

Phytotoxic effects of essential oils from *Nepeta glocephalata* Rech.f. and *N. ispanhanica* Boiss. on selected weed species

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Phytotoxic effects of essential oils from *Nepeta glocephalata* Rech.f. and *N. ispanhanica* Boiss. on selected weed species

Abstract: In the present study the bioherbicidal activity of essential oils hydrodistilled from *Nepeta glocephalata* Rech.f. and *N. ispanhanica* Boiss. were investigated on four weed species (barnyard grass (*Echinochloa crus-galli* (L.) Beauv), redroot pigweed (*Amaranthus retroflexus* L.), lambsquarters (*Chenopodium album* L.) and canary grass (*Phalaris canariensis* L.)). A total of 37 components were identified from the essential oils of *N. glocephalata* and *N. ispanhanica* constituting approximately 98.61 % and 96.1 % of the oils, respectively. In laboratory bioassay different concentrations (0, 1, 2, 4 and 8 $\mu\text{l ml}^{-1}$) of two *Nepeta* essential oils on germination, root and shoot length were studied. Results showed by increasing the concentration of oils, all studied traits of the weeds were decreased compared with control. In a glass house bioassay post-emergence application of *Nepeta* essential oils (1.25 %, 2.5 %, 5 % and 10 %, v/v) on 3-week-old weed plants caused visible injury (7-days after spray) ranging from chlorosis to necrosis of plant weeds. In foliar application under glasshouse conditions, both *Nepeta* essential oils reduced the seedling dry mass and concentrations of chlorophyll a chlorophyll b. The study concludes that *Nepeta* essential oils have phytotoxic effects and could be used as bioherbicides but the selectivity of these compounds should be considered also.

Key words: *Nepeta glocephalata* Rech.f.; *N. ispanhanica* Boiss.; bioherbicide; 1, 8-cineole; chlorophyll a; weed seed germination; root length

Fitotoksični učinki eteričnih olj iz dveh vrst mačje mete (*Nepeta glocephalata* Rech.f. in *N. ispanhanica* Boiss.) na izbrane vrste plevelov

Izvleček: V raziskavi je bila preučevana bioherbicidna aktivnost vodnih destilatov eteričnih olj iz dveh vrst mačje mete (*Nepeta glocephalata* Rech.f. in *N. ispanhanica* Boiss.) na štiri plevelne vrste (navadna kostreba (*Echinochloa crus-galli* (L.) Beauv), navadni (srhkodlakavi) ščir (*Amaranthus retroflexus* L.), bela metlika (*Chenopodium album* L.) in kanarska čužka (*Phalaris canariensis* L.)). Celokupno je bilo v eteričnih oljih obeh vrstah določenih 37 sestavin, ki so predstavljale 98,61 % oziroma 96,1 % olja. V laboratorijskem poskusu so bili preučevani učinki različnih koncentracij (0, 1, 2, 4 in 8 $\mu\text{l ml}^{-1}$) eteričnih olj iz obeh vrst mačje mete na kalitev, dolžino korenin in poganjkov izbranih plevelov. Rezultati so pokazali, da so se vrednosti vseh merjenih parametrov plevelov zmanjševale s povečevanjem koncentracije eteričnih olj. V poskusu v rastlinjaku so bile preučevane vidne poškodbe uporabe eteričnih olj iz obeh vrst mačje mete (1,25 %, 2,5 %, 5 % and 10 %, v/v) na tri tedne starih sejankah plevelov, sedem dni po škropljenju z eteričnimi olji, ki so se pojavile kot kloroze in nekroze. Pri foliarni uporabi eteričnih olj obeh vrst mačje mete v rastlinjaku se je zmanjšala suha masa sejank plevelov, zmanjšale so se tudi vsebnosti klorofila a in b. Na osnovi raziskave lahko zaključimo, da imajo eterična olja obeh vrst mačje mete fitotoksične učinke in bi lahko bile uporabljene kot bioherbicidi vendar je pri tem potrebno upoštevati selektivne učinke njihovih sestavin.

Ključne besede: *Nepeta glocephalata* Rech. f.; *N. ispanhanica* Boiss.; bioherbicid; 1, 8-cineol; klorofil a; kalitev semen plevelov; dolžina korenin

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1 INTRODUCTION

Herbicide-resistant weeds and environmental concerns have led researchers to consider using alternative ways to manage weeds (Vyvyan, 2002; Ashraf et al., 2017). Allelopathy is one of these ways (Weston, 1996). Allelopathic compounds can reduce the use of synthetic herbicides and thus reduce environmental pollution and lead to more safe crops (Singh et al., 2002, 2003, 2005a, b). Among the natural plant products, essential oils constitute an important group of that provide a characteristic odor to the aromatic plants (Singh et al., 2002). Earlier studies have documented that essential oils and their constituents inhibited seed germination and retard plant growth (Barney et al., 2005; Batish et al., 2006; Ens et al., 2009). The allelopathic activities of some essential oils and their monoterpenes on seeds germination or seedling growth at several species have been shown in previous studies (Dudai et al., 1999; Abraham et al., 2000; Tworkoski, 2002; Singh et al., 2004; Dudai et al., 2004; Armirante et al., 2006; Kordali et al., 2006; Kordali et al., 2007). Allelopathic properties of essential oils from different aromatic plants belonging to Lamiaceae, Compositae, Myrtaceae, Cupressaceae, Rutaceae and Verbenaceae families have been reported (Dudai et al., 1999; Angelini et al., 2003; Kaur et al., 2010; Amri et al., 2013; Verdeguer et al., 2011). Also allelopathic potential of the essential oil of many plants from family Lamiaceae such as *Salvia apiana* Jeps. and *Salvia leucophylla* Greene (Muller et al., 1964), *Satureja hortensis* L. and *Thymus vulgaris* L. (Tworkoski 2002), *Rosmarinus officinalis* L., *Satureja montana* L. (Angelini et al., 2003), *Lavandula* spp. and peppermint (*Mentha × piperita* ‘Mitcham’) (Campiglia et al., 2007; Mahdavi and Saharkhiz, 2015), *Zataria multiflora* Boiss and its different chemotypes (Saharkhiz et al., 2010), *Satureja khuzestanica* Jamzad, *Satureja bachtiarica* Bunge, *Satureja rechingeri* Jamzad and *Satureja spicigera* (K.Koch) Boiss. (Taban et al., 2013) have been previously reported.

Genus *Nepeta* is one of the largest genera of the Lamiaceae family that comprises about 300 herbaceous perennial and annual species (Formisano et al., 2011). The greatest diversity and richness of species is found in Southwestern Asia, (especially Iran and Turkey), and the Western Himalayas. There are seventy-nine species of *Nepeta* in Iran and about 39 of them are endemics (Jamzad, 2012). Much research was done on diversity, species richness and chemical properties of *Nepeta* species. Most *Nepeta* species are rich in essential oils. Diverse biological activities of *Nepeta* oil such as feline attractant, canine attractant, insect repellent, arthropod defense (Tucker and Tucker, 1988, Wagner and Wolf, 1977), antibacterial, antifungal and antiviral activities (Tucker and Tucker,

1988) have been reported previously. There are several reports on the chemical composition of the essential oils of the genus *Nepeta* found in Iran (Sefidkon, 2004, 2005; Sajjadi, 2005; Sonboli et al., 2005; Jamzad, 2012). Allelopathic potential of this genus was revealed. Phytotoxicity of *Nepeta* essential oils has been mainly tested (Kobaisy et al., 2005, Eom et al., 2006, Mancini et al., 2009, Mutlu et al., 2011, Kekec et al., 2012, Bozari et al., 2013, Živković, 2013). Allelopathy of water extracts has been studied by Mutlu and Atici (2009) and Babaahmadi et al. (2013). No bioassays or field experiments had been done to study the allelopathic potential of *Nepeta glocephalata* Rech.f and *N.ispahanica* Boiss., Endemic plants of Iran.

The aim of the present study was to study the essential oil composition of *N. glocephalata* and *N. ispahanica* in order to know if these compositions have phytotoxic effects on germination, seedling growth injury and photosynthesis of barnyard grass (*Echinochloa crus-galli* (L.) Beauv), a most important weed in rice (*Oryza sativa* L.), redroot pigweed (*Amaranthus retroflexus* L.) and lambsquarters (*Chenopodium album* L.), annual plants seriously influencing summer crops and canary grass (*Phalaris canariensis* L.), serious weed of wheat (*Triticum aestivum* L.) fields in Iran.

2 MATERIALS AND METHODS

2.1 PLANT MATERIAL

Above ground parts (leaves and flowers/inflorescences) of *N. glocephalata* rech.f. were collected from natural sites of Kashan, Esfahan Province, at an altitude of 1600 m and the above ground parts of *N. ispahanica* Boiss. were collected from north-west of Tehran, at an altitude of 1800 m during the flowering period in July 2015 in Iran. The air-dried of the plant were powdered and hydrodistilled in a Clevenger-type apparatus for 3 h. The essential oils were dried over anhydrous sodium sulphate and stored at 3 °C in a dark before analysis.

2.2 GC AND GC/MS ANALYSES

The oils were analyzed by GC and GC/MS. The GC analyses were performed using a Perkin-Elmer (UK) 8500 gas chromatograph equipped with Flame Ionization Detector (FID) and a DB-5 fused silica column (30 m × 0.25 mm, film thickness 0.25 µm). Oven temperature was held at 60 °C for 3 min and programmed to 275 °C at a rate of 3 °C/min; injector temperature (split: 1: 25) 250 °C; detector temperature, 280 °C; carrier gas, N₂ at 12 psi. Varian 3700 chromatography equipped with a CP-

Sil5CB column (25 m×0.25 mm i.d., film thickness 0.39 µm) combined with a Varian MAT 44S, ionization energy 70eV. The carrier gas was He and injector temperature was 270 °C. Approximately, 0.1 µl of neat oil was injected under split condition (100:1) and the oven temperature was held at 60 °C for 5 min., programmed at 5 °C min⁻¹. to 220 °C and then holds at this temperature for 20 min.

2.3 IDENTIFICATION OF COMPONENTS

The compounds in the oil were identified by comparison of their retention indices (RI, HP-5) with those reported in the literature as well as by comparing their mass spectra with the Wiley GC-MS Library, Adams Library, Mass Finder 2.1 Library data, and published mass spectra data (McLafferty and Stauffer, 1989; Adams, 2007).

2.4 GERMINATION AND SEEDLING GROWTH BIOASSAY

Seeds of two monocotyledon weeds (barnyard grass (*Echinochloa crus-galli* (L.) Beauv) and canary grass (*Phalaris canariensis* L.) and two dicotyledon weeds (redroot pigweed (*Amaranthus retroflexus* L.) and lamb-quarters (*Chenopodium album* L.)) were collected from weeds growing in the summer crops. The germination tests were done in petri dishes (9 cm dia) in a germination chamber at 30 °C (day) and 20 °C (night) for barnyard grass, canary grass and redroot pigweed and at 20 °C and 10 °C for canary grass, respectively. For each essential oil, an oil-in-water emulsion was prepared at 1, 2, 4 and 8 µg ml⁻¹ concentrations. Distilled water used as control. Each Petri dish contained 25 weed seeds placed on two layers of filter paper (Whatman® No.5) wetted with 6 ml oil-in-water emulsion. To prevent evaporation, petri dishes were sealed with parafilm (16). After 14 days, all germinated seeds were counted. Seeds showing root emergence (2 mm) were recorded as germinated. After 14 days no seed germinations were observed. The germination percentages were determined. Root and shoot length were measured by scientific ruler.

2.5 GLASS HOUSE STUDIES

In another experiments, the effects of *Nepeta* species oils on 3-week-old weed plants raised under controlled conditions in experimental glass house were studied. Plants of the four weed species were raised from the seeds in plastic pots 12-cm in diameter. Pots were filled with

730 g garden soil (soil: sand: manure: 3:1:1, w/w) and ten seeds of each weed species were sown per pot. Pots were thinned to 5 equal-sized healthy plants per pot at one-week after sowing. Plants were watered every other day. Studied treatments in this experiment were 1.25, 2.5, 5 and 10 % (v/v) solution of essential oil or distilled water (control) at 3-week-old plants. A hand pressure sprayer filled with flooding nozzle was used for spraying at a rate of 400 l ha⁻¹. The weed plants were examined for visible injury levels in terms of percent chlorotic and necrotic areas at 7-days after spray (DAS). Fresh leaves of all weed species (100 mg fresh leaf samples) were homogenized in 80 % aqueous acetone (5 ml). The homogenate was filtered through Whatman filter paper no. 1. The final volume was adjusted to 5 ml by acetone (80 %). Chlorophyll a and chlorophyll b contents were determined spectrophotometrically using Unico 1200-Spectrophotometer at 663 nm for chlorophyll a and 647 nm for chlorophyll b. Calculations were completed using Lichtenthaler's equation (Lichtenthaler, 1987) and expressed as mg g⁻¹ dry mass. Also dry mass of plant were measured after were oven-drying at 750 °C for 48 h.

2.6 STATISTICAL ANALYSIS

All the experiments were repeated and the presented data are average of the two experiments. The experimental design used for both experiments was completely randomized in a 5 × 2 factorial scheme (5 concentrations × 2 *Nepeta* essential oils), with four replications. ANOVA was used to test for significant differences between the means of each *Nepeta* species and each essential oil concentration. For all statistical analysis, the SAS ver 9.1 program was used. The means were compared by Tukey's HSD post hoc test ($p < 0.05$).

3 RESULTS

3.1 CHEMICAL COMPOSITIONS OF THE EXAMINED ESSENTIAL

The chemical compositions of the two *Nepeta* essential oils compounds were listed in Table 1. Total of 35 compounds were identified in *N. ispanhanica* and *N. glocephalata* essential oils by GC/MS analysis. Eighteen components were identified, representing more than 96.1 % of the total oil components of *N. ispanhanica* essential oil detected. The major components of *N. ispanhanica* oil were 1,8-cineole (66.4 %), β-pinene (10.7 %) and α-pinene (3.1 %). Twenty-nine compounds reached 98.6 % of the total *N. glocephalata* es-

sential oils. The main components of *N. glocephalata* oil were 1,8-cineole (34.1 %), β -pinene (21.5 %), α -pinene (8.1 %) sabinene (7.8 %), (Z)- β -ocimene (7.6 %)

and (E)- β -ocimene (6.9 %). Other components were present in amounts less than 3 %.

Table 1: Percentage composition of the essential oils of *Nepeta* species

No	Compound	IR	%	
			<i>N. ispanhanica</i>	<i>N. glocephalata</i>
1	α -Thujene	935	-	0.8
2	α -Pinene	940	3.1	8.1
3	Camphene	954	-	0.2
4	Sabinene	981	1.9	6.6
5	β -Pinene	986	10.7	21.5
6	Myrcene	998	-	1.7
7	δ -3-Carene	1011	-	0.5
8	α -Terpinene	1024	-	0.2
9	<i>p</i> -Cymene	1034	-	0.8
10	1,8-Cineole	1041	66.4	34.1
11	(Z)- β -Ocimene	1046	-	7.1
12	(E)- β -Ocimene	1056	-	6.5
13	γ -Terpinene	1066	-	0.3
14	<i>trans</i> -Sabinene-hydrate	1075	0.4	0.8
15	<i>cis</i> -Sabinene hydrate	1088	0.4	-
16	Tepinolene	1095	-	0.3
17	Linalool	1107	-	0.4
18	<i>trans</i> -Pinocarveole	1129	1.1	-
19	<i>cis</i> - <i>p</i> -menth-2-en-1-ol	1131	-	0.2
20	Verbenol	1134	0.6	-
21	Allo-ocimene	1137	-	0.2
22	<i>trans</i> -Sabinole	1149	-	0.5
23	Pinovarvone	1172	0.9	0.2
24	Myrtenal	1175	1.0	-
25	δ -Terpineole	1177	1.1	0.5
26	Myrtenol	1184	1.0	-
27	Terpinen-4-ol	1187	1.0	1.8
28	Cryptone	1196	-	0.2
29	α -Terpineole	1200	2.0	2.9
30	Myrthanol	1207	-	0.5
31	4 α ,7 α ,7 α -Nepetalactone	1422	0.1	-
32	β -caryophyllene	1434	0.2	0.1
33	Germacrene D	1496	-	1.2
34	Bicyclogermacrene	1512	-	0.3
35	4 α β ,7 α ,7 α -Nepetalactone	1575	2.1	-
36	β -caryophyllene oxide	1585	2.1	-
37	Spathulenole	1595	-	0.1
Total		-	96.1	98.6

Retention Indices (The retention indices were determined on CPSil5CB column)

3.2 GERMINATION AND SEEDLING GROWTH BIOASSAY

The effect of *Nepeta* species essential oils against seed germination, root length and shoot length of barnyard grass, canary grass, redroot pigweed and lambsquarters is shown in Figs. 1–3. Significant differences were found among control and all concentrations of *Nepeta* species essential oil tested. The essential oils of two *Nepeta* species reduced the germination of all studied weeds. Furthermore the difference between the control and the lowest concentration was significant for all weed species. At $1 \mu\text{g ml}^{-1}$ of *N. ispanica* germination reduction compare to control was 25 %, 11 % and 44 % and 49 % for barnyard grass, canary grass, redroot pigweed and lambsquarters, respectively. Also germination reduction of barnyard grass, canary grass, redroot pigweed and lambsquarters was 21.5 %, 8 % and 29 % and 44.75 % at $1 \mu\text{g ml}^{-1}$ of *N. glocephalata*, respectively (Fig. 1). Redroot pigweed and lambsquarters were most sensitive to *N. ispanica* essential oil, their germination was completely inhibited by it at concentration $4 \mu\text{g ml}^{-1}$. At highest concentration $8 \mu\text{g ml}^{-1}$ germination percentages were 6.5 % and 10.20 % for canary grass by *N. ispanica* and *N. glocephalata*, respectively. Barnyard grass seeds germinated 3 % and 11.75 % at highest concentration of *N. ispanica*

and *N. glocephalata* essential oils, respectively. The regression lines between seed germination and essential oil concentrations confirm the different susceptibility of weed species (Fig. 1). There were significant differences among control and all concentrations, and between two *Nepeta* species essential oils for each weed species. The regression analysis between oil concentrations and root length showed that increasing concentration of essential oil increased the inhibitory effects on weed root length till a lethal dose (Fig. 2). When the root length of redroot pigweed and lambsquarters was completely inhibited by essential oils of *N. ispanica* at $4 \mu\text{g ml}^{-1}$, the root length was reduced respectively to 70 % for barnyard grass and to 73 % for canary grass, which explain that monocots weeds were more resistant than dicots. No significant differences among control and all concentrations, and between two *Nepeta* essential oils observed for shoot length of barnyard grass and canary grass. The shoot reduction of barnyard grass compared with control was the 29 % and 28 % with *N. glocephalata* and *N. ispanica* essential oils at the highest concentration, respectively. Canary grass showed a reduction of the 28 % with *N. glocephalata* and 25 % with *N. ispanica* essential oils at the same concentration. Shoot length of redroot pigweed and lambsquarters reached to zero at 4 and $8 \mu\text{g ml}^{-1}$ of *N. ispanica* at $8 \mu\text{g ml}^{-1}$ of *N. glocephalata*.

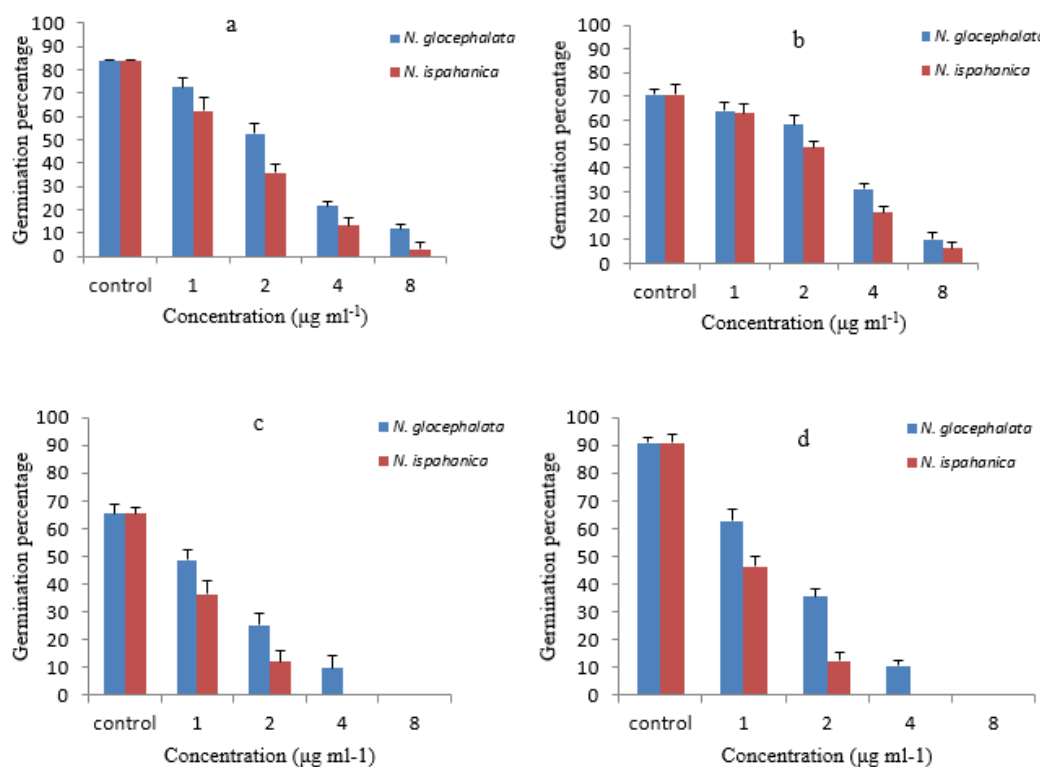


Figure 1: Effect of *N. ispanica* and *N. glocephalata* essential oils on (a) barnyard grass, canary grass (b) redroot pigweed (c) and lambsquarters (d) germination measured after 2 weeks. Vertical bars along each data point represent the standard error

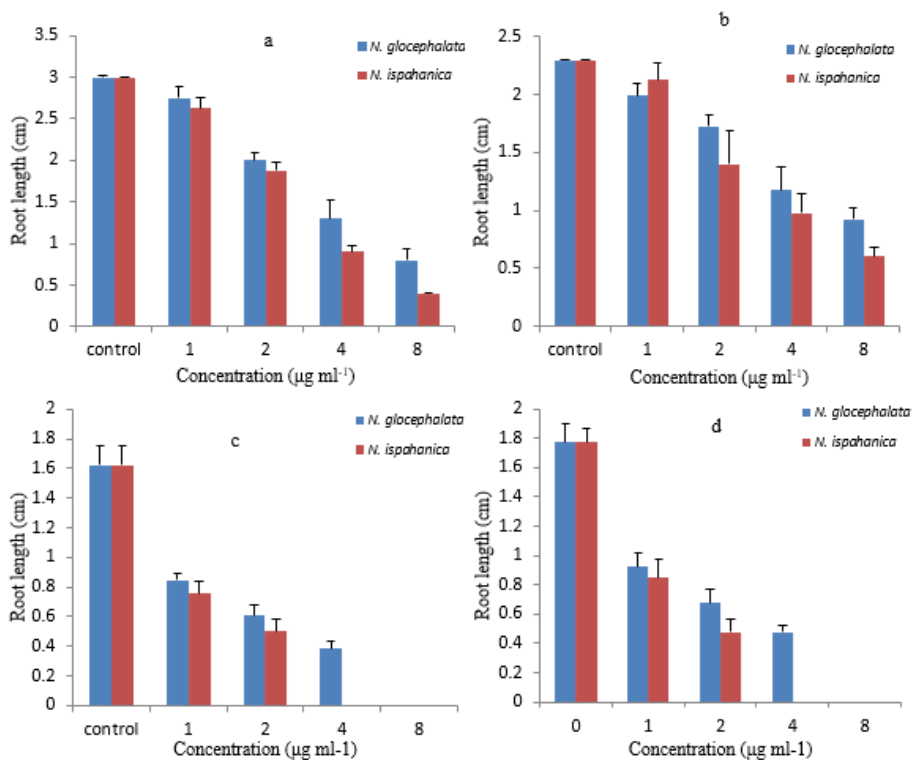


Figure 2: Effect of *N. ispahantica* and *N. glocephalata* essential oils on (a) barnyard grass, canary grass (b) redroot pigweed (c) and lambsquarters (d) root length measured after 2 weeks. Vertical bars along each data point represent the standard error

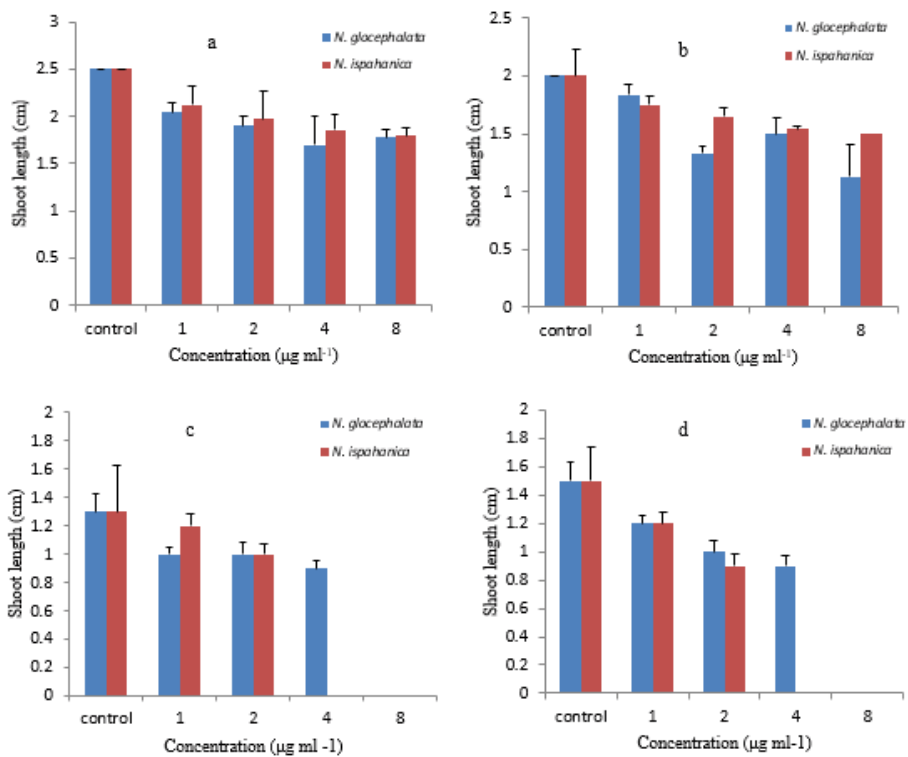


Figure 3: Effect of *N. ispahantica* and *N. glocephalata* essential oils on (a) barnyard grass, canary grass (b) redroot pigweed (c) and lambsquarters (d) shoot length measured after 2 weeks. Vertical bars along each data point represent the standard error

3.3 GLASS HOUSE STUDIES

For more investigation of herbicidal activity of *Nepeta* essential oils, an experiment was done on 3-week-old weeds. The mature plants of test weeds were damaged upon spray of *Nepeta* essential oils and showed visible injury ranging from chlorosis to necrosis of plants. In general, the visible injury symptoms observed 7 days after spraying increased with increasing concentrations of both *Nepeta* essential oils (Table 2). At the lowest concentration 1.25 % of both *Nepeta* essential oils, all the test weeds showed sign of injury. At the highest concentration (10 v/v) visible injury by *N. ispahanica* essential oil were 44 % 45.5 % 59.25 % and 51.62 % for barnyard grass, canary grass, redroot pigweed and lambsquarters, respectively. While visible injury of barnyard grass, canary grass, redroot pigweed and lambsquarters caused by *N. glocephalata* essential oil were 39 % 36.62 % 44 % and 41.5 %, respectively at 7 days after spraying that did not have significant difference each other's (Table 2).

Increasing *Nepeta* essential oils concentration decreased dry mass of all weed species. The inhibition rates of barnyard grass dry mass ranged from 24.89 % to 75.21 %, and from 16.75 to 61.17 % in *N. ispahanica* and *N. glocephalata*, respectively. In canary grass the inhibition rates of dry mass, ranged from 22.49 to 63.26 %, and from 13.95 to 56.56 % at concentrations for *N. ispahanica* and *N. glocephalata*, respectively. Essential oil of *N. ispahanica* caused dry mass inhibition of redroot pigweed from 37.5 % to 90 % while *N. glocephalata* reduced it from 23.75 % to 81.5 %. The inhibition rates of lambsquarters dry mass ranged from 19.75 % to 86 %, and from 14.75 to 76 % in *N. ispahanica* and *N. glocephalata*, respectively. There was a significant difference in the inhibition of dry mass among concentrations and the the highest inhibition of dry mass caused by *N. ispahanica* at concentration of 10 v/v that was significantly different from other treatments.

Increasing essential oil concentration decreased chlorophyll a and chlorophyll b at all studied weeds. For example contents of chlorophyll a in barnyard grass were reduced 12.77 %, 25.40 %, 44.38 % and 49.62 % by *N. ispahanica* at concentrations of 1.25 %, 2.5 %, 5 % and 10 % v/v, respectively. *N. glocephalata* concentrations of 1.25 %, 2.5 %, 5 % and 10 % inhibited contents chlorophyll a in barnyard grass, 11.65 %, 15.56 %, 29.1 % and 43.56 %, respectively that the difference between the concentrations of 1.25 v/v and 2.5 v/v was not significant. The highest inhibition of chlorophyll a in canary grass was caused by *N. ispahanica* at concentration of 10 v/v which was not significantly different from *N. glocephalata*. In redroot pigweed, *N. ispahanica* at the concentration of 1.25 v/v decreased chlorophyll a by 22.05 % and

decreased further by increasing concentration. In lambsquarters, no significant difference was observed between *N. ispahanica* and *N. glocephalata* at a concentration of 2.5 v/v (Table 4).

N. ispahanica essential oil inhibited chlorophyll b of barnyard grass by 11.30 %, 21.59 %, 29.13 % and 36.79 % at concentrations of 1.25 %, 2.5 %, 5 % and 10 %, respectively. At concentrations of 1.25 %, 2.5 %, 5 % and 10 % *N. glocephalata* essential oil reduced chlorophyll b by 7.86 %, 12.09 %, 22.21 % and 33.35 %, respectively in barnyard grass. The highest of inhibition of chlorophyll b in canary grass and redroot pigweed was caused by *N. ispahanica* at a concentration of 10 v/v which was not significantly different from *N. glocephalata* in redroot pigweed. In lambsquarters, there was no significant difference between *N. ispahanica* and *N. glocephalata* at a concentration of 1.25 v/v, but at the highest concentration, a significant difference was observed between these two species (Table 5). In this studies chlorophyll a decreased more than chlorophyll b in all species weeds (Tables 4 and 5).

4 DISCUSSION

Many researchers reported the presence of nepetalactones in several *Nepeta* species in relatively high concentrations (Sefidkon and Shaabani, 2004; Rustaiyan et al., 2000, Rustaiyan and Nadji, 1999, Sajjadi and Khatamsaz, 2000) but no nepetalactones were detected in *N. glocephalata* essential oil. 1, 8-cineole, which was the first major component of the studied oils, has been reported in the oil of some *Nepeta* species from Iran (Rustaiyan et al., 2000; Rustaiyan and Nadji, 1999; Sajjadi and Khatamsaz, 2000). 1, 8-Cineole was also reported previously to be the main compound of *N. ispahanica* oil (Sefidkon et al., 2005). β -pinene has also been found in the oils of some *Nepeta* species (Thappa et al., 2001; Baser et al., 2000; Rustaiyan et al., 2000; Rustaiyan and Nadji, 1999; Sefidkon et al., 2002) but the concentrations of it found in this study was the most in comparison with previous studies. β -pinene and α -pinene are typical in most *Nepeta* species (Gkinis et al., 2003; Thappa et al., 2001, Baser et al., 2000; Rustaiyan and Nadji, 1999; Sefidkon et al., 2002).

The herbicidal activity of both *Nepeta* essential oils were due to the high percentage of 1,8-Cineole. This is in agreement with Zunino and Zygadlo (2004) who reported that monoterpenes such as 1,8-cineole, thymol, geraniol and camphor have been reported to inhibit root growth in maize (*Zea mays* L.). In a study with 27 monoterpenes, against seed germination and primary root growth of radish (*Raphanus sativus* L.) and garden cress (*Lepidium sativum* L.), only 1, 8-cineole, inhibited

Table 2: Effects of *Nepeta* essential oils on visible injury of barnyard grass, canary grass, redroot pigweed and lambsquarters at 7 days after spraying

Concentration (v/v)	Barnyard grass		Canary grass		Redroot pigweed		Lambsquarters	
	<i>N. ispahantica</i>	<i>N. glocephalata</i>	<i>N. ispahantica</i>	<i>N. glocephalata</i>	<i>N. ispahantica</i>	<i>N. glocephalata</i>	<i>N. ispahantica</i>	<i>N. glocephalata</i>
1.25	19 ± 1.63 d	13.75 ± 1.89 e	17.87 ± 2.01 d	10.97 ± 0.41 e	21.5 ± 3.76 e	11.125 ± 1.10 f	20.25 ± 2.28 d	12.43 ± 0.47 e
2.5	32 ± 2.16 c	19.5 ± 0.57 d	28.01 ± 1.31 c	20.01 ± 1.07 d	31.5 ± 1.08 cd	25.875 ± 2.78 de	31.75 ± 1.48 c	22.68 ± 1.21 d
5	40.75 ± 0.9 ab	29.25 ± 0.95 c	37.28 ± 0.4 b	29.01 ± 0.81 c	43.75 ± 1.04 b	36.5 ± 1.22 c	42.25 ± 0.45 b	32.87 ± 0.93 c
10	44 ± 3.36 a	39 ± 1.82 b	45.55 ± 2.65 a	36.6 ± 2.47 b	59.25 ± 6.7 a	44 ± 2.61 b	51.62 ± 3.01 a	41.5 ± 0.54 b

Values are means ± standard error of four replicates. Within each species, different letters indicate that means are different at the 95 % level of probability (Tukey's HSD post hoc test)s

Table 3: Effects of *Nepeta* essential oils on dry weights inhibition % of barnyard grass, canary grass, redroot pigweed and lambsquarters at 7 days after spraying

Concentration (v/v)	Barnyard grass		Canary grass		Redroot pigweed		Lambsquarters	
	<i>N. ispahantica</i>	<i>N. glocephalata</i>	<i>N. ispahantica</i>	<i>N. glocephalata</i>	<i>N. ispahantica</i>	<i>N. glocephalata</i>	<i>N. ispahantica</i>	<i>N. glocephalata</i>
1.25	24.89 ± 1.67 g	16.74 ± 0.56 h	22.49 ± 4.53 f	13.95 ± 2.18 g	37.5 ± 1.73 f	23.75 ± 2.21 g	19.75 ± 2.5 f	14.75 ± 2.21 f
2.5	39.55 ± 1.84 e	28.58 ± 0.96 f	32.55 ± 2.23 d	22.15 ± 0.80 e	53 ± 2.16 e	39.25 ± 0.95 f	41 ± 2.58 d	29.5 ± 2.64 e
5	69.13 ± 1.70 c	51.74 ± 1.84 d	52.60 ± 7.06 b	43.95 ± 3.30 c	77.5 ± 2.08 c	63 ± 2.44 d	61.5 ± 3 c	59 ± 4.6 c
10	75.21 ± 0.61 a	67.17 ± 0.24 b	63.26 ± 4.52 a	56.56 ± 2.8 ab	90 ± 0.1 a	81.5 ± 1.29 b	86 ± 1.41 a	76 ± 1.82 b

Values are means ± standard error of four replicates. Within each species, different letters indicate that means are different at the 95 % level of probability (Tukey's HSD post hoc test)

Table 4: Effects of *Nepeta* essential oils on Chlorophyll a inhibition% of barnyard grass, canary grass, redroot pigweed and lambsquarters at 7 days after spraying

Concentration (v/v)	Barnyard grass		Canary grass		Redroot pigweed		Lambsquarters	
	<i>N. ispahantica</i>	<i>N. glocephalata</i>	<i>N. ispahantica</i>	<i>N. glocephalata</i>	<i>N. ispahantica</i>	<i>N. glocephalata</i>	<i>N. ispahantica</i>	<i>N. glocephalata</i>
1.25	12.77 ± 6.79c	11.65 ± 0.55c	13.80 ± 3g	8.71 ± 1.36g	22.05 ± 1.02d	14.47 ± 1.45f	20.27 ± 5.85cd	11.65 ± 0.51e
2.5	25.40 ± 1.31b	15.56 ± 3.57c	31.59 ± 5.92ef	19.09 ± 2.43e	37.80 ± 1.97d	26.47 ± 5.13e	32.90 ± 5.74 c	27.56 ± 0.69 c
5	44.38 ± 5.0a	29.1 ± 5.74b	39.13 ± 4.41bc	32.21 ± 1.33cd	52.47 ± 2.17b	44.69 ± 3.78c	54.38 ± 5.72 a	41.6 ± 4.53 b
10	49.62 ± 2.5a	43.56 ± 2.43a	46.79 ± 3.57a	41.60 ± 1.77ab	59.32 ± 1.0a	53.07 ± 1.85b	59.62 ± 2.54 a	54.81 ± 0.56 a

Values are means ± standard error of four replicates. Within each species, different letters indicate that means are different at the 95 % level of probability (Tukey's HSD post hoc test)

Table 5: Effects of *Nepeta* essential oils on Chlorophyll b inhibition % of barnyard grass, canary grass, redroot pigweed and lambsquarters at 7 days after spraying

Concentration (v/v)	Barnyard grass		Canary grass		Redroot pigweed		Lambsquarters	
	<i>N. ispahantica</i>	<i>N. glocephalata</i>	<i>N. ispahantica</i>	<i>N. glocephalata</i>	<i>N. ispahantica</i>	<i>N. glocephalata</i>	<i>N. ispahantica</i>	<i>N. glocephalata</i>
1.25	11.30 ± 2.20c	7.86 ± 2.12cd	17 ± 2.37d	8.937 ± 1.46e	19.54 ± 3.42ef	15.11 ± 4.87f	15.43 ± 1.25d	11.04 ± 1.20e
2.5	21.59 ± 5.93b	12.09 ± 5.99c	28.75 ± 1.49c	20.68 ± 1.91d	29.63 ± 2.71cd	23.52 ± 2.52de	25.80 ± 1.38c	18.91 ± 3.19d
5	29.13 ± 4.41ab	22.21 ± 1.34b	39.25 ± 0.46ab	25.87 ± 2.13c	38.27 ± 2.99ab	33.18 ± 1.11bc	32.90 ± 1.73b	26.21 ± 0.48c
10	36.79 ± 3.58a	33.35 ± 2.88a	42.12 ± 2.21a	37 ± 3.36b	45.11 ± 4.2a	40 ± 2.37ab	40.50 ± 2.60a	37.34 ± 2.35a

Values are means ± standard error of four replicates. Within each species, different letters indicate that means are different at the 95 % level of probability (Tukey's HSD post hoc test)

their root elongation at the lowest concentrations (10^{-5} M, 10^{-6} M) applied (De Martino et al., 2010). Romagni et al. (2000) have shown that 1, 8-cineole, and its natural analogue 1, 4-cineole, both suppress the growth of several weeds. 1, 8-cineole inhibits the germination, speed of germination, seedling growth, chlorophyll content and respiratory activity of *Ageratum conyzoides* L. 1753 not Hieron. 1895 nor Sieber ex Steud. 1840. Singh et al. (2002) and De Feo et al. (2002) have investigated the herbicidal activity of 10 volatiles compounds from *Ruta graveolens* L. essential oils and showed that 1,8-cineole significantly inhibits the germination and radical elongation of radish.

The effects of the allelochemicals in studied traits directly dependent on the concentration and *Nepeta* species. The germination and root length decreased with increasing concentrations of essential oils. These results are in agreement with that of Ibáñez and Blázquez (2017) who reported that there are significant effects in shoot and/or shoot + root length of weeds depending on the weed and dose. *N. ispanhanica* oil exerted the more inhibitory effect than *N. glocephalata* for all weed species. It can be due to higher concentration of 1,8-cineole in *N. ispanhanica*. While the inhibition is not similar between the *Nepeta* species oils, weed species differed in their response to the toxic effect of each oil. It was reported that the degree of allelopathic interference can even vary within species (Li et al. 2009).

My observations in glass house indicated that both *Nepeta* oils can act as contact herbicides. These observations are in agreement with previous studies showing that volatile oils and even their monoterpenes exhibit herbicidal activity (Tworkoski, 2002; Singh et al., 2005, 2006). Batish et al. (2004, 2007) concluded that the 5 % essential oil from *E. citriodora* caused 50–80 % visible injury in *A. viridis*, *P. minor* and *E. crus-galli*. In addition Poonpaiboonpipat et al. (2013) reported that the essential oil lemon grass (*Cymbopogon citratus* (DC ex Nees) Stopf) applied on barnyardgrass in greenhouse caused leaf wilting. The reduction in seedling dry mass, chlorophyll a and chlorophyll b content observed in my study is in agreement with previous reports indicating that the monoterpenes had a potential to reduce chlorophyll content (Chowhan et al. 2011; Kaur et al. 2010; Gouda et al., 2016). It may be due to inhibition of biosynthesis of chlorophyll and/or degradation of chlorophyll.

5 CONCLUSION

From the present study, it could be concluded that *Nepeta* essential oils strongly inhibited the germination and root length of all weeds. Dicot weeds (lambsquarters

and redroot pigweed) were significantly more sensitive than monocot weeds (barnyard grass and canary grass). Indeed, at the dose of $4 \mu\text{g ml}^{-1}$, germination of lambsquarters and redroot pigweed was totally inhibited by *N. ispanhanica* essential oil. Further studies are required to investigate the herbicidal potential of *Nepeta* essential oils under field conditions and determine the effects on crop species and other weed species. This study is considered the first study regarding of herbicidal effects of *N. glocephalata* and *N. ispanhanica* but the selectivity of these compounds should be considered.

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