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American Journal of Essential Oils and Natural Products

Available online at www.essencejournal.com

AJEONP

American Journal of Essential Oils and Natural Products

ISSN: 2321 9114
AJEONP 2016; 4(1): 40-45

Received: 19-11-2015
Accepted: 21-12-2015

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Essential oil analysis and phytotoxic activity of catnip (*Nepeta cataria* L.)

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Abstract
This study was conducted to assess the phytotoxic activity of essential oil (EO) from aerial parts of Catnip (*Nepeta cataria* L.) on some noxious weeds and field crops with a view to explore the possibility of production of natural herbicides. The EO of *N. cataria* was extracted by hydrodistillation and the composition of the volatile oil was characterized by GC-FID and GC-MS. The inhibitory effects of EO at concentrations of 0, 150, 300, 600, and 1200 μlL^{-1} on seed germination and seedling growth of *Hordeum spontaneum* Koch, *Taraxacum officinale*, *Avena fatua* L. and three crop seeds including *Lipidium sativum*, *Nepeta cataria* and *Ocimum basilicum* were tested. The examined concentrations of *N. cataria* EO showed different phytotoxic as well as selective properties on the germination and growth of the studied species. The studied EO could be considered as an allelochemical agent in formulation of natural herbicides in future weed control.

Keywords: *Nepeta cataria*, Essential oil, Nepetalactone components, phytotoxicity.

1. Introduction
Potential damage to human health and to the environment from herbicides is regarded as a real problem [4, 22]. Excessive use of chemical herbicides causes contamination in soil and groundwater and increases weed resistance [1]. The use of artificial herbicides can also enhance the risk of toxic residues in agricultural products. It has resulted in an increased interest in alternative strategies leading to the development of biodegradable compounds [6, 9, 1]. The phenomenon of allelopathy, where a plant species chemically interferes with germination, growth and development of vicinity plant species has been known for a long time [19]. allelopathy plays an important role in both natural and agro-ecosystems. Suitable manipulation of the allelopathy towards improvement of crop productivity and environmental protection through eco-friendly control of weeds, pests, crop diseases, conservation of nitrogen in crop land, and synthesis of novel agrochemicals based on natural products have gained prominent attention of scientist engaged in allelopathy research. Especially, allelopathy has potential in integrated weed management [8]. The production and accumulation of secondary metabolites, which inhibit or stimulate germination of seeds and development of plants, are important mechanisms in the interactions between plants [14, 22]. Aromatic plants, which known to be rich in active principles, can play an important role in plant-plant interactions and constitute a primary source of potential allelochemicals [27, 14, 21]. Most of germination and growth inhibitors produced by perennial angiosperms identified by Rice (1984) were phenolic compounds or derivatives of cinnamic acid. Other authors also have found EOs, coumarins, flavonoids, alkaloids, cyanoglycosides, proteins, and amino acids among the inhibitory compounds [14]. Evidence for allelopathic interactions in nature from plants containing EOs has been frequently described [3, 6, 22, 30]. The biological activity of EOs depends on the synergistic or antagonistic effects of constituent types present in different concentrations [7, 14]. Therefore, EOs present in aromatic plants cause a number of positive or negative effects [27]. Application of EOs to dry seeds such as wheat (*Triticum aestivum* L.), black mustard (*Brassica nigra* (L.) Koch), palmer amarant *(Amaranthus palmeri* S. Watson), and *Arabidopsis thaliana* for germination inhibition has been reported by several workers [9, 29, 30]. The allelopathic competence of aerial parts and roots of many plants and trees is also well documented in both laboratory and greenhouse experiments. Tworkoski, (2002) determined the phytotoxicity effects of some EOs e.g. red thyme (*Thymus vulgaris*), summer savory (*Satureja hortensis*) as post emergence growth inhibitors on several weeds [26]. Singh *et al*., (2005) reported the phytotoxic activity of volatile oils from *Eucalyptus citriodora* on *Parthenium hysterophorus* [25] and Saharkhiz *et al*., (2009) showed the allelopathic effects of four EOs to control seed germination and seedling growth of three genera within the Poaceae family [21].

Nepeta is a genus of annual or perennial herbs belongs to the Lamiaceae family which includes approximately 250 species. These plants are native to central and southern Europe, Asia, the Middle East, northern Africa, and to tropical mountains in Africa [12, 13]. *Nepeta* species are used in the traditional medicine of many countries and have a large ethnobotanical effect: diuretic, diaphoretic, vulnerary, antitussive, antispasmodic, antiasthmatic, tonic, febrifuge, emmenagogue and carminative [12, 16]. Previous researches have studied the composition and content of the EO and extracts in *Nepeta* species including *N. meyeri*, because of the marvelous biological activities of the *Nepeta* species and their use in traditional medicine but there are no available findings on phytotoxic effects of *N. cataria* up to now. As far as the authors are aware, few published reports are available regarding the weed control with EOs. The present study was conducted to assess the phytotoxic activity of EO from aerial parts of *N. cataria* on some noxious weeds such as *H. spontaneum*, *T. officinale*, *A. fatua* along with three crop seeds including *L. sativum*, *N. cataria,* and *O. basilicum* were tested. This research could also shed some light towards a better understanding of allelopathic competence of *N. cataria* EO.

2. Materials and methods

2.1. Plant Material

Nepeta cataria was used for extraction of EO considering allelopathic effects. To test the allelopathic potential of this plant, three weeds, *Hordeum spontaneum*, *Taraxacum officinale*, *Avena fatua* and three crops including *Lipidium sativum*, *Nepeta cataria* and *Ocimum basilicum* were selected as plants to be exposed to EO. The plant species, from which the EO was obtained, had been collected from Experimental Field, Collage of Agricultural, Shiraz University. The species was identified and authenticated by A.R. Khosravi, a plant taxonomist at the Shiraz University Herbarium, Shiraz, Iran. Voucher specimen (SUH 24995) has been deposited in the herbarium.

2.2. Essential oil isolation and analysis

At full flowering stage, the aerial parts of *N. cataria* were hydro-distillated (4 times) for 2.5 hr, using an all-glass Clevenger-type apparatus. Sample oils were dried over anhydrous sodium sulphate and stored in sealed vials at 4 °C before GC and GC-MS analysis and allelopathic assessments.

GC and GC-MS analysis: The oils were analyzed by GC-MS. The analysis was carried out on a Thermoquest- Finnigan Trace GC/MS instrument equipped with a DB-5 fused silica capillary column (60 m × 0.25mm i.d., film thickness 0.25 µm). The oven temperature was programmed to increase from 60 to 250 °C at a rate of 4 °C min and finally held for 10 min; transfer line temperature was 250 °C. Helium was used as the carrier gas at a flow rate of 1.1mL min $^{-1}$ with a split ratio equal to 1/ 50. The quadrupole mass spectrometer was scanned over the 35 - 465 amu with an ionizing voltage of 70 eV and an ionisation current of 150 mA.

GC-FID: Analyses of the oils were conducted using a Thermoquest-Finnigan instrument equipped with a DB-5 fused silica column (60 m × 0.25mm i.d., film thickness 0.25 µm). Nitrogen was used as the carrier gas at the constant flow rate of 1.1mL.min^{-1}; the split ratio was the same as for GC/MS. The oven temperature was raised from 60 to 250 °C at a rate of 4 °C min $^{-1}$ and held for 10 min. The injector and detector (FID) temperatures were kept at 250 and 280 °C, respectively. Semi quantitative data were obtained from FID area percentages without the use of correction factors. Retention indices (RI) were calculated using retention times of n-alkanes (C6–C24) that were injected after the oil at the same temperature and conditions. Compounds were identified by comparison of their RI with those reported in the literature [2], and their mass spectrums were compared with the Wiley Library (Wiley 7.0).

2.3. Bioassay of inhibition induced by *N. cataria* EO

In order to detect the allelopathic effects of the studied *Nepeta cataria*, four concentrations of EOs (150, 300, 600, 1200 µlL^{-1}) and a control (0 µlL^{-1}) were used. Four replications of 50 seeds were used for each treatment. Whatman no. 2 filter paper was sterilized in oven at 72 °C for 48 hr and placed in 9 cm diameter Petri dishes. The seeds of the experimental plants were sterilized with sodium hypochlorite (5% v/v) for 10 min, washed under running tap water (for 15 min) followed by distilled water, then placed in Petri dishes and supplemented with 4 ml of the EO solution. To prevent evaporation, Petri dishes were sealed with parafilm and placed in a phytotron at 25 ± 2 °C with a 16 hr photoperiod. They were monitored daily and moistened with the EO solution as needed. The number of germinated seeds was recorded daily in all experiments. Final germination percentages were calculated for each trial. After 14 days, all germinated and non-germinated seeds were counted. The seeds showing radicle emergence were recorded as germinated. Moreover, root and stem length, root and stem fresh and dry weights were measured.

2.4. Statistical Analyses

The design of the experiment was a Completely Randomized Design (CRD) arrangement with 4 replications. Data were analyzed using SAS and mean comparisons were made following Duncan test at $P \leq 0.05$.

3. Results and Discussion

The hydro-distillation of 30 g (four replications) of aerial parts of catnip at full flowering stage showed that the mean of essential oil content was 0.9 % based on dry weight (% w/w). The composition of EO are shown in Table 1 along with the retention indices of the identified components that are arranged in the order of their elution from a DB-5 column. A total of 14 compounds representing 99 % of the total were detected. The results showed that 4a-α,7-α,7a-β-nepetalactone (55%), 4a-α,7-β,7a-α-nepetalactone (31.2%), α-pinene (4.6%), and β-pinene (1.6%) were the major oil components.

Table 1: Essential oil compositions (%) of Catnip (*N. cataria*) which used in the phytotoxic assessments

NO	Component	RI*	%
1	α-Pinene	936	4.6
2	Sabinene	957	0.1
3	β-Pinene	978	1.6
4	1-cyclohexen-1-yl-methyl ketone	980	0.7
5	Triplal	1023	0.4
6	Thymol	1294	0.6
7	4a-α,7-α,7a-β-nepetalactone	1332	55
8	4a-α,7-β,7a-α-Nepetalactone	1342	31.2
9	trans Caryophyllene	1430	2.1
10	α-Humulene	1446	0.9
11	11-Dodecenol	1500	1.1
12	Spathulenol	1580	0.3
13	Caryophyllene oxide	1569	0.1
14	6,10-Dimethyl-2-undecane	1907	0.3
	Total		99
	Essential oil yield (w/w %)		0.9

*The retention Kovats indices were determined on DB-5 capillary column.

In phytotoxic studies of the present work, the phytotoxic effects of the EO from *N. cataria* on the germination percentage, seedling length, biomass weight of various weeds and crop species were evaluated after 14 days ($P \leq 0.05$). As shown in (Fig.1) the control treatment exhibited the maximum germination percentage but the results showed that germination percentage of weeds and crops altered with different concentrations of the EO. On the other hand, different concentrations of EO exhibited various effects in controlling germination. The results indicated that germination percentage decreased with increasing concentration of oils in all seeds. Suppression effects of the volatile oil are generally assigned to its components. At 1200 μlL^{-1}, seed germinations were observed completely stopped in all plants except in *H. spontaneum*. The 1200 μlL^{-1} concentration of *N. cataria* resulted in decreasing *H. spontaneum* seed germination from 100 to 26%. Suppression effect of EO at 300μlL^{-1} was shown in *N. cataria* seed germination. However, at 600μlL^{-1} the germination of A. *fatua* and *T. officinale* and at 1200 μlL^{-1} the germination percent of *L. sativum* and *O. basilicum* were completely stopped (Fig.1). In spite of the results indicated that there was no significant difference between control to 300 μlL^{-1} in *H. spontaneum, O basilicum,* and *L. sativum*, a diverse significant seed germination reduction in *A. fatua and N. cataria* was observed (Fig.1). Likewise, there were equality with 150 and 300 μlL^{-1} in all studied species, however a significant difference with control treatment observed in higher concentrations. In general the desired plants were much less sensitive to the 150 μlL^{-1} concentration compare with other concentrations of EO but they exhibited significant difference with control in *N. cataria* and *T. officinale* (Fig.1). The trends in seedling length were shown in (Fig.2) which presented that, inhibitions were relatively enhanced with the increasing amount of EO concentration in seedling length character as like as seed germination. At 1200 μlL^{-1} the most inhibition in seedling elongation occurred. At 600μlL^{-1} seedling elongation of *A. fatua and T. officinale* prohibited completely, at 150 μlL^{-1} concentration there was no distinct difference in compare with control treatment in all samples. Moreover, the trends in fresh and dry weight of various weeds and crop species were shown in Fig 3 and 4. As shown in (Fig.3) fresh weight was decreased with increasing EO concentration in all samples. Highest fresh weight was shown in control and lowest one was appeared at 1200 μlL^{-1} in all seed samples. In most samples there was no significant difference between different fresh weights in all concentrations of EO, except in *A. fatua* and *H. spontaneum.* Results in (Fig.4) showed that dry weight was decreased with increasing EO concentration in all samples. Highest dry weight was shown in control and lowest dry weight was appeared at 1200 μlL^{-1} in all samples. In most cases there was no significant difference between different dry weights in all concentrations of EO, except in *H. spontaneum.*

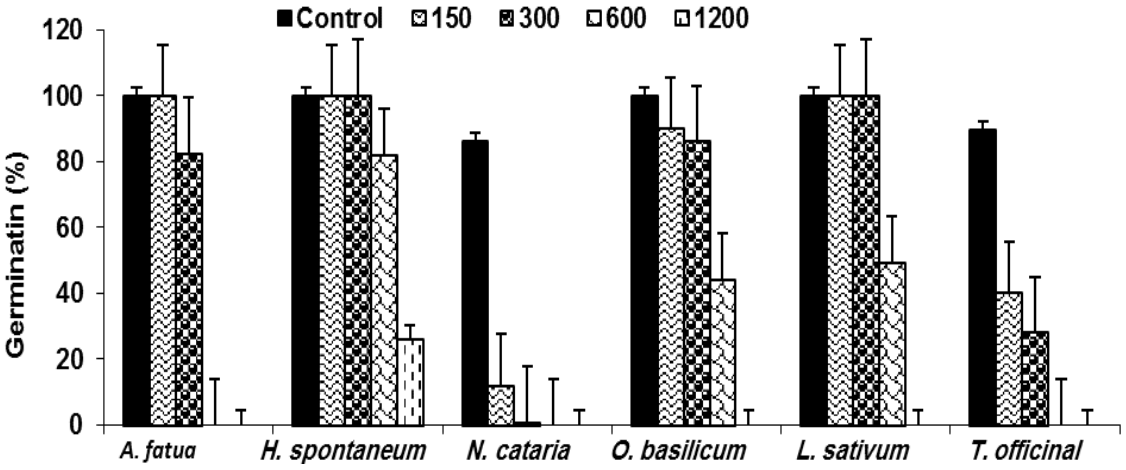

Fig 1: Effect of different concentrations of *N. cataria* essential oil on seed germination of the examined species

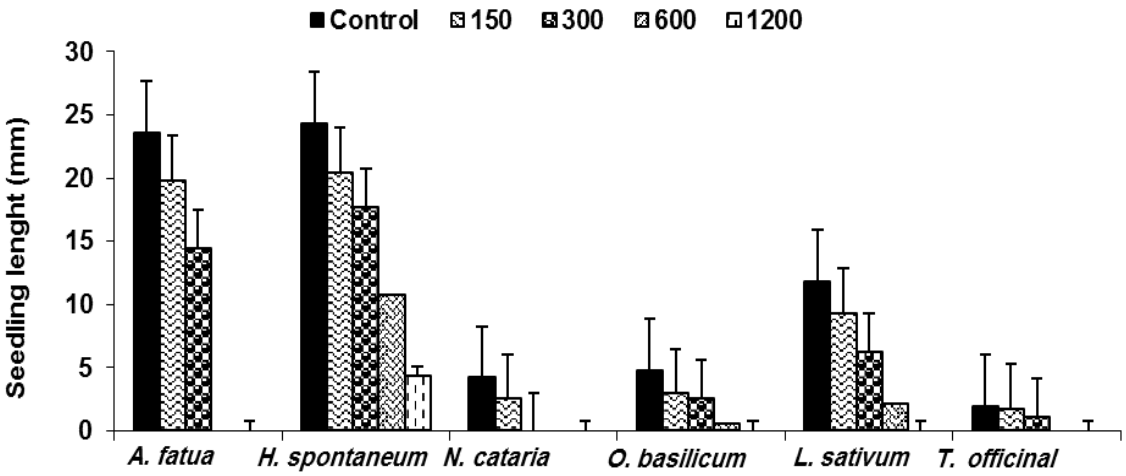

Fig 2: Effect of different concentrations of *N. cataria* essential oil on seedling length of the examined species.

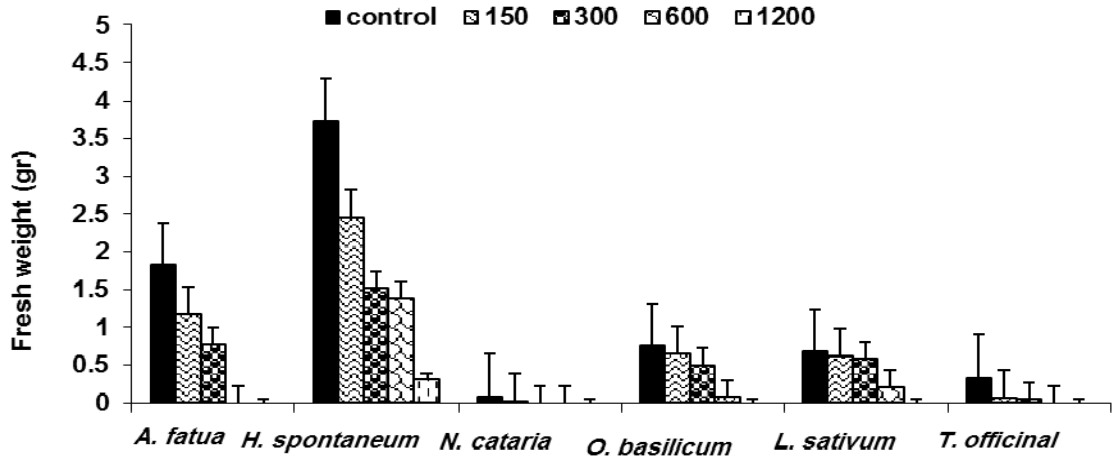

Fig 3: Effect of different concentrations of *N. cataria* essential oil on fresh weight of the examined species

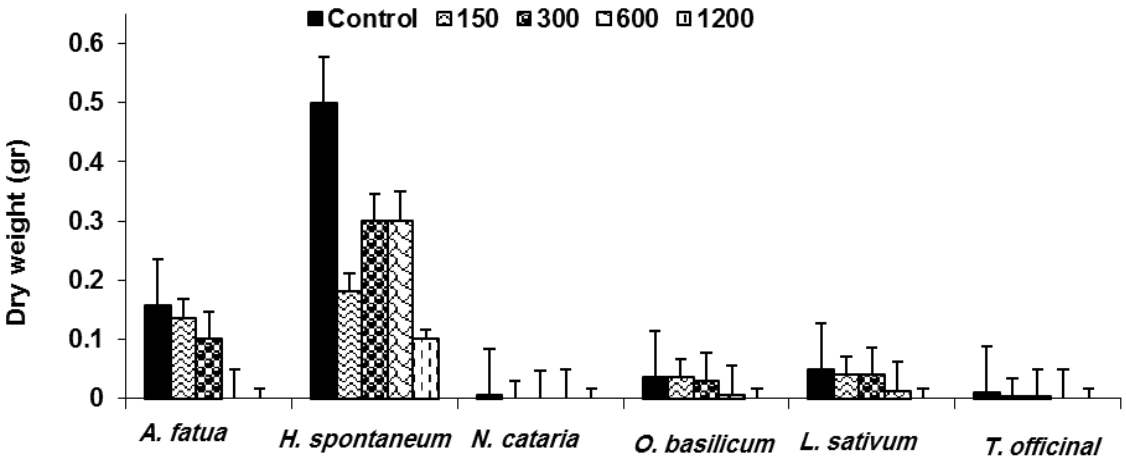

Fig 4: Effect of different concentrations of *N. cataria* essential oil on dry weight of the examined species.

Resent investigations indicate medicinal and aromatic plants or their secondary metabolites derivatives could be potentially used as natural agents in organic agricultural systems as pesticides, herbicides, fungicides and/or natural food preservatives, colors, and antioxidants. The findings of the present study showed that *N. cataria* oil possess some remarkable phytotoxic impacts which depends on the exposed species and oil concentrations. It was found that EO showed a selective phytotoxic effect on the studied species and the susceptibility of the species was as follow: *N. cataria*> *T. officinale, A. fatua*> *L. sativum, O. basilicum*> *H. spontaneum*. In this order, *N. cataria* showed the most vulnerability to its own oil and *H. spontaneum* exhibited highest tolerance to the phytotoxic potency of the applied EO. In addition to oil concentration, the oil components are very crucial for phytotoxic impacts. In the present study, nepetalactone isomers, which are the main *N. cataria* EO compounds, were determined in the EOs of other *Nepeta* species [11]. Eom *et al*., in 2006 detected three forms of nepetalactone in *N.* × *faassenii* foliar volatiles. They showed the most abundant component was 4α,7,7α-nepetalactone which is similar to the EO composition results of our study [10].

Predominate oils of *Nepeta* species contain nepetalactone as the major components, but dissimilar oil compositions have been detected in several *Nepeta* species [5, 11, 23, 24]. Differential response of various allelopathic compounds and their concentrations has been reported in prior probes [17, 22, 28]. Mutlu and Atici in 2009 studied the allelopathic effects of *Nepeta meyeri* extracts on seed germination and seedling growth of some crop plants [16]. In their studies both the root and leaf extracts of *N. meyeri* caused general phytotoxic effects on the seed germination and seedling growth at all concentrations. The allelopathic potential of *N. meyeri* depended on whether; the extract was derived from the leaf or root parts of the plant. The determined prohibitions were further for leaf extracts than root extracts, and were more pronounced at increasing concentrations of the leaf extract. Also, in our previous work on phytotoxic activity of different chemotypes of *Zataria multiflora* (Lamiaceae family), it was found that the EOs component types, their concentrations and the species exposed to the oils, all were imperative for growth inhibition and natural herbicide formulation goals [22]. The mode of the action of the EOs against germination and plant growth is not quite clear. However, some studies have indicated that volatile monoterpenes are potent inhibitors of cell mitosis [14, 20]. It has been reported that EOs from cinnamon (*Cinnamomum zeylanicum* Blume.) and red thyme (*Thymus vulgaris* L.) reduced sprout growth in potato (*Solanum tuberosum*), possibly by killing meristematic cells [22]. Tworkoski (2002) suggested that EOs altered membrane permeability based on the electrical conductivity measured by electrolyte leakage in leaf discs of dandelion. However, this aspect merits further investigations [26].

4. Conclusion

Based on the results reported herein, it could be concluded that EO from *N. cataria*, especially at 600 and/or 1200 m μlL^{-1}, shows strong phytotoxic effects on controlling the seed germination and seedling growth of examined crops and weeds. Inhibitory effects of this oil are generally due to its concentration, components, and also depend on the species exposed to the oil. From the practical application point of view, the selective property of the oil is of great interest as a proper natural herbicide for future weed management.

The findings of this study can be considered the first step towards a possible practical application of EOs as potentially natural herbicides. Further studies on the more phytotoxic aspects of the studied oil, its selective degree (phytotoxic effects on the more field crops and weeds) and especially on the formulation of EO are required before this technique could be commercially recommended to control weeds under field conditions.

5. Acknowledgments

The authors wish to extend their thanks and appreciation to the Shiraz University Research and Technology Council and NACE (Natural Antimicrobial Center of Excellence) for their financial supports.

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