1.0	37.33±3.71 f 17.33±3.71 e	-7.67 50.01*	36.79±1.04 f 18.00±1.61 cde	-10.15 46.11*	26.16±1.05 f 18.15±1.16 de	-166.67* -85.32*
Controls	2.0	11.33±4.67 bcde	67.32*	17.76±1.97 cde	46.83*	24.06±1.82 ef	-145.26*
Trifluralin	0.5 1.0 2.0	4.00±2.00 ab 7.33±1.33 abcd 5.33±2.91 abc	88.46* 78.86* 84.63*	4.67±0.33* ab 5.09±0.56 ab 2.44±0.63 ab	86.02* 84.76* 92.69*	4.67±0.3* ab 3.91±0.46* ab 3.11±0.63* ab	52.40* 60.14* 68.30*
DMSO	-	34.67±2.40 f	-	33.40±2.39 f	-	9.81±0.42 bc	-
C. album							
Essential Oil	0.5 1.0 2.0	0.00±0.00 a 0.00±0.00 a 0.00±0.00 a	100.00* 100.00* 100.00*	0.00±0.00 a 0.00±0.00 a 0.00±0.00 a	100.00* 100.00* 100.00*	0.00±0.00 a 0.00±0.00 a 0.00±0.00 a	100.00* 100.00* 100.00*
Extracts Hexane	0.5 1.0	20.00±2.31 ef 17.33±2.91 def	50.00* 56.68*	28.83±1.84 c 6.73±0.63 a	-1.76 76.24*	13.50±0.80 fgh 4.73±0.21 b	-72.41* 39.59*
	2.0	10.67±1.33 bcd	73.33*	26.94±3.56 bc	4.91	18.00±2.93 i	-129.89*
Chloroform	0.5	23.33±2.67 f	41.68*	26.00±1.19 bc	8.22	15.43±0.88 ghi	-97.06*
	1.0 2.0	14.67±2.40 cde 6.67±2.40 abc	63.33* 83.33*	7.95±1.36 a 6.30±1.97 a	71.94* 77.76*	5.45±0.60 bc 9.20±0.83 cde	30.40* -17.50
Aaatona						$12.62 \pm 0.64$ efg	-61.17*
Acetone	0.5 1.0	42.00±3.46 g 14.67±4.06 cde	-5.00 63.33*	24.95±1.23 bc 9.18±1.21* a	11.93 67.57*	$7.18\pm0.85$ bcd	8.30
	2.0	13.33±1.76 cde	66.68*	19.25±2.18 b	32.05*	11.15±0.65 def	-42.40*
Methanol	0.5	36.67±0.67 g	8.33	27.27±0.95 bc	2.68	17.09±0.67 hi	-118.26*
	1.0	19.33±3.71 ef	51.68*	8.44±0.71 a	70.21*	9.34±0.63 cde	-19.28
	2.0	20.00±4.00 ef	50.00*	23.63±1.69 bc	16.59*	10.93±0.43 def	-39.59*
Controls							
Trifluralin	0.5	38.67±2.40 g	3.33	3.97±0.17 a	85.99*	5.05±0.13 b	35.50*
	1.0	6.67±0.67 abc	83.33*	4.40±0.40 a	84.47*	4.40±0.40 b	43.81*
DMSO	2.0	3.33±1.76 ab 40.00±5.29 g	91.68* -	0.83±0.17 a 28.33±2.27 bc	97.07* -	4.33±1.02 b 7.83±0.32 abc	44.70* -

**Table 3**. Contact inhibitory effects of the essential oil and extracts isolated from the aerial parts of *N. meyeri* on seed germinations and seedling growths of *A. retroflexuas* and *C. album.* 

Conc.: Concentration.

Means in the column with the same letter are not significantly different using Duncan's test ( $\alpha$ =0.05).

\*Statisticaly different from the negative control according to LSD test.

Treatments	Conc. (mg/mL)	Germination (%)	Inh. (%)	Root length (mm)	Inh. (%)	Radicle length (mm)	Inh. (%)
C. arvense							
Essential Oil	0.5	0.00±0.00 a	100.00*	0.00±0.00 a	100.00*	0.00±0.00 a	100.00*
	1.0	0.00±0.00 a	100.00*	0.00±0.00 a	100.00*	0.00±0.00 a	100.00*
	2.0	0.00±0.00 a	100.00*	0.00±0.00 a	100.00*	0.00±0.00 a	100.00*
Extracts	0.5	26.00+2.46.5	25.01*	22.05+1.50 1.5	21.02*	12 10 10 72 1	() 77*
Hexane	0.5 1.0	26.00±3.46 fg 17.33±2.40 de	25.01* 50.01*	22.95±1.59 cdef 20.00±2.67 cde	31.02* 39.89*	12.10±0.72 de 10.19±0.81 bcde	62.77* 68.65*
	2.0	$17.33 \pm 2.40$ de 13.33 \pm 1.76 cd	61.55*	$24.20\pm3.42$ cdef	27.26*	11.10±0.85 bcde	65.85*
	2.0	15.55±1.76 cd	01.55	24.20±3.42 cuci	27.20	11.10±0.85 bede	05.05
Chloroform	0.5	24.00±2.31 efg	30.78*	17.36±1.33 cde	47.82*	12.78±1.02 de	60.68*
	1.0	16.00±2.00d	53.85*	16.04±2.50 bcd	51.79*	11.38±0.83 bcde	64.99*
	2.0	7.33±2.91 abc	78.86*	30.18±5.64 ef	9.29	12.91±1.48 de	60.28*
Apotono	0.5	26.67.1.76		26 40 1 20 1 6	20.65*		(0.00*
Acetone	0.5	36.67±1.76 i	-5.77	$26.40 \pm 1.28 \text{ def}$	20.65*	12.73±0.59 de	60.83* 76.77*
	1.0	14.67±0.67 cd	57.69* 84.63*	12.55±3.17 abc 19.13±6.83 cde	62.28* 42.50*	$7.55 \pm 1.23$ abcde	76.77* 56.02*
	2.0	5.33±0.67 ab	04.03**	19.15±0.85 cde	42.50*	14.00±1.86 e	56.92*
Methanol	0.5	28.00±3.46 gh	19.24	22.88±2.00 cdef	31.23*	10.76±0.49 bcde	66.89*
	1.0	25.33±1.76 fg	26.94*	$26.92\pm2.34$ def	19.09*	11.92±0.89 cde	63.32*
	2.0	$10.67 \pm 1.33$ bcd	69.22*	$11.44\pm2.04$ abc	65.61*	9.94±1.49 bcde	69.42*
Controls							
Trifluralin	0.5	16.00±1.15 d	53.85*	3.83±0.35 ab	88.49*	3.75±0.37 ab	88.46*
	1.0	18.67±1.76 def	46.15*	4.68±0.61 ab	85.93*	5.00±0.24 abcd	84.62*
	2.0	4.00±1.05 ab	88.46*	1.29±0.29 a	96.12*	3.85±0.77* abc	88.15*
DMSO	-	34.67±6.36 hi	-	33.27±2.95 f	-	32.50±2.77 f	-
S. arvensis							
Essential Oil	0.5	0.00±0.00 a	100.00*	0.00±0.00 a	100.00*	0.00±0.00 a	100.00*
	1.0	0.00±0.00 a	100.00*	0.00±0.00 a	100.00*	0.00±0.00 a	100.00*
	2.0	0.00±0.00 a	100.00*	0.00±0.00 a	100.00*	0.00±0.00 a	100.00*
Extracts							
Hexane	0.5	18.67±1.76 b	49.09*	20.50±3.57 cdef	63.45*	11.14±1.08 cdef	46.95*
	1.0	14.00±1.15 b	61.82*	8.10±2.36 abc	71.81*	5.81±0.93 abc	72.33*
	2.0	4.00±2.00 a	89.09*	11.43±3.89 abcd	60.22*	6.43±1.43 bcd	69.38*
Chloroform	0.5	20.22+1.76 -	20.02*	42 27 2 (1 -	50 (1*	12.05 + 0.70 J-f	42 (2*
Chlorotothi	0.5	29.33±1.76 c	20.02*	43.27±2.61 g	-50.61*	12.05±0.70 def	42.62*
	1.0	16.67±2.40 b	54.54*	18.40±2.61 bcdef	35.96*	9.20±0.69 cd	56.19*
	2.0	4.00±2.30 a	89.09*	42.14±3.40 g	-46.68*	15.71±2.68 efg	25.19*
Acetone	0.5	28.67±1.76 c	21.82*	27.91±2.13 defg	2.85	10.35±0.75 cde	50.71*
	1.0	16.00±1.15 b	56.37*	14.46±1.98 abcde	49.67*	6.33±0.51 bcd	69.86*
	2.0	0.00±0.00* a	100.00*	0.00±0.00 a	100.00*	0.00±0.00 a	100.00*
	2.0	0.00-0.00 a	100.00	0.00-0.00 a	100.00	0.00-0.00 a	100.00
Methanol	0.5	17.33±2.67 b	52.74*	19.29±2.74 bcdef	32.86*	11.04±1.02 cdef	47.43*
	1.0	15.33±2.67 b	58.19*	10.78±2.45 abc	62.48*	5.96±0.68 abcd	71.62*
	2.0	5.33±0.67* a	85.47*	32.13±5.28 fg	-11.83	16.63±3.35 fg	20.81*
Controls				U		C	
Trifluralin	0.5	15.33±1.76 b	58.19*	2.52±0.36 ab	91.23*	2.43±0.34 ab	88.43*
	1.0	4.00±1.15 a	89.09*	1.00±0.00 a	96.52*	$1.33\pm0.21$ ab	93.67*
	2.0	1.33±0.67 a	96.37*	5.00±2.87 abc	82.60*	6.00±3.05 abcd	71.43*
DMSO	-	36.67±3.71 d	-	28.73±1.97 efg	-	21.00±1.02 g	-

**Table 4**. Contact inhibitory effects of the essential oil and extracts isolated from the aerial parts of *N. meyeri* on seed germinations and seedling growths of *C. arvense* and *S. arvensis*.

Conc.:Concentration.

Means in the column with the same letter are not significantly different using Duncan's test ( $\alpha$ =0.05).

\*Statisticaly different from the negative control according to LSD test.

<b>T</b>	Conc.	Germination	Inh.	Root length	Inh.	Radicle length	Inh.
Treatments	(mg/mL)	(%)	(%)	(mm)	(%)	(mm)	(%)
A. retroflex	cus						
Extracts							
Hexane	0.5	16.00±3.06 cd	53.85*	40.83±3.80 e	-22.25*	10.63±0.55 def	-8.47
	1.0	2.00±1.00 a	94.23*	$7.00 \pm 3.00$ ab	79.04*	8.40±4.06 bcde	14.24
	2.0	1.33±0.67 a	96.16*	5.00±2.89 ab	85.03*	5.00±2.89 abcd	48.98*
Chloroform	0.5	5.33±1.33 ab	84.63*	29.00±2.82 cde	13.17	17.75±1.76 g	-81.12*
	1.0	6.00±3.06 ab	82.69*	22.50±4.43 bcd	32.63*	19.00±3.71 g	-93.88*
	2.0	1.33± 0.67 a	96.16*	5.00±2.89* ab	85.03*	5.00±2.89 abcd	48.98*
Acetone	0.5	10.00±3.06 bc	71.16*	31.00±3.66 de	7.19	12.00±1.36 ef	-22.45
	1.0	6.00±2.16 ab	82.69*	$30.50\pm5.45$ de	8.68	15.70±2.39 fg	-60.20*
	2.0	0.00±0.00 a	100.00*	0.00±0.00 a	100.00*	$0.00\pm0.00$ a	100.00*
	0.7		11051	22.10.2.10.1	1.00		
Methanol	0.5	19.33±0.67 d	44.25*	33.10±2.18 de	1.00	9.69±0.61 cde	1.12
	1.0	7.33±1.71 ab	78.95*	$13.33 \pm 2.64$ abc	60.09*	8.75±1.09 bcde	10.71
Controls	2.0	0.67±0.67 a	98.07*	10.67±2.75 ab	60.05*	5.00±2.89 abcd	48.98*
Trifluralin	0.5	4.00±2.00 ab	88.46*	4.67±0.33* ab	86.02*	4.67±0.33 abcd	52.65*
	1.0	7.33±2.31 ab	78.95*	5.09±0.56 ab	84.63*	$3.91\pm0.46$ abc	60.10*
	2.0	5.33±2.91 ab	84.63*	2.44±0.63 a	92.70*	3.11±0.63 ab	68.27*
DMSO	-	34.67±2.40 e	-	33.40±2.39de	-	9.80±0.42 cde	-
C. album							
Extracts							
Hexane	0.5	40.67±2.40 b	-1.68	36.56±1.62 fg	-19.05*	8.18±0.31 bc	-7.35
	1.0	11.33±1.76 a	71.68*	28.85±5.99 defg	6.06	8.38±1.00 bc	-9.97
	2.0	3.33±0.67* a	91.68*	28.00±6.04 cedfg	8.82	16.40±3.08 e	-115,22*
Chloroform	0.5	40.67±2.90 b	-1.68	34.34±1.87 efg	-11.82	8.51±0.41 c	-11.68
	1.0	11.33±1.76 a	71.68*	24.06±4.05 cedf	21.65	8.18±0.71 bc	-7.35
	2.0	6.67±1.33 a	83.33*	15.70±3.00 bc	48.88*	12.70±1.54 d	-66.67*
Acetone	0.5	45.22 4.061	10.00	26 25 1 45 6	10.04	0.05 0.51	20 52*
ACCIUIC	0.5	45.33±4.06 b	-13.33	36.25±1.45 fg	-18.04	9.87±0.71 cd	-29.53*
	1.0	12.67±3.71 a	68.33*	27.47±3.89 cdef	10.55	9.00±0.61 c	-18.11
	2.0	4.00±1.31 a	90.00*	20.71±4.14 cd	32.56	9.57±2.48 cd	-25.59*
Methanol	0.5	40.00±5.14 b	0.00	40.78±1.84 g	-32.79*	9.25±0.77 c	-21.39*
	1.0	6.00±2.31 a	85.00*	19.44±7.24 cd	36.70*	9.44±1.00 cd	-23.92*
	2.0	6.00±1.15 a	85.00*	22.77±8.78 cde	25.86*	11.00±1.01 cd	-44.36*
Controls							
Trifluralin	0.5	3.97±0.17 a	90.08*	3.97±0.17 ab	87.07*	5.05±0.13 ab	33.73
	1.0	4.40±0.40 a	89.00*	4.40±0.40 ab	85.67*	4.40±0.40 a	42.26

 Table 5.
 Contact inhibitory effects of the essential oil and extracts isolated from the roots of *N. meyeri* on seed germinations and seedling growths of *A. retroflexus* and *C. album*.

Conc.: Concentration.

DMSO

2.0

Means in the column with the same letter are not significantly different using Duncan's test ( $\alpha$ =0.05).

-

98.00\*

 $0.80{\pm}0.20$  a

30.71±2.15 defg

97.40\*

-

4.00±1.18 a

 $7.62 \pm 0.28$  bc

47.51\*

-

\*Statisticaly different from the negative control according to LSD test.

0.80±0.20 a

40.00±5.29 b

Treatments	Conc. (mg/mL)	Germination (%)	Inh. (%)	Root length (mm)	Inh. (%)	Radicle length (mm)	Inh. (%)	
C. arvense								
Extracts								
Hexane	0.5	21.33±3.71 cd	38.48*	34.06±2.34 e	-2.38	7.63±0.54 ab	76.52*	
	1.0	7.33±0.67 ab	78.86*	16.09±3.81 cd	51.64*	8.00±0.84 ab	75.38*	
	2.0	4.67±0.67 a	86.53*	27.86±4.48 de	16.26*	17.14±2.14 cd	47.26*	
Chloroform	0.5	26.67±2.40 de	23.08	27.75±1.94 de	16.59*	8.43±0.50 abc	74.06*	
	1.0	6.67±3.52 ab	80.76*	15.00±2.52 bcd	54.91*	5.72±0.82 ab	82.40*	
	2.0	3.33±1.76 a	90.40*	16.67±4.41 cd	49.90*	14.67±4.01 bcd	54.86*	
Acetone	0.5	21.33±3.52 cd	38.48*	39.68±2.50 e	-19.27*	17.88±1.59 d	44.99*	
	1.0	4.67± 1.33 a	86.53*	$10.78 \pm 3.54$ abc	67.60*	5.44±1.21 ab	76.34*	
	2.0	0.00±0.00* a	100.00*	0.00±0.00 a	100.00*	0.00±0.00 a	100.00*	
Methanol	0.5	21.33±5.21 cd	38.48*	30.47±2.57 e	8.42	6.94±0.42 ab	78.65*	
	1.0	8.67±2.40 ab	74.99*	$12.85 \pm 1.80$ abc	61.38*	$7.69\pm0.72$ ab	76.34*	
	2.0	$0.00\pm0.00$ a	100.00*	3.33±0.33 ab	89.99*	$3.33\pm3.33$ a	89.75*	
Controls	2.0	0.00-0.00 u	100.00	5.55-0.55 ab	07.77	5.55 <u>-</u> 5.55 u	07.15	
Trifluralin	0.5	16.00±1.15 bc	53.85*	3.83±0.35 ab	88.49*	3.75±0.37 a	88.46*	
	1.0	18.67±1.76 cd	46.15*	4.68±0.61 ab	85.93*	5.00±0.24 ab	84.62*	
	2.0	4.00±2.05 a	88.46*	1.29±0.29 a	96.12*	3.85±0.77 a	88.15*	
DMSO	-	34.67±3.36 e	-	33.27±2.95 e	-	32.50±2.77 e	-	
S. arvensis								
Extracts								
Hexane	0.5	18.67± 2.91 c	49.09*	13.04±1.81 abcd	54.61*	6.21±0.39 bc	70.43*	
	1.0	6.67±2.40 b	81.81*	9.40±2.17 abcd	67.28*	6.90±1.09 bc	67.14*	
	2.0	0.00±0.00 a	100.00*	0.00±0.00 a	100.00*	0.00±0.00 a	100.00*	
Chloroform	0.5	24.67±2.40 d	32.72*	22.57±2.19 de	21.44*	8.78±0.62 c	58.19*	
	1.0	4.67±0.67 ab	87.27*	15.71±5.17 bcde	45.32*	6.43±0.92 bc	69.38*	
	2.0	0.00±0.00 a	100.00*	0.00±0.00 a	100.00*	0.00±0.00 a	100.00*	
Acetone	0.5	20.67±2.40 cd	43.63*	15.65±1.48 bcde	45.53*	6.84±0.46 bc	67.43*	
	1.0	5.33±1.90 ab	85.47*	12.78±3.34 abcd	55.52*	9.44±2.82 c	55.05*	
	2.0	$0.00 \pm 0.00$ a	100.00*	0.00±0.00 a	100.00*	0.00±0.00 a	100.00*	
Methanol	0.5	25.33±1.76 d	30.92*	17.11±1.52 cde	40.45*	8.61±0.59 c	59.00*	
	1.0	7.33±1.76 b	80.01*	9.46±3.66 abcd	67.07*	6.55±0.87 bc	68.81*	
	2.0	0.00±0.00 a	100.00*	0.00±0.00 a	100.00*	0.00±0.00* a	100.00*	
Controls	2.0	0.00-0.00 a	100.00	5.00-0.00 a	100.00	0.00±0.00 a	100.00	
Trifluralin	0.5	15.33±1.76 c	58.20*	2.52±0.36 ab	91.23*	2.43±0.34 ab	88.43	
	1.0	4.00±1.15 ab	89.09*	1.00±0.00 a	96.52*	$1.33\pm0.21$ ab	93.67	
	2.0	1.33±0.67 ab	96.37*	5.00±2.89 abc	82.60*	6.00±3.05 bc	71.43	
DMSO	_	36.67±3.71 e	-	28.73±1.97 e	-	21.00± 1.02 d	_	

**Table 6.** Contact inhibitory effects of the essential oil and extracts isolated from the roots of *N. meyeri* on seed germinations and seedling growths of *C. arvense* and *S. arvensis*.

Conc.: Concentration.

Means in the column with the same letter are not significantly different using Duncan's test ( $\alpha$ =0.05).

\*Statisticaly different from the negative control according to LSD test.

	Phytotoxic eff	ect (% mean death)
	24. hour	48. hour
A. retroflexus		
The oil	42.67±8.35 b	64.00±5.29 b
Extracts		
Hexane	36.67±5.70 b	63.33±5.81 b
Chloroform	30.00±2.31 b	58.67±1.76 b
Acetone	38.00±5.03 b	64.67±4.81 b
Methanol	40.67 ±5.46 b	66.00±7.57 b
Controls		
Trifluralin	38.67±1.33 b	63.33±3.33 b
DMSO	5.33±2.40 a	11.33±5.33 a
C. album		
The oil	36.00±3.06 b	61.33±6.57 ef
Extracts		
Hexane	31.33±4.37 b	54.00±7.02 def
Chloroform	31.33±1.33 b	62.67±1.76 ef
Acetone	34.67±3.71 b	51.33±5.33 cde
Methanol	35.33±6.36b	45.33±6.36 bcd
Controls		
Trifluralin	39.33±6.57 bc	67.33±1.33 ef
DMSO	7.33±0.67a	11.33±2.40 a
C. arvense		
The oil	34.00±6.11 cd	58.67±6.77 ef
Extracts		
Hexane	27.33±1.33 bc	51.33±2.40 cde
Chloroform	33.33±5.21 cd	60.67±6.57 ef
Acetone	28.00±2.31 bc	54.67±4.06 def
Methanol	29.33±5.81 bc	52.00±12.22 cdef
Controls		
Trifluralin	39.33±8.35 cde	70.67±5.81 f
DMSO	8.00±1.15 a	11.33±2.67 ab
S. arvensis		
The oil	28.67±6.67 bc	53.33±6.36 b
Extracts		
Hexane	32.00±5.03 c	59.33±4.67 b
Chloroform	22.00±1.14 b	51.33±7.06 b
Acetone	31.33±6.96 c	50.00±5.77 b
Methanol	28.00±2.00 bc	54.00±1.15 b
Controls		
Trifluralin	33.33±4.67 c	67.33±5.93 bc
DMSO	6.00±2.00 a	10.67±1.76 a

**Table 7.** Phytotoxic effects of *N. meyeri* essential oil and its extracts against *A. retroflexus, C album, C. arvense* and *S. arvensis.* 

Means in the same column by the same letter are not significantly different to the test of Duncan ( $\alpha$ =0.05)

	Table 8	8. Soil Ana	alysis.										
Soil sample	%					me/100gr			mg/ kg=ppm				
	рН	CaCO <sub>3</sub>	Organic substance	Ν	Р	К	Ca	Mg	Na	Fe	Cu	Mn	Zn
*	7.31	6.0	1.18	1.72	3.48	2.48	19.35	7.32	0.62	1.75	3.20	2.40	1.69
**	6.81	2.1	2.58	3.16	5.72	3.12	14.25	6.40	1.24	3.12	5.18	6.23	4.18

\* N. meyeri growing region;\*\* N. meyeri non-growing region

The nutrient analysis of *N. meyeri* was also conducted, and the amount of nutrients was given in Table 9. The amount of N was % 3.15; and the amount of P was 2715 mg/kg, that of K was 12138 mg/kg, that of Ca was 4200 mg/kg, that of Mg was 2316 mg/kg. The amounts of Fe, Cu, Mn and Zn were 152 mg/kg, 48 mg/kg, 39 mg/kg and 26 mg/kg, respectively.

**Table 9.** Minerals analysis of *N. meyeri*.

	mg/kg=ppm								
N (%)	Р	К	Ca	Mg	Fe	Cu	Mn	Zn	
3.15	2715	12138	4200	2316	152	48	39	26	

# 4. Conclusion

Although there are different methods for the use of the management of weeds, the development of natural herbicides and pesticides would help to decrease the negative impact of synthetic agents, such as residues, resistance and environmental pollution. In this respect, natural pesticides and herbicides may be effective, selective, biodegradable, and less toxic to environment. The oil and extracts of *N. meyeri* showed potent herbicidal and phytotoxic effects against *A. retroflexus, C. album, C. arvense* and *S. arvensis*. Based on the present results, the oil and extracts could be suggested as alternative bio-herbicides. However, further studies are required to determine the cost, applicability, safety and phytotoxicity against the cultured plants of these agents as potential herbicides.

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#### References

- [1] J. Fletcher, C. Bender, B. Budawle, W.T. Cobb, S.E. Gold, C.A. Ishimaru, D. Luster, U. Melcher, R. Murch, H. Scherm, R.C. Seen, J.L. Sherwood, B.W. Sobral and S.A. Tolin (2006). Plant pathogen forensics: capabilities needs, and recommendations, *Microbial. Mol. Biol. Rev.* 70, 450–471.
- [2] S.O. Duke, F.E. Dayan, J.G. Romagni and A.M. Rimando (2000). Natural products as sources of herbicides: current status and future trends, *Weed Res.* **40**, 99–111.
- [3] N. Dudai, A. Poljakoff-Mayber, A.M. Mayer, E. Putievsky and H.R. Lerner (1999). Essential oils as allelochemicals and their potential use as bioherbicides, *J. Chem. Ecol.* **25**, 1079–1089.
- [4] S. Kordali, R. Kotan and A. Cakir (2007a). Screening of in vitro antifungal activities of 21 oxygenated monoterpenes as plant disease control agents, *Allelopathy J.* **19**, 373–392.
- [5] S. Kordali, A. Cakir and S Sutay (2007b). Inhibitory effects of monoterpenes on seed germination and seedling growth, *Z. Naturforsch. C.* **62**, 207–214.
- [6] S. Kordali, A. Cakir, H. Ozer, R. Cakmakci, M. Kesdek and E. Mete (2008). Antifungal, phytotoxic and insecticidal properties of essential oil isolated from Turkish *Origanum acutidens* and its three components, carvacrol, thymol and *p*-cymene, *Bioresource Technol.* **99**, 8788–8795.
- [7] S. Kordali, A. Cakir, T.A. Akcin, E. Mete, A. Akcin, T. Aydin and H. Kilic (2009) Antifungal and herbicidal properties of essential oils and *n*-hexane extracts of *Achillea gypsicola* Hub-Mor. and *Achillea biebersteinii* Afan. (Asteraceae), *Ind. Crop. Prod.* **29**, 562–570
- [8] K.H. Rechinger (1982). *Nepeta*, In: Flora Iranica, No.150, Akademische Druck and Verlagsanstalt, Graz, Austria, pp. 108–216.
- [9] K. Javidnia, A.R. Mehdipour, B. Hemmateenejad, S.R. Rezazadeh, M. Soltani, A.R. Khosravi and R. Miri (2011) Nepetalactones as chemotaxonomic markers in the essential oils of *Nepeta* species, *Chem. Nat. Comp.* 47, 843–847.
- [10] A. Zargari (1990). Medicinal Plants. Vol. 4, Tehran University Publication, pp. 106–109.
- [11] G.H. Amin (1991). Popular Medicinal Plants of Iran. Iranian Research Institute of Medicinal Plants, Tehran, pp. 55.
- [12] B. Tepe, D. Daferera, A.S. Tepe, M. Polissiou and A. Sokmen (2007). Antioxidant activity of the essential oil and various extracts of *Nepeta flavida* Hub. Mor., *Food Chem.* **103**, 1358–1364.
- [13] S.E. Sajjadi (2005) Analysis of the essential oil of Nepeta sintenisii Bornm. from Iran, Daru. 13, 61-64.
- [14] N. Micelia, M.F. Taviano, D. Giuffrida, A. Trovato, O. Tzakouc and E.M. Galatia (2005). Anti-inflammatory activity of extract and fractions from *Nepeta sibthorpii* Bentham, *J. Ethnopharmacol.* **97**, 261–266.
- [15] Z. Aytac and G. Yildiz (1996). A new record for the Flora of Turkey, *Turk. J. Bot.* 4, 385–387.
- [16] T. Dirmenci (2005). A new subspecies of Nepeta (Lamiaceae) from Turkey, Bot. J. Linn. Soc. 147, 229-233
- [17] S. Mutlu and O. Atici (2009). Allelopathic effect of *Nepeta meyeri* Benth. extracts on seed germination and seedling growth of some crop plants, *Acta Physiol. Plant.* **31**, 89–93.
- [18] S. Mutlu, O. Atici and M. Esim (2010) Bioherbicidal effects of essential oil of *Nepeta meyeri* Benth. on weed spp., *Allelopathy J.* **26**, 291–299.

- [19] R.M. Inderjit Callaway (2003). Experimental designs for the study of allelopathy, *Plant Soil*. 256, 1–11.
- [20] R.P. Adams (2007). Identification of essential oil components by gas chromatography/mass spectrometry. Allured Publishing Co. Carol Stream, Illinois, USA.
- [21] N. Dudai, D. Chaimovitsh, O. Larkov, R. Fischer, Y. Blaicher and A. Mayer (2009). Allelochemicals released by leaf residues of *Micromeria fruticosa* in soils, their uptake and metabolism by inhibited wheat seed, *Plant Soil*. 314,311-317.
- [22] M.L. Jackson (1962). Soil Chemical Analysis. Prentice Hall Inc. Eng. Cliffs. N. I., USA.
- [23] F. Matloubi Moghadam and M. Hosseini (1996). Composition of the essential oil from *Nepeta crassifolia* Boiss.
   & Buhse, *Flavour Fragr. J.* 11, 113–115.
- [24] K. Javidnia, R. Miri, F. Safavi, A. Azarpira and A. Shafiee (2002). Composition of the essential oil of *Nepeta persica* Boiss. from Iran, *Flavour Fragr. J.* 17, 20–22.
- [25] F. Sefidkon and A. Akbari-nia (2003). Essential Oil Composition of Nepeta pogonosperma Jamzad et Assadi from Iran, J. Essent. Oil Res. 15, 327–328.
- [26] M. Dabiri and F. Sefidkon (2003a). Chemical composition of the essential oil of *Nepeta racemosa* Lam. from Iran, *Flavour Frag. J.* 18, 157–158.
- [27] M. Dabiri and F. Sefidkon (2003b). Chemical composition of *Nepeta crassifolia* Boiss. & Buhse. oil from-Iran, *Flavour Fragr. J.* **18**, 225–227.
- [28] F. Sefidkon and A. Shaabani (2004). Essential oil composition of *Nepeta meyeri* Benth. from Iran, *Flavour Frag. J.* **19**, 236–238.
- [29] A. Esmaeili, A. Rustaiyan, S. Masoudi and K. Nadji (2006). Composition of the essential oils of *Mentha aquatica* L. and *Nepeta meyeri* Benth. from Iran, *J. Essent. Oil Res.* **18**, 263–265.
- [30] Z.A. Mamedova, R.M. Mammadov and V.N. Gadzhiev (2011). Studying essential oil of *Nepeta meyeri* Benth. of flora in Azerbaijan, *Khimiya Rastitel'nugo. Syr'ya.* **4**, 135–138.
- [31] M.O. Karryev, M.V. Artemeva, A.A. Meshcheryakov and L.I. Rozhkova (1981). Content of biologicaly active compounds in useful plants of Turkmenia, *Izvestiya Akademii Nauk Turkmenskoi SSR*, Seriya Biologicheskikh Nauk. 4, 54–66.
- [32] G. Kekec, S. Mutlu, L. Alpsoy, M.S. Sakcali and O. Atici (2013). Genotoxic effects of catmint (*Nepeta meyeri* Benth.) essential oils on some weed and crop plants, *Toxicol. Ind. Health.* 29, 504–513.
- [33] Y. Cigremis, Z. Ulukanli, A. Ilcim and M. Akgoz (2010). In vitro antioxidant and antimicrobial assays of acetone extracts from *Nepeta meyeri* Bentham, *Eur. Rev. Med. Pharmaco. Sci.* 14, 661–668.
- [34] K.H.C. Baser, N. Kirimer, M. Kürkçüoğlu and B. Demirci (2000). Essential oils of *Nepeta* species growing in Turkey, *Chem. Nat. Comp.* 4, 356–359.
- [35] A. Adiguzel, H. Ozer, M. Sokmen, M. Gulluce, M. Sokmen, H. Kilic, F. Sahin and O. Baris (2009). Antimicrobial and antioxidant activity of the essential oil and methanol extract of *Nepeta cataria*, *Pol. J. Microbiol.* 58, 69–76.
- [37] J.J. Zhu, D.R. Berkebile, C.A. Dunlop, A. Zhang, D. Boxler, K. Tangtrakulwanich, R.W. Behle, F. Baxendale and G. Brewer (2012). Nepetalactones from essential oil of *Nepeta cataria* represent a stable fly feding and oviposition repellent, *Med. Vet. Entomol.* **26**, 131–138.
- [38] T. Tsuruoka, D. Bekh-Ochir, F. Kato, S. Sanduin, A. Shataryn, A. Ayurzana, T. Satou, W. Li and K. Koike (2012). The essential oil of Mongolian *Nepeta sibirica*: a single component and its biological activities. J. *Essent. Oil Res.* 24, 555–559.
- [39] M.A. Birkett, A. Hassanali, S. Hoglund, J. Pettersson and J.A. Pickett (2011). Repellent activity of catmint, *Nepeta cataria*, and iridoid nepetalactone isomers against Afro-tropical mosquitoes, ixodid ticks and red poultry mites, *Phytochemistry*. 72, 109–114.
- [40] G. Ghikines, B. Bozin, N. Mimica-Dukic and O. Tzakou (2010). Antioxidant activity of *Nepeta nuda* L. ssp. *nuda* essential oil rich in nepetalactones from Greece, *J. Med. Food.* **13**, 1176–1181.
- [41] M.A. Birkett, T.J.A. Bruce and J.A. Pickett (2010). Repellent activity of *Nepeta grandiflora* and *Nepeta clarkei* (Lamiaceae) against the cereal aphid, *Sitobion avenae* (Homoptera: Aphididae), *Phytochem. Lett.* **3**, 139–142.
- [42] G. Ghikinis, O. Tzakou, D. Iliopoulou and V. Roussis (2003). Chemical composition and biological activity of *Nepeta parnassica* oils and isolated nepetalactones, Z. *Naturforsch. C* 58, 681–686.
- [43] H.D. Skaltsa, D.M. Lazari, A.E. Loukis and T. Constantinidis (2000). Essential oil analysis of *Nepeta argolica* Bory & Chaub. subsp. *argolica* (Lamiaceae) growing wild in Greece, *Flavour Frag. J.* 15, 96–99.
- [44] A. Rustaiyan and K. Nadji (1999). Composition of the essential oil of *Nepeta ispahanica* Boiss. and *Nepeta binaludensis* Jamzad from Iran, *Flavour Frag. J.* **14**, 35–37.
- [45] U. Suschke, F. Sporer, J. Schneele, H.K. Geiss and J.Reichling (2007). Antibacterial and cytotoxic activity of Nepeta cataria L., N. cataria var. citriodora (Beck.) Balb and Melissa officinalis L. essential oils, Nat. Prod. Commun. 2, 1277–1286.
- [46] E. Kalpoutzakis, N. Aligiannis, A. Mentis, S. Mitaku and C. Charvala (2001). Composition of the essential oils of two *Nepeta* species and in vitro evaluation of their activity against *Helicobacter pylori*, *Planta Med.* 67, 880– 883.

- [47] F.M. Moghaddam and M. Hosseini (1996). Composition of the essential oil from *Nepeta crassifolia* Boiss. & Buhse, *Flavour Frag. J.* **11**, 113–115.
- [48] F. Sefidkon (2001). Essential oil composition of *Nepeta glomerulosa* Boiss from Iran, *J. Essent. Oil Res.* 13, 422–423.
- [49] F. Sefidkon, M. Dabiri and A. Alamshahi (2001). Analysis of the essential oil of Nepeta fissa C. A. May from Iran, Flavour Frag. J. 17, 89–90.
- [50] E. Hanlidou, R. Karousou and D. Lazari (2012). Essential oils of three taxa of the *Nepeta argolica* Aggregate from Greece, Chem. Biodivers. **9**, 1559–1566.
- [51] S.E. Sajjadi, N. Ghassemi and M. Marini (2012). Chemical constituents of the essential oil of *Nepeta prostrata* from Iran, *J. Essent. Oil Bear. Pl.* **15**, 557–560.
- [52] F. Senatore and M. Ozcan (2003). Composition of the essential oil of Nepeta betonicifolia CA Meyer (Lamiaceae) from Turkey, J. Essent. Oil Res. 15, 200–201.
- [53] G. Kokdil, S. Kurucu and A. Yildiz (1998). Essential oil composition of *Nepeta nuda* L. ssp. *nuda*, *Flavour Frag. J.* **13**, 233–234.
- [54] S. Mutlu, O. Atici, N. Esim and E. Mete (2011). Essential oils of catmint (*Nepeta meyeri* Benth.) induce oxidative stres in early seedlings of various weed species, *Acta Physiol. Plant.* **33**, 943–951.
- [55] E. Mancini, N.A.Arnold, V. De Feo, C. Farmisano, D. rigano, F. Piozzi and F. Senatore (2009). Phytotoxic effects of essential oils of *Nepeta curviflora* Boiss. and *Nepeta nuda* L. subsp *albiflora* growing wild in Lebanon, *J. Plant Interact.* 4, 253–259.
- [56] M. Kobaisy, M.R. tellez, F.E. dayan, L.K. Mamonov, G.S. Mukanova, G.T. Sitpaeva and N.G. Gemejieva (2005). Composition and phytotoxic activity of *Nepeta pannonica* L. essential oil, *J. Essent. Oil Res.* 17,704– 707.
- [57] R. Koitabashi, T. Suzuki, T. Kawazu, A. Sakai, H. Kuroiwa and T. Kuroiwa (1997). 1, 8-Cineole inhibits root growth and DNA synthesis in the root apical meristem of *Brassica campestris* L., *J. Plant Res.* **110**, 1–6.
- [58] M.P. Zunino and J.A. Zygadlo (2004). Effect of monoterpenes on lipid oxidation in maize, *Planta*. 219, 303–309.
- [59] N. Nishida, S. Tamotsu, N. Nagata, C. Saito and A. Sakai (2005). Allelopathic effects of volatile monoterpenoids produced by *Salvia leucophylla*: inhibition of cell proliferation and DNA synthesis in the root apical meristem of *Brassica campestris* seedlings, *J. Chem. Ecol.* **31**, 1187–1203.



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