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Effect of water stress on plant growth and thymol and carvacrol concentrations in Mexican oregano grown under controlled conditions

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Abstract

Herb consumption in United States has grown significantly over the last twenty years. A review of the overall herb imports into the U.S. shows that oregano import is the largest in quantity and in dollar value. Thymol and carvacrol are the two major compounds in the essential oil obtained from Mexican oregano. These compounds are of special interest due to their antioxidant and antimicrobial properties. In this study, the effect of moisture on growth of Mexican oregano (*Lippia berlandieri* Schauer) and its thymol and carvacrol composition was examined in greenhouse tests. The crop yield increased significantly with increasing moisture and age of the plants. Although on an average the older plants contained less oil than the younger plants, the differences were not statistically significant. Total thymol and carvacrol content of oregano oils obtained from younger plants was higher than that of the mature plants. The amount of water received by the plant did not have a significant effect on the thymol and carvacrol content of the oil extracted from Mexican oregano.

Key words: Carvacrol, essential oil, *Lippia berlandieri* Schauer, Mexican oregano, moisture, plant growth, thymol.

Introduction

Demand for spices/herbs has increased rapidly during recent years in the U.S. According to the American Spice Trade Association (ASTA) statistics, U.S. annual oregano imports increased from 2,800 tons to 6,200 tons between the years 1980 and 2000 (ASTA, 2000). Turkey, Mexico and Greece are the major countries exporting oregano to the U.S.

Mexican oregano (*Lippia graveolens*, *Lippia berlandieri* Schauer) belongs to the Verbenaceae family. It is quite distinct from its European counterparts. Mexican oregano has a much stronger and robust flavour that is described as "wild". The leaves of the Mexican oregano are larger and somewhat a darker shade of green and its strong flavour is attributed to its higher essential oil content than other varieties.

Microorganisms in spices can lead to spoilage or disease if contaminated spices are used as ingredients in food products. Utilization of the essential oils and oleoresins extracted from spices minimizes microbial contamination. Furthermore, flavour variations that may result from varying quality and source of the spice can be minimized by utilizing standardized spice extracts to ensure a reliable flavour supply for food product formulation.

There are a few studies published on the chemical composition and pharmacological properties of Mexican oregano oil (Compadre *et al.*, 1987; Dominguez *et al.*, 1989; Pino *et al.*, 1989; Uribe-Hernandez *et al.*, 1992). Pino *et al.* (1989) identified 33 compounds in the *Lippia graveolens* HBK species. Thymol and carvacrol were the two major compounds in the essential oil fraction. These compounds are of special interest due to their antioxidant and antimicrobial properties (Baricevic and Bartol, 2002). The presence of α-*p*-dimethylstyrene, aromadenrene, α-humulene and eremophilene in the volatile oil was also reported (Pino *et al.*, 1989).

Duration of daylight, temperature, water stress and the plant growth phase affect the development of the oregano plant and its essential oil composition (Tucker and Maciarello, 1994). Recently, we reported the effect of moisture and plant growth phase on the essential oil composition of Mexican oregano in field trials (Vazquez and Dunford, 2005). The experimental results indicated that plant growth has a significant effect on the essential oil composition. The amount of water received by the plant did not have a significant effect on thymol and carvacrol concentrations in the essential oil obtained from Mexican oregano. Our previous study was carried out on samples obtained from the field where there was not good control over the water received by the plants. It is conceivable that natural moisture precipitation on the plants during the experimental period (110 days) may have affected the experimental results to a certain degree. It was perceived that greenhouse tests would allow better control of the experimental parameters. Hence, these tests would reflect the treatment effects to a better extent than the field trial.

Therefore, the main objective of this study was to examine the effect of moisture on plant growth and thymol and carvacrol contents of Mexican oregano grown under controlled conditions.

Materials and methods

The experiments were carried out in green house of Department of Plant and Soil Sciences at Oklahoma State University.

Greenhouse roof was an opaque fiberglass material that blocks some of the sunlight. The greenhouse ambient temperature was maintained at 22-24°C. The photoperiod was 16 h daylight and 8 h night. The seeds were collected from plants grown in Chihuaha, Mexico (*Lippia berlandieri* Schauer). Seedlings were developed in small perforated plastic tubes (5 oregano seeds/tube) in the same greenhouse (October 2002). Two-month old seedlings were transplanted (December 2002) into individual plastic pots (1 plant/pot in 25 cm i.d. and 23 cm deep pots). The pots were filled with a commercial peat moss/bark mixture (Metro-Mix Soil Mix, American Plant Product and Services, Oklahoma City, OK). Initial height of the seedlings was 15 cm. Experiments were set up as a 3 x 4 factorial, randomized complete block design. There were 4 watering schemes (0.2, 0.4, 0.8 and 1.2 L water/pot/15 days) and 3 growth phases (seedling = 30 days after transplant (S), full bloom = 60 days after transplant (F) and maturity = 90 days after transplant (M)). Five replicates were carried out for each treatment. Each replicate consisted of one plant. There was no fertilizer application during the plant growth tests.

All oregano leaves harvested from each plant were dried on a laboratory bench at room temperature. Essential oil was extracted from harvested plant material using a laboratory scale steam distillation unit. The extraction unit consisted of two Erlenmeyer flasks, one containing water and the other containing dry oregano leaves, placed in a hot water bath. The steam generated in the first flask was directed into the flask containing oregano leaves. The steam + oil mixture from the second flask was cooled through a Graham type condenser. Extraction time was 3h. The condensate was collected in a separatory funnel to allow water and oil phase separation. The oil fraction was recovered from the water phase using petroleum ether. Oil samples were analyzed by a HP 6890 Plus gas chromatography system equipped with a flame ionization detector (FID) (HP Company, Wilmington, DE). A Perkin Elmer PE-1 capillary column (30 m, 0.25 mm i.d. and 0.25 mm film thickness) was used for separation of the oil components. Essential oil standards (thymol and carvacrol, 1,8-cineole, *p*-cymene and γ-terpinene) were purchased from Sigma (Sigma-Aldrich, St. Louis, MO). The helium carrier gas flow rate was 26 cm/s. The injector temperature was maintained at 250°C. A temperature program with total run time of 25 min was used. The column temperature, after an initial isothermal period of 1 min at 55°C, was increased to 95°C at a rate of 3°C min^{-1}, and maintained at this temperature for 1 min. Then the column temperature was further increased to 220°C at a ramp rate of 20°C min^{-1} and maintained at this temperature for 3.42 min. The detector conditions were as follows: temperature 250°C, H_2 flow 40 mL/min, air flow 400 ml min^{-1} and make-up gas (He) 30 ml min^{-1}. Oil samples (1 µl) were injected by an autosampler (HP 7683, HP Company, Wilmington, DE). Peak areas were calculated and data collection was managed using an HP Chemstation (Revision. A.09.01, Agilent Technologies, Palo Alto, CA).

Statistical Analysis: All extraction runs and analyses were carried out at least in duplicate and in randomized order with the mean values being reported. Analysis of variance (ANOVA) of the results was performed using General Linear Model procedure of SAS (Software Version 8.1. SAS Institute Inc., Cary, NC). Multiple comparison of the various means were carried out by LSD (Least Significant Difference) test at $P = 0.05$.

Results and discussion

The volumes of water used for the treatment of oregano plants were based on the author's estimation of natural moisture precipitation on plants in Chihuahua, Mexico. Furthermore, relatively low water volumes used for plant treatment allowed us to evaluate the effect of water stress on the plant growth and essential oil composition. Both moisture and the plant growth phase had a significant effect on the plant material production (Table 1). As expected, plants that received more water produced a higher amount of fresh and dry matter. Mature plants, which received 1.2 l water in 2 week period produced the largest amount of plant material. Mature plants also produced significantly higher amount of leaves than stems (dry leaves/dry stems, w/w, ratio is about 2) as compared to the younger plants (dry leaves/dry stems, w/w, ratio is about 1). The height of the plants also increased with increasing amount of water application (Table 1). These results indicate that water stress on the plants may significantly reduce the production capacity of Mexican oregano.

Table 1. Effect of moisture and plant growth on plant material production

Treatment	Fresh matter (g)	Dry leaves (g)	Dry stems (g)	Dry total matter (g)	Plant height (cm)
S0.2	6.3^{f}	0.8^{f}	0.7^{g}	1.4^{f}	19.4^{k}
S0.4	7.3^{f}	0.9^{f}	0.6^{g}	1.4^{f}	24.6^{j}
S0.8	12.1^{e}	1.0^{f}	$1.1^{f,g}$	$2.0^{e,f}$	30.4^{i}
S1.2	12.1^{e}	$0.8^{e,f}$	$1.4^{f,g}$	$2.3^{e,f}$	38.6^{h}
F0.2	7.3^{f}	$1.4^{e,f}$	$1.1^{f,g}$	$2.2^{e,f}$	43.6^{g}
F0.4	13.0^{e}	1.9^{e}	$1.6^{e,f}$	3.5^{e}	48.0^{f}
F0.8	24.3^{d}	2.7^{d}	$2.9^{c,d}$	5.7^{d}	62.4^{d}
F1.2	32.3^{c}	2.8^{d}	3.8^{c}	6.9^{d}	72.6^{c}
M0.2	11.9^{e}	3.2^{d}	$2.3^{d,e}$	5.5^{d}	53.0^{e}
M0.4	23.1^{d}	6.6^{a}	3.3^{c}	9.9^{c}	61.2^{d}
M0.8	44.6^{b}	11.5^{b}	5.8^{b}	17.4^{b}	82.4^{b}
M1.2	56.6^{a}	13.8^{a}	8.0^{a}	21.8^{a}	91.2^{a}

Means in the same column with the same alphabet are not significantly different at P=0.05. First part of the treatment labels refers to plant growth phase and second part is for the amount of water applications; *i.e.* S-0.2: Seedlings received 0.2 l water 2 $weeks^{-1}$.
S = Seedling stage (30 days old), F = Full boom (60 days old), M = Maturity (90 days old).

Essential oil content of the oregano plants varied between 0.7 to 2.5% (w/w) (Table 2). Although on the average the younger plants contained more oil than that of the older plants the differences were not statistically significant (P>0.05). This is partly due to the large deviation in oil content among the individual plants. For example the average oil content for the treatment M-0.2 (mature plant received 0.2 l moisture/2 weeks) was much higher than that in the rest of the mature plants. This was due to one plant among the 5 replicates having extremely high oil content (4.2%) as compared to the other plants within the same treatment group (1.6-1.9%). Same phenomena was observed among the other treatment replicates. A few plants among the same treatment replicates had either extremely low or high oil content. The variations might have been due to genetic variability of the germplasm and/or the seeds used for the greenhouse tests.

Volatile oils from Mexican oregano mainly consisted of thymol,

carvacrol, *p*-cymene, cineole and γ-terpinene (Vazquez and Dunford, 2005). Oregano oil from younger plants contained more of these compounds (thymol + carvacrol + *p*-cymene + cineole + γ-terpinene = 60-80% of the oil, w/w) than that of the mature plants (40-60%, w/w). Our research focused on thymol and carvacrol because of their bioactivity in *in vitro* systems. It is well established that these compounds possess antioxidant and antimicrobial properties (Pascual *et al.*, 2001). Furthermore thymol and carvacrol are the major components in the Mexican oregano essential oils.

Table 2. Effect of moisture and plant growth on the thymol and carvacrol concentration (%, w/w) and oil content (%, w/w) of Mexican oregano

Treatment[1]	Oil Amount	Thymol	Carvacrol
S0.2	$2.3^{a,b,c}$	$43.4^{a,b}$	25.0^{b}
S0.4	2.5^{a}	$37.1^{a,b}$	25.5^{b}
S0.8	$2.0^{a,b,c,d}$	$46.7^{a,b}$	24.5^{b}
S1.2	$2.4^{a,b}$	$40.2^{a,b}$	21.1^{b}
F0.2	$1.5^{b,c,d,e}$	$27.8^{a,b}$	50.0^{a}
F0.4	$1.9^{a,b,c,d}$	$38.1^{a,b}$	24.6^{b}
F0.8	$1.8^{a,b,c,d}$	$38.1^{a,b}$	$29.0^{a,b}$
F1.2	$1.8^{a,b,c,d}$	53.9^{a}	$29.9^{a,b}$
M0.2	$2.3^{a,b,c}$	22.7^{b}	$29.0^{a,b}$
M0.4	$1.2^{d,e}$	$45.5^{a,b}$	$31.9^{a,b}$
M0.8	0.7^{e}	$40.9^{a,b}$	19.1^{b}
M1.2	$1.5^{d,e,c}$	$43.2^{a,b}$	15.7^{b}

Means in the same column with the same alphabet are not significantly different at P=0.05. [1]Please see Table 1 for the explanation of treatment labels.

The greenhouse tests did not reveal a clear trend for the effect of moisture and plant growth on the thymol and carvacrol content of the oil (Table 2). Although the thymol concentration in the oil decreased as plants aged at the lowest water application level (S-0.2, F-0.2 and M-0.2, plants received 0.2 l of water every two weeks), this trend was not consistently observed at higher water application levels. Furthermore, the differences were not statistically significant (P=0.05). In general, carvacrol content of Mexican oregano oil was less than that of thymol. However, there was no apparent trend for the effect of treatments on the carvacrol content in the oil. The ANOVA of the thymol and carvacrol concentration data also indicated that there was no significant main effect (plant growth phase/thymol: P = 0.6094, moisture/thymol: P = 0.4559, plant growth phase/carvacrol: P = 0.2112 and moisture/carvacrol: P = 0.0877) and moisture by plant growth phase interaction (thymol: P = 0.5231 and carvacrol: P = 0.1685).

The amount of moisture received by the plants does not affect the Mexican oregano essential oil composition significantly. These results confirm the findings of the field trials that were carried out using the same oregano species (Vazquez and Dunford, 2005). The greenhouse tests also allowed us to examine the variations among individual plants that received the same treatment in a controlled environment. Oregano plants that contained a very large amount of either thymol or carvacrol were identified. Currently these plants are being tested for stability of their chemical composition as a function of time.

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