Journal of Novel Applied Sciences
Available online at www.jnasci.org
©2013 JNAS Journal-2013-2-2S/974-977
ISSN 2322-5149 ©2013 JNAS

Quantitative and Qualitative Comparison of Dracocephalom moldavica L. Essance under Drought Stress and its Interaction with Gibberellin and Ascorbate

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ABSTRACT: Moldavian balm(*Dracocephalom moldavica* L.) is a resistant aromatic annual plant containing variable amounts of propanoids, monoterpenes and sesquiterpenes. It has antibacterial properties and is used for toothache. In the present study under a completely random design with 4 replications effects of drought stress and its interaction with gibberellic and ascorbic acids were studied on *Dracocephalom moldavica* L. Treatments included drought stress at 2 levels(daily irrigation and no irrigation for 7 days), gibberellic and ascorbic acids at 2 levels(0 and 10 mM). Plant samples were collected and dried for extraction and the chemical compounds were submitted to GC and GC/MS equipments for analysis. Results revealed that drought stress increased percent of essence concentration while the quality of the essence decreased. Gibberellin and ascorbate on the other hand reduced essence concentration while improving its performance. The main component of the essence was Citral(27.55%). Drought stress reduced Geranyl acetate and Geranial contents while gibberellin and ascorbate increased these compounds.

***Keywords*:** Ascorbate, *Dracocephalom moldavica* L., Essence, Gibberellin.

INTRODUCTION

The global interest in medicinal plants and their natural compounds in pharmaceutical and cosmetics industry followed by a trend in using these plants by the government, local people and industry make it necessary to carry out extensive basic and applied research. The problems associated with chemical compounds in synthetic drugs, pesticides, additives, preservatives, essences and artificial flavorings are widely acknowledged to the point that almost all chemical compounds are being gradually excluded from food, pharmaceutical, cosmetic and hygienic products in industrial countries. Thorough research and wise exploitation of medicinal plants seem to be necessary when their worldwide use in pharmaceutical, cosmetics, hygienic and food industry is being accelerated (Mohammadian, 2010). Of about 8000 plant species growing in Iran, 2000 species are exclusive to this country(Mohammadian, 2010). While Iran is an important source of basic natural herbal essences, unfortunately the use of artificial essences and flavorings such as banana, orange and strawberry are on the increase.

Dracocephalom moldavica L. is a resistant aromatic annual plant with green leaves belonging to *Lamiaceae* family(Said-Al Ahl and Abdou, 2009). *D. moldavica* is widely used as a sedative and for treating kidney pains. Its extract is also used for toothache, frostbite, and rheumatism. In rats and rabbits under laboratory conditions it has been used against tumors(Said-Al Ahl and Abdou, 2009).

Plant essences which are used as flavoring are considered as secondary metabolites. They are a mix of propanoids monoterpenes and sesquiterpenes. The essences and compounds in various plants organs are in fact stored in their specialized secondary structures. The largest part of the matter present in *Dracocephalom moldavica*

L. essence is a monoterpene containing oxygen (55.8% – 88.4%) and its main components include Geranial (22.82% - 55.8%), Geranyl acetate (9.75% - 31.48%), Neral (16.08 – 22.02) and Geraniol (0.42% - 16.59%). The produced essence is classified as the aromatic essences (Said-Al Ahl and Abdou, 2009).

Water is an abiotic factor that has significant effects on medicinal plants growth and their effective compounds (Misra and Srivastava, 2000). Drought stress significantly increases secondary metabolites and essence contents in plants. Due to chemical reactions and metabolic changes in effective compounds produced by medicinal plants, lack of humidity has particularly important effects on these plants(Sunka *et al.,* 2003). Ascorbic acid is the most widespread antioxidant in plants that plays an important role in plants growth and metabolism, electron transfer system, glucose metabolism and production of secondary metabolites (El-Gengaili *et al.,* 1995). It is now hypothesized that there exists a relation between various levels of gibberellic acid and the plants' resistance against abiotic stresses. In fact gibberellic acid can help fight against abiotic stresses through affecting genetic paths of secondary metabolites biosynthesis (Pouryousef *et al.,* 2007).

The aim of the present study was to identify the compounds in *Dracocephalom moldavica* L. essence analyzing the quantity and quality of the essence and the effective compounds in the plant under severe drought stress and its interaction with gibberellic and ascorbic acids.

MATERIALS AND METHODS

The study was carried out in Pakdasht region in Varamin, southeastern Tehran in sandy-loamy soils. The experiment was conducted in pots based on a completely random design with 4 replications. Irrigation was applied at two levels, normal (no stress) and no irrigation (severe stress). Treatments with ascorbic and gibberellic acids were also applied at 2 levels (0 and 10 mM). Seeds were sown in late March. Drought stress and ascorbic and gibberellic acids treatments were applied after 6 weeks when plants were at 6-7 leaf stage. After 10 days, twigs were cut and dried in shadow. The samples were then chopped and using a Clevenger apparatus the essence was extracted for 3 h by distillation. For every 20 g dried sample, 200 ml distilled water was added (1:10 w/v). GC and GC-MC chromatography equipments were used to identify chemical compounds in the essence. After dehydration with sodium sulfate, the essence was diluted with chloroform solution (1-10 v/v) and injected into the apparatus. The GC apparatus (plu 6890) was equipped with DB-5 column 30 m length, 0.32 mm internal diameter and 0.5 µm stable phase layer depth. The gas chromatography apparatus was connected to a mass spectrophotometer GC-MC (Quadrople). The chemical compounds were identified through comparison with the standard compounds available at the computer data base library. Analysis of variance and comparison of the means in the treatment samples were carried out with SAS ver. 16 using Duncan test ($p \leq 0.05$).

RESULTS AND DISCUSSION

Results

The obtained essence was yellow in color with especially strong odor. Results showed a significant difference ($p \leq 0.05$) in concentration of essence between various levels of drought stress (Table 1). Average concentrations of essence under severe drought stress and favorable irrigation conditions were 0.62% and 0.34, respectively (Table 2). As Table 1 shows, the effect of ascorbate acid on the essence concentration was significant ($p \leq 0.01$). In fact, application of 10 mM ascorbate reduced essence concentration to 0.34% (Table 1). Effect of gibberellin on essence concentration (Table 2) was also significant ($p \leq 0.05$). Application of 10 mM gibberellin under severe drought stress reduced essence concentration to 0.43% (Table 2). Moreover, interaction of effects of drought stress, gibberellin and ascorbate on the essence concentration (Table 1) was significant ($p \leq 0.01$). Comparison of average interaction of effects of the 3 factors under study suggests that drought stress and application of 10 mM ascorbate and gibberellin reduced essence concentration to 0.36% (Table 2).

Results of the study showed a significant difference ($p \leq 0.01$) in essence performance under various study conditions (Table 1). Mean essence performance under severe drought stress and favorable irrigation conditions were 7.46 mL and 16.44 mL, respectively (Table 2). Various levels of ascorbate made significant difference ($p \leq 0.01$) in essence performance (Table 1). Application of 10 mM ascorbate resulted in 14.47 mL essence performance (Table 2). Also various levels of gibberellin had significant effect ($p \leq 0.01$) on essence performance (Table 1). Application of 10 mM gibberellin increased essence content to 10.30 mL (Table 2). The highest essence performance (17.84 mL) was observed with interaction of effects of 17.84 mL ascorbate and gibberellin under severe drought stress (Table 2).

Table 1. Analysis of Variance of the effect of drought stress, ascorbate and gibberellin on the features under study

Variations	df	Essence (%)	Essence Performance (mL)
repetition	3	**2.93	**2.76
drought stress	1	*2.22	**2.62
gibberellin × drought stress	1	*0.39	**0.08
ascorbate × drought stress	1	**0.24	**0.08
drought stress × ascorbate × gibberellin	1	**0.32	**0.06
error	16	0.001	0.006
Variation factor (%)		1.61	3.76

ns: non significant, *: significant at ($p \leq 0.05$), **: significant at ($p \leq 0.01$)

Table 2. Comparison of mean effect of drought stress, ascorbate and gibberellins on the features under study

Sources of Variations	Essence (%)	Essence Performance (mL)
drought stress (irrigated)	0.34[c]	16.44[b]
drought stress (non-irrigated)	0.62[a]	7.46[c]
ascorbate 10 mM	0.43[b]	14.47[d]
gibberellins 10 mM	0.43[b]	10.30[d]
drought stress × ascorbate × gibberellin	0.36[c]	17.84[a]

a, b, c, d: various levels of significance of the treatments

Compounds detected in the essence of *D. moldavica* are tabulated in Table 3. Eighteen compounds were determined in the essence with Citral comprising the highest concentration (27.55%) in the control plants (no stress). Application of severe drought stress drastically reduced geranial and Geranyl acetate concentrations while the other compounds increased. Gibberellin and ascorbate alone reduced essence content in the plants under study; however, simultaneous application of gibberellin and ascorbate resulted in the conditions very close to the control.

Table 3. Compounds determined in the essence of D. moldavica under drought stress, gibberellins and ascorbate treatments

	Compounds	Control	Non-irrigated	non-irrigated × ascorbate	non-irrigated × gibberellins	non-irrigated × ascorbate× gibberellin
1	Citral	27.55	30.66	27.82	27.05	27.63
2	Citrol	18.53	23.37	20.18	20.36	18.94
3	Geranial	15.77	1.59	14.95	15.01	15.98
4	Geraniol	2.28	8.36	3.84	3.09	2.77
5	Geranial ester	13.32	19.49	14.37	13.42	13.31
6	Geranyl acetate	7.89	0.72	4.32	6.82	7.34
7	Trans-Geraniol	3.06	3.83	3.09	2.87	2.35
8	Octane	2.14	2.32	2.41	2.73	2.67
9	Nerolacetate	3.38	3.95	3.34	2.34	2.89
10	2-4-6-trimethyl1-3-cyclohexene-1-carboxaldehyde	2.18	3.08	2.38	2.34	2.21
11	Methyl geraniate	0.2	1.1	0.8	0.43	0.301
12	Allo-aromadendrene	0.18	0.21	0.19	0.189	0.176
13	β-Bourbonene	0.14	0.1	0.12	0.128	0.1299
14	β-Damascenone	0.4	0.39	0.32	0.245	0.276
15	Bornyl acetate	0.04	0.11	0.09	0.1	0.114
16	Linalool	0.17	0.173	0.17	0.175	0.171
17	Camphene	0.5	0.64	0.51	0.54	0.521
18	Mytenol	0.2	.0.15	0.142	0.148	0.146

Discussion

D. moldavica is an annual plant belonging to the *Lamiaceae* family which naturally grows wild. This study was conducted to investigate the behavior of *D. moldavica* plants under drought stress and treated with an antioxidant (ascorbate) and a plant hormone (gibberellic acid) comparing the quantity and quality of the plant essence under the study conditions. Mean concentration percentage and performance of the essence produced by the plants under irrigation and non-irrigation conditions and also application of ascorbate and gibberellin showed that with a cut in irrigation, the essence percent increased from 0.34% to 0.64% which was statistically meaningful. Essence performance on the other hand reduced from 16.44 mL to 7.46 mL which is in agreement with the findings reported by Said-Al Ahl and Abdou (2009) on *D .moldavica*.

D .moldavica essence contains mainly Geranyl acetate, phenolic acids of Rosmarinic acid and Caffeic acid, tannin, and di and tri terpenes such as Oleanolic acid (Li and Ding, 2003). In the present study, drought stress reduced two compounds of the essence namely, Geranial and Geranyl acetate while the other compounds increased compared with the normally irrigated plants. These findings support the results of the study reported by (Said-Al Ahl and Abdou, 2009). Application of gibberellin and ascorbate alone reduced the percentage of the compounds under severe drought stress while simultaneous use of these compounds resulted in the treated plants with qualities close to those of the control plants.

Water is an abiotic factor that plays the main role in the medicinal plants' growth and their essential oils (Omidbaigi et al., 2003). These same researchers studying moisture stress in basil found that as the soil moisture reduced, so did the essence performance of the plants while the percentage of essence increased. Fatima et al, (2000) observed an increase in the percentage of the essence compounds in Jawa plants under drought stress. Li and Ding, (2003) studying *D. Kotschyi* samples reported that Limonene (29%) was the highest essence content and Myrtenal (30.8%) was the main compound in study earth the plants' essence. Percentage of essence in *D. polychaetum bornm* was reported as 98.75% and the main compound were monoterpenes (Kakasy et al., 2002). The essence percentage in the same plant is reported as more than 1% compared with the other species of *Dracocephalom* genus (Kakasy *et al.,* 2002). The presence of Luteolin and Apigenin Flavons compounds in the species of *Dracocephalom* genus such as *D. moldavica, D. Kotschyi*, *D. grandiflorum* and *D. integifolium* results in their therapeutic effects (Wang, 2000). Application of gibberellin under stress condition reduced the essence percent in the plants which is in line with the findings of Fatima *et al*, (2000) on Citronella java *Cymbopogon winterianus*) where essence percent was reported to increase under salt stress while the application of gibberellin reduced essence percent and improved its performance. Reduction in essence was also reported in safflower plants treated with gibberellin (Hasan, 2009).

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