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Impacts of harvest time and water stress on the growth and essential oil components of horehound (*Marrubium vulgare*)

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ABSTRACT

The study investigated the effect of water stress and harvesting frequency on the growth and essential oil (EO) content and components of *Marrubium vulgare* grown under Egyptian conditions. Two-month old seedlings of *Marrubium vulgare* were transplanted into pots and irrigation treatments (4, 8 and 12 days) were applied one month after transplanting. Growth and chemical component response variables were determined at flowering stage using plants harvested at 80, 150 and 210 days after transplanting. Essential oil was obtained by hydro-distillation and expressed as ml 100 g^{-1} fresh herb. Chemical composition of the EO was determined using liquid chromatography linked to mass spectrometry (GC–MS). The results showed that *Marrubium* plants irrigated every 4 days and harvested at 210 days gave the highest plant height, number of branches and fresh weight, whereas their lowest values were obtained when harvested at 210 days and irrigated every 12 days. On the other hand, the EO content of *Marrubium* plants irrigated every 12 days and harvested at 80 days was the highest, and the lowest was obtained when harvested at 210 days and irrigated every 4 days. Eighty compounds were identified in the essential oil of *Marrubium vulgare*. Thymol (29.6–60.7%), carvacrol (0.5–19.3%), m-cymene (1.0–14.2%), γ-terpinene (1.1–12.1%), thymol methyl ether (0.4–10.4%) and α-himachalene (0.0–10.3%) were the major marker compounds, whereas 14 compounds were minor and 60 compounds were considered as traces. In summary, water stress and harvesting frequency affected growth and caused quantitative changes in the essential oil components. It can be concluded that irrigating *Marrubium vulgare* plants every 4 days and harvesting at 210 days from the transplanting date is essential to maximize production, whereas irrigating every 12 days and harvesting 80 days after transplanting is recommended to maximize essential oil content.

1. Introduction

Marrubium vulgare L. (horehound, white horehound) belongs to the *Lamiaceae* family. It is a perennial, herbaceous medicinal plant native to Europe, northern Africa, and southwestern and central Asia. This plant was frequently employed as a folk medicine to treat a variety of ailments related to upper respiratory tract infections. Nowadays, the plant is widely used as an herbal medicine to treat liver diseases, biliary tract disorders, bronchial asthma and nonproductive cough (Verma et al., 2012). It possesses tonic, stimulant, expectorant, antispasmodic, antidiabetic, diaphoretic, and diuretic properties (Boudjelal et al., 2012; Vergara-Galicia et al., 2012). Essential oils are appreciated for their bioactive efficacy as fungicides, bactericides (Zarai et al., 2011), antioxidants (Vergara-Galicia et al., 2012) and other biological activities.

Plant production and essential oil content can be influenced independently by changes in harvesting time or by environmental factors (Hay and Waterman, 1993). Environmental stresses are among the factors most limiting to plant productivity. The environmental factors can also influence the growth and biosynthesis of secondary metabolites in medicinal and aromatic plants. Essential oil yields have been affected by osmotic stress (Charles et al., 1990). Water deficit in plants may lead to physiological disorders, such as a reduction in photosynthesis and transpiration (Sarker et al., 2005). Water stress in plants influences many metabolic processes, and the extent of its effects depends on drought severity. The optimization of irrigation for the

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production of fresh herbs and essential oils is important, since water is a major component of the fresh produce and affects both weight and quality (Jones and Tardien, 1998). In aromatic plants, soil moisture may cause significant changes in the yield and composition of essential oils; for example, water deficit decreased the oil yield of rosemary (*Rosmarinus officinalis* L.) (Singh and Ramesh, 2000) and anise (*Pimpinella anisum* L.) (Zehtab-Salmasi et al., 2001). By contrast, water stress caused a significant increase in oil yield of citronella grass (*Cymbopogen winterianus* Jowitt.) expressed on the basis of plant fresh mass (Fatima et al., 2000) with the severity of the water stress response varying with cultivar and plant density in parsley (*Petroselinum crispum*L.) (Petropoulos et al., 2008). Said-Al Ahl and Abdou (2009) on dragonhead and Said-Al Ahl et al. (2009a, 2009b) showed that both essential oil content (%) and yield significantly decreased with increasing water stress levels of *Origanum vulgare* and *Melissa officinalis,* respectively.

In most perennial aromatic plants, reduction in biomass yield due to repeated harvesting affects essential oil yield. Repeated harvests can either be beneficial or detrimental to oil production, depending on other environmental factors. For example, herbage yield is usually high at first harvest, then becomes constant, and then declines with repeated harvests (Murtagh, 1996; Weiss, 1997). Kothari et al. (2004) found that biomass yield was higher in the first harvest and gradually declined in the subsequent harvests of *Ocimum tenuiflorum.* Contrary to the decrease in biomass yield, essential oil content was lower in the first harvest and increased gradually in subsequent harvests to reach maximum in the fourth harvest (Kothari et al., 2004). The essential oil in geranium is mostly contained in the leaves; therefore, the higher the proportion of leaves in the harvested produce the better the yield of oil is (Rao et al., 1990). Determination of the correct harvesting time is extremely important both for maximizing yield and oil quality as it exerts remarkable influences on the oil yield of essential oil content of crops (Weiss, 1997). Doimo et al. (1999) reported that not only the harvest time affected oil yield, but that the geographic area where these crops were grown also influenced yield. The chemical composition of the essential oil was also observed to be influenced by environmental changes (i.e., different soil water content, temperature and photoperiod). These environmental conditions may increase or decrease different terpenoids in the crop.

However, studies on irrigation intervals and harvesting frequency on yield and essential oil of *Marrubium vulgare* have not been investigated. Therefore, this study aimed to evaluate the effect of irrigation intervals on the fresh herb yield and essential oil content and their main constituents of *Marrubium vulgare* L. harvested at three times.

2. Materials and methods

2.1. Plant material

Seeds of *Marrubium vulgare* were obtained from the HEM ZADEN B.V Venhuizen − The Netherlands. Seeds were sown in the nursery on March 15 of 2014 and 2015. Two months after seed sowing, the seedlings were transplanted into pots (30 cm diameter, 50 cm depth) filled with 10 kg of air dried soil on May 15 of each year. The pots contained three seedlings each and were placed in full sun light. The experiment was carried out under the natural conditions of the greenhouse of the National Research Center, Dokki, Giza, Egypt. The soil texture was sandy loam, having a physical composition of: 45.00% sand, 28.25% silt, 26.75% clay and 0.85% organic matter. Chemical analyses of the soil showed: pH = 8.40; E.C. = 0.79 dsm^{-1}; total nitrogen = 0.13%; available phosphorus = 2.18 mg/100gram; potassium = 0.02 mg/100gram. All properties were determined according to the standard methods (Jackson, 1973).

Irrigation interval treatments (4, 8 and 12 days), of two liters of water were applied per pot a month after transplanting. Growth characters (plant height [cm], number of branches/plant, herb fresh weight [g/plant]) and chemical constituents were determined at the flowering stage. Plants from each water stress level were harvested at three times; 80, 150 and 210 days after transplanting. The essential oil percentage was determined in the fresh herb using modified Clevenger apparatus (Guenther, 1961. Essential oil (%) was expressed as (ml 100 g^{-1} fresh herb). The resulted essential oil of each experimental unit was collected and dehydrated over anhydrous sodium sulphate and kept in refrigerator until GC–MS analyses.

2.2. GC–MS analyses and identification of components

GC–MS analyses were carried out on a Varian 450-GC connected with a Varian 220-MS. Separation was achieved using a Factor Four TM capillary column VF 5 ms (30m × 0.25 mm i.d., 0.25 μM film thickness). Injector type 1177 was heated to a temperature of 220 °C. Injection mode was splitless (1 μL of a 1:1000 *n*-hexane solution). Helium was used as a carrier gas at a constant column flow rate of 1.2 mL min^{-1}. Column temperature was programmed: initial temperature was 50 °C for 10 min, then increased to 100 °C at 3 °C min^{-1}, maintained isothermal for 5 min and then increased to 150 °C at 10 °C min^{-1}. The total time for analysis was 46.7 min. The mass spectrometer trap was heated to 200 °C, manifold 50 °C and transfer line 270 °C. Mass spectra were scanned every 1 s in the range 40–650*m/z*. Components were identified by comparison of their mass spectra with those stored in NIST 02 (software library) or with mass spectra from the literature (Jennings and Shibamoto, 1980; Adams, 2007), as well as by comparison of their retention indices with standards.

2.3. Statistical analysis

Repeated Measures Analysis (RMA) of a Randomized Blocks Design with the factor of interest being Irrigation (3 levels: 4, 8, and 12 days interval), and harvesting repeatedly at three time points (Day 80,150, and 210 after transplanting) was completed to determine the effect of irrigation on height, number of branches, weight, and volatile oil of Marrubium, and how this effect evolved over the harvesting times. Since the whole experiment was conducted during two seasons (2014 and 2015), Season was used as a blocking factor. Since the response measurements were measured at different harvest times from the same experimental unit (pot), the values measured repeatedly are expected to be dependent on each other. The most appropriate covariance structure (dependence) for each response variable was determined using the Akaike Information Criterion (Littell et al., 1998). The validity of model assumptions (normal distribution and constant variance of the error terms) were verified by examining the residuals as described in Montgomery (2013). All analyses were completed using the Mixed Procedure of SAS (SAS Institute Inc., 2014). For significant (p-value < 0.05) or marginally significant ($0.05 < p\text{-value} < 0.1$) effects, multiple means comparisons were completed by comparing the least squares means of the corresponding treatment combinations. Letter groupings were generated using a 5% level of significance for the main effects and using a 1% level of significance for interaction effects to protect Type I experimentwise error rate from over inflation.

3. Results and discussion

3.1. Growth parameters

The interaction effect of Irrigation interval and Harvest day was significant on plant height, number of branches, and herb fresh weight of *Marrubium vulgare* (Table 1), which suggests that the effect of irrigation interval was not consistent at all harvest days after transplanting.

The plants irrigated every 4 days gave the highest height, number of branches, and weight at 210 days after transplanting (Fig. 1). The lowest mean height, number of branches, and weight was obtained from those irrigated every 12 days and harvested on day 80 after transplanting (Fig. 1). However, at 80 days after transplanting,