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Essential Oil Content and Composition of German Chamomile (*Matricaria chamomilla* L.) at Different Irrigation Regimes

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Abstract: The essential oil content and chemical composition of German chamomile (*Matricaria chamomilla* L.) were determined at different irrigation regimes (irrigation at 100, 85, 70 and 55% of field capacity, respectively). Essential oil was obtained by hydro-distillation of dried flower and was analyzed by GC-MS. Results indicated that the yield of dry flower and essential oil per pot, essential oil percent and its composition vary with irrigation regimes. Highest amount of essential oil percent, yield of dry flower and essential oil per pot were obtained from irrigation at 85% of the field capacity. However, it was not significantly different from irrigation at 70% of field capacity. Lowest amount of essential oil percent were obtained when the plants irrigated with 100 and 55% of field capacity. Minimum yield of dry flower and essential oil per pot were observed when the plants irrigated with 55% of field capacity, but it was not significantly different from irrigation at 100% of field capacity. Major constituents of the essential oil for all irrigation treatments were azulene-7-ethyl-1,4-dimethyl, limonene, bisabolol oxides A and B, bisabolone oxide, trans-β-farnesen and isobornyl isobutyrate<8-isobutyryloxy>.

Key words: Azulene, essential oil, irrigation, *Matricaria chamomilla*

INTRODUCTION

German chamomile, *Matricaria chamomilla* L. (syn. *Chamomilla recutita* (L.) Rauschert), which belongs to the Asteraceae family, is a very important medicinal plant species (Ivens, 1979; Salamon, 1992). Chamomile is a native of south and east Europe. However, at present it has spread over nearly all the European continent. The plants can be found in north Africa, Asia, north and south America, Australia and New Zealand (Ivens, 1979). The flowers of German chamomile accumulate blue essential oil from 0.2 to 1.9% (Bradley, 1992; Mann and Staba, 1992). As a medicinal plant, its dried flowers are an old age remedy, known in ancient Egypt, Greece and Rome (Isaac, 1989). Nowadays, in phytotherapy, flower anthodia are mainly used. The chamomile drug is included in the pharmacopoeia of 26 countries (Pamukov and Achtardziev, 1986). Pharmacological properties includes anti-inflammatory, antiseptic, carminative, healing, sedative and spasmolytic activity (Salamon, 1992). However, *M. chamomilla* has exhibited both positive and negative bactericidal activity with *Mycobacterium tuberculosis*, *Salmonella typhimurium* and *Staphylococcus aureus*. About 120 chemical constituents have been identified in chamomile as secondary metabolites, including 28 terpenoids, 36 flavonoids and 52 additional compounds with potential pharmacological activity (Mann and Staba, 1992). Components such as alpha-bisabolol and cyclic ethers are antimicrobial. Unibelfiferone is fungistatic while chamazulen and alpha-bisabolol are antiseptic (Duke, 1985). The chamomile extracts was found to have the most effective antileishmanial activity (Shnitzler *et al.*, 1996).

According to Yanive and Palevitch (1982) and Bernath (1986), essential oil content and composition of essential oil in plants varies and is due to the genetic and environmental factors. Drug yield is changeable during individual years, but regularly ranges between 300 to 500 kg dry flower per hectare land area. Optimization of agroecosystems, knowledge of specific ecophysiological requirements and selection of specific types can lead to produce 1 to 1.2 t ha^{-1} of dry flowers (Salamon, 1992). Well irrigated chamomile plants produced flowers with high content of essential oil (Kerekes, 1962) and flower yield (Hornok, 1992).

Based on our knowledge, information about the response of this species to irrigation are scarce. Therefore, the main objective of the present study was to find out the effect of different irrigation regimes on the essential oil content and composition of German chamomile.

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MATERIALS AND METHODS

Plant material: Seeds of *Matricaria chamomilla* tetraploid variety Bodegold, a chamazulene-high mixed type with a high content of bisabolol, provided from Germany, were used in the present study. Potted seedlings of *M. chamomilla* were subjected to study the effect of different irrigation regimes on the essential oil content and composition at the experimental field of Faculty of Agriculture, Urmia University, Urmia, (1320 m above sea level, latitude 37°, 32' north, altitude 45°, 5' east), Iran. The seeds were sown in spring 2004 in pots. Irrigation treatments were arranged in a randomized complete block design with 5 replications. Irrigation regimes were: 100, 85, 70 and 55% of field capacity, respectively. Irrigation was done when field capacity reduced to these points and then it was increased to 100% of field capacity. The soil was clay-loam with 22.5% of field capacity. The harvested crop consisted of typical freshly gathered flower heads, with approximately = 10% of flowers containing fragments of the small flower stalks, which were up to 30 mm long (Bottcher *et al.*, 2001). Flower heads were picked when fully developed and dried at a shady place (Letchamo, 1993). Flowers were hand harvested at the medium stage of development (Letchamo, 1990) and dried at 25°C for 72 h.

Isolation of essential oil: The air-dried parts of chamomile (15 g of the dry sample) were hydro-distilled in a Clevenger-type apparatus in 1000 mL round bottomed flask with 600 mL deionized water for 4 h (Hoelzl and Demuth, 1975; Letchamo, 1993). The essential oil was stored in a dark glass bottle and kept at 3°C until analysis.

GC-MS analysis: A HP-6890N GC, with MSD 5973N, equipped with a Split/Splitless injector and a HP-5 MS column (30.0 m length, 0.25 mm Id, 0.25 μm film thickness) was used. The temperature program of the GC, started at 45.0°C and was held for 1 min. Then the column was sequentially heated at a rate of 3°C min^{-1} to 115°C and thereafter heated at a rate of 2°C min^{-1} to 180°C and then heated at a rate of 5°C min^{-1} to 280°C and held for 2.0 min. The solvent delay time was set at 3 min. Both the transfer line temperature and the injector temperature were programmed at 280, 250°C, respectively.

Identification of components: The components of the oil were identified by comparison of their mass spectra with those of a computer library or with authentic compounds and confirmed by comparison of their retention indices either with those of authentic compounds or with data published in the literatures (Davies, 1990; Shibamoto, 1987). Data were analysed using MSTATC statistical package (Nissan, 1989).

RESULTS

Essential oil content: Results (Table 1) showed that different irrigation regimes have significant effect on the essential oil percent, yield of dry flower and essential oil yield of German chamomile. The highest amount of essential oil (0.754% w/w) accumulated in the flowers of plants when irrigated with 85% of field capacity. However, there was no significant difference between essential oil percent of plants which received 85 and 70% of field capacity. The lowest amount of essential oil (0.626% w/w) belonged to the plants when irrigated with 100% field capacity. However, there was no significant difference between essential oil content of plants which received 100 and 55% of field capacity. Maximum yield of dry flower (5.226 g/pot) and essential oil (39.514 mg/pot) obtained from irrigation at 85% field capacity, but these values were not significantly different from the values from 70% of field capacity. Lowest yield of dry flower (3.814 g/pot) and essential oil (24.232 mg/pot) were observed in irrigation at 55% field capacity.

Oil components: The constituents of essential oil of German chamomile obtained at different irrigation regimes are shown in Table 2. The main components of the essential oil for all irrigation treatments were azulene-7-ethyl-1, 4-dimethyl (chamazulene), limonene, bisabolol oxides A and B, trans-β-farnesen, Bisabolone oxide and isobornyl isobutyrate <8-isobutyryloxy>. Other components were included in low magnitudes. Based on mean yield of essential oil and percent of its constituents, we calculated yield of oil components per pot. Yield of main components of essential oil at irrigation regimes of 100, 85, 70 and 55% of field capacity were: azulene-7-ethyl-1, 4-dimethyl (chamazulene) as an important constituent, 4.68, 6.59, 5.79 and 4.67 mg/pot, limonene, 0.33, 0.41, 0.38 and 0.24 mg/pot, bisabolol oxide A, 8.90, 6.90, 12.93 and 7.57 mg/pot, bisabolol oxide B, 4.17, 14.08, 8.07 and 4.79 mg/pot, trans-β-farnesene, 0.74, 1.57, 0.59 and 0.65 mg/pot, bisabolone oxide, 2.43, 1.99, 2.30 and 1.19 mg/pot, isobornyl isobutyrate <8-isobutyryloxy>, 3.51, 4.54, 4.08 and 3.40 mg/pot, respectively. Therefore, at 85% field capacity higher amounts were obtained for components such as chamazulene, limonene,

Table 1: Essential oil percent, yield of dry flower and essential oil yield of *Matricaria chamomilla* L. at different irrigation regimes

Irrigation regimes	Oil percent w/w (%)	Dry flower yield (g/pot)	Essential oil yield (mg/pot)
I_1 = 100% of field capacity	0.626b	4.290b	26.752b
I_2 = 85% of field capacity	0.754a	5.226a	39.514a
I_3 = 70% of field capacity	0.751a	4.908a	36.554a
I_4 = 55% of field capacity	0.635b	3.814b	24.232b

Means followed by same letters in each column are not significantly different

Table 2: Composition (%) of the essential oils of *Matricaria chamomilla* L. at different irrigation regimes

	Irrigation regimes (% of field capacity)			
Compounds	I_1(100)	I_2(85)	I_3(70)	I_4(55)
Hexyl, 2-methyl butyrate	0.15	-	-	
Myrcene	-	0.005	-	-
α-terpinene	-	0.01	-	-
Octane	0.13	0.11	0.17	0.12
Butanoic acid, 2-methyl-, ethyl ester	0.02	0.01	0.07	0.02
β-bisabolene	-	-	-	0.01
Octane, 2-methyl	0.07	-	-	-
Octane, 3-methyl	0.08	0.06	0.08	0.07
1-butanol, 3-methyl-, acetate	0.05	0.03	0.04	0.04
Nonane	0.04	0.03	0.03	0.03
α-pinene	0.01	0.01	0.01	0.01
Sabinene	0.04	0.08	0.03	0.09
Yomogi alcohol	0.02	0.03	0.03	0.02
Para cymene	0.03	0.03	0.04	0.02
Limonene	1.22	1.04	1.03	0.99
1, 8-cineole	0.07	0.09	0.09	0.09
Trans-β-ocimene	0.07	0.10	0.07	0.05
Butanoic acid, 2-methyl butyl ester	-	0.01	-	-
Butanoic acid, 3-methyl butyl ester	0.09	-	-	0.08
Artemisia ketone	0.18	0.19	0.20	0.22
Artemisia alcohol	0.06	0.08	0.07	0.11
Champhor	-	0.01	-	-
Neral	-	0.04	-	-
γ-terpinene	-	-	0.10	-
β-guaiene	-	-	0.03	-
Epi-α-cadinol	-	-	0.06	-
Borneol	0.03	0.05	-	0.04
β-elemene	0.03	0.03	0.02	0.01
Trans-β-caryophyllene	0.06	0.06	0.02	0.03
Trans-β-farnesene	2.76	3.97	1.62	2.67
Germacrene-D	0.57	0.79	0.49	0.62
β-selinene	0.02	0.05	0.02	0.02
Bicyclogermacrene	0.24	0.56	0.18	0.34
Germacrene-A	0.02	0.03	0.02	0.01
(E, E)-farnesene	0.01	0.10	0.02	-
α-copaene	-	0.005	-	-
Delta-cadinol	-	-	-	0.01
Delta-cadinene	0.02	0.02	0.02	0.02
Nerolidol B (trans)	0.40	-	0.30	-
Nerolidol acetate <E >	-	0.05	-	-
Spathulenol	0.58	0.69	0.42	0.61
Caryophyllene oxide	0.05	-	-	-
Lepidozene	0.07	-	-	-
Calarene	0.06	-	-	-
Lavandulol	-	0.02	-	-
Terpin-4-ol	-	0.02	-	-
α-terpineol	-	0.05	-	0.01
Delta elemene	-	0.01	-	-
α-humulene	-	0.01	-	-
Bisabolol oxide B	15.58	35.63	22.07	19.77
Bisabolone oxide A <α>	9.10	5.04	6.30	4.91
(E, Z)- farnesol	0.06	-	-	-
Gama-trans-bisabolene	-	0.02	-	-
Bergamotol <z-α>	-	0.13	0.04	-
Azulene-7-ethyl-1, 4-dimethyl (Chamazulene)	17.48	16.68	15.83	19.27
Bisabolol oxide A	33.27	17.46	35.38	31.25
β-costol	0.07	0.13	-	0.08
1, 1'-biphenyl, 3, 3'-dimethyl	0.16	0.14	0.08	0.10
2-pentadecanone, 6, 10, 14-trimethyl	0.07	0.05	0.05	0.04
Isobornyl isobutyrate <8-isobutyryloxy>	13.11	11.48	11.15	14.03
Linoleic acid	0.14	0.09	0.10	0.12
Tricosane	0.07	0.06	0.06	0.06
Tetracosane	-	0.04	0.01	0.05
Hexanedioic acid, dioctyl ester	0.08	-	-	-
Pentacosane	0.75	0.47	0.50	0.54
Di–(2-ethylhexyl)-phthalate	0.31	0.14	0.15	0.18
Hexacosane	0.02	0.02	0.01	0.01
Heptacosane	0.21	0.14	0.12	0.13
Nonacosane	0.07	0.04	0.03	0.03
Cis-osimene	-	0.02	-	-
Valencene	-	0.03	-	-

trans-β-farnesene, bisabolol oxide B and isobornyl isobutyrate <8-isobutyryloxy>. On the other hand, highest yield was observed for bisabolol oxide A at 70% field capacity. For bisabolone oxide, largest amounts was obtained from irrigations at 100 and 70% field capacity, respectively.

DISCUSSION

Low amount of essential oil yield at 100% field capacity could be attributed to excess water. This condition may reduce oxygen supply to the roots, which in turn limits respiration, nutrient uptake and other critical root functions (Hopkins, 1995). However, the lowest yield obtained at 55% field capacity is due to water deficit. Damage resulting from drought stress is related to the detrimental effects of dessication on protoplasm and to an increase in solute concentration as the protoplast volume shrinks, which may itself have serious structural and metabolic consequences. The integrity of membranes and proteins is also affected by dessication, which in turn leads to metabolic dysfunctions. In addition to membrane damage, numerous studies have shown that cytosolic and organellar proteins may undergo substantial loss of activity or complete denaturation when dehydrated. Photosynthesis can be affected by water stress in two ways, closure of the stomata and effects on the structural integrity of the photosynthetic machinery (Hopkins, 1995). Both electron transport and photophosphorylation were reduced in the chloroplasts isolated from sunflower leaves with leaf water potential below about -1.0 MPa. These effects reflect damage to the thylakoid membranes and ATP synthetase protein (Rao *et al*., 1987). The consequence of all these events is a general disruption of metabolism in the cell and reduction of yield and yield components (Hopkins, 1995).

Omidbaigi *et al*. (2003) measured essential oil content in *Ocimum basilicum* under different irrigation regimes. Percentage of essential oil were 1.12, 1.04, 1.26 and 0.99 in plants irrigated with 100, 85, 70 and 55 % field capacity, respectively (Omidbaigi *et al*., 2003). In another study, maximum yield of root biomass in *Atropa belladonna* were observed in plants grown under the highest water supply (35% of water depletion) as compared to 55, 65 and

95% of water depletion. But highest percent of hyoscyamine and of scopolamine in roots were obtained under severe water stress condition (95% depletion of available soil water) (Baricevic *et al.*, 1999). A study on *Glycyrrhiza glabra* L. showed that, root yields ranged, on the average, from 14.6 t ha^{-1}, under rainfed conditions, to 20.0 t ha^{-1} under irrigated conditions. Irrigation favoured higher rooting of cuttings and root weight, from 69% in the control to 81% under irrigation and from 0.48 to 0.56 kg, respectively (Marzi *et al.*, 1993). Water deficit have reduced content of essential oil and phenolic compounds of *Rosmarinus officinalis* (Solinas *et al.*, 1996), essential oil content of *Matricaria chamomilla* L. (Kerekes, 1962) and yield of essential oil of *Cymbopogon* sp. (Chaterjee *et al.*, 1995). Omidbaigi *et al.* (2003) also reported that the amount of main constituents of the oil of *Ocimum basilicum* such as linalool, methyl chavicol, 1, 8-cineole affected by water stress.

In conclusion it seems that irrigation at 70% field capacity is a suitable irrigation regime for German chamomile, but when water supply is sufficient, irrigation at 85% field capacity may be practiced to produce components such as chamazulene, limonene, bisabolol oxide B, trans-beta farnesene and isobornyl isobutyrate <8-isobutyryloxy>.

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