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Irrigation dose and plant density affect the essential oil content and sensory quality of parsley (*Petroselinum sativum*)

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ABSTRACT

In the current study the influence of 3 irrigation treatments (ID0 as a control, ID1, and ID2), and 3 plant density treatments (PD0 as a control, PD1, and PD2) were investigated on the production (total yield), volatile composition of essential oil, and sensory quality of parsley (*Petroselinum sativum*). The results showed that the highest plant yield was obtained when using the highest values of both irrigation dose (ID2 = 1788 m^3 ha^{-1}) and plant density (PD2 = 7.41 plants m^{-2}). Hydrodistillation technique was used to extract the essential oil of parsley shoots and GC–MS and GC-FID were used to identify and quantify the components of the essential oil, respectively. The results showed that the main compounds of the essential oil were β-phellandrene, *1,3,8-p*-menthatriene, myristicin, myrcene, terpinolene, limonene, α-pinene, *and* α-phellandrene. The treatment ID1 (861 m^3 ha^{-1}) led to the highest concentrations of most of the main compounds: 1,3,8-*p*-menthatriene (150 mg kg^{-1}), myristicin (46.8 mg kg^{-1}), and myrcene (33.7 mg kg^{-1}); a similar pattern was found for the plant density PD0 (5.56 plants m^{-2}), with contents being *1,3,8-p*-menthatriene (143 mg kg^{-1}), *β*-phellandrene (130 mg kg^{-1}), and myristicin (38.1 mg kg^{-1}). Aroma attributes, such as parsley-like, citrus, and green grass significantly had the highest intensities in ID1 and PD0 plants. The final recommendation based on all data generated is to use the irrigation dose of 861 m^3 ha^{-1} (ID1) and the plant density of 5.56 plants m^{-2} (PD0) for better yield and quality of the final product under the assayed conditions.

1. Introduction

Parsley is a very popular herb, native of the Mediterranean region, and widely used as herb or spice in Europe, America, and Middle Eastern countries. The varieties of parsley differ according to which organs or parts of the plant are used. There are plain and curly-leafed varieties, which are basically used for their leaves, as well as turnip-rooted varieties, such as Hamburg type varieties, which are used for their fleshy roots (Petropoulos et al., 2006, 2008; Najla et al., 2012). Parsley is native to Europe and Western Asia and is cultivated worldwide as an annual crop for its aromatic and attractive leaves (Simon and Quinn, 1988; Zhang et al., 2006). Parsley, leaves and seeds, is commonly used as food condiment in many dishes, such as meats, fishes, salads, creams, or soups. It is also a rich source of essential oil, which can be also extracted from leaves and seeds and used as a flavoring agent or fragrance in perfumes (Zhang et al., 2006). There are different techniques for the extraction of essential oils from parsley, for instance, Vokk et al. (2011) used hydrodistillation with Clevenger apparatus to isolate the essential oils of dried plant material of dill and parsley, while Petropoulos et al. (2004) employed simultaneous distillation–extraction (SDE) for the same objective. However, other isolation techniques have been successfully applied in aromatic herbs, such as hydrodistillation with Deryng apparatus (Calín-Sánchez et al., 2015). Moreover, microwave extraction process was applied by Costa et al. (2014) for the extraction of essential oil of mint and volatile compounds of fresh coriander leaves, which were extracted using solid-phase microextraction (SPME) (Fan and Sokorai, 2002).

Vokk et al. (2011) reported that the major constituents in the essential oil of Estonian parsley leaves were myristicin, β-phellandrene, *p*-1,3,8-menthatriene and β-myrcene; while the results obtained by Petropoulos et al. (2004) indicated that the

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main compounds of essential oil of Greek parsley plants were β-phellandrene, 1,3,8-*p*-menthatriene, *p*-dimethylstyrene, myristicin, β-myrcene, and apiole.

In fact, there are many factors affecting the composition of essential oil and also the yield of herbs, such as irrigation dose, plant density, sowing date, climate of the area, among others. For instance, Khazaie et al. (2008) studied the effect of irrigation frequency and planting density on herbage biomass and oil production of thyme; Callan et al. (2007) studied the effect of plant density on the dill oil composition, and Petropoulos et al. (2004) studied the effect of sowing date on the parsley essential oil composition.

The optimization of irrigation for the production of parsley fresh leaves is essential because, as in other horticultural crops, water is a major component of the fresh plant material, and significantly affects both weight and quality (Jones and Tardieu, 1998; Petropoulos et al., 2008). Providing a permanent source of water is a priority to increase the production and improve the quality of the cultivated vegetables; besides, parsley is classified as a sensitive plant to water stress (Najla et al., 2012). Khazaie et al. (2008) reported that optimum planting density is a key factor to achieve maximum crop production, especially when water is a limiting factor, as it is the case of the Spanish agriculture. Several authors discussed the effect of plant density on the yield production and essential oil yield. For instance, Shalaby and Razin (1992) reported that herbage biomass and essential oil production of thyme increased at lower planting distances (Khazaie et al., 2008). El-Gendy et al. (2001) showed that the lowest planting distance (15 cm) resulted in higher biomass and essential oil yield compared to a 45 cm planting distance in sweet basil (Khazaie et al., 2008).

The aim of this study was to optimize the irrigation dose and plant density to obtain the highest production (yield, kg ha^{-1}) and highest quality (essential oil content and sensory quality) of parsley.

2. Material and methods

2.1. Plant material, irrigation doses and plant density

Parsley seeds (*Petroselinum sativum* L.), cultivar *Gigante Italiano Darkness* (plain type) were sown on the 19th of September 2014 in expanded polystyrene (EPS) trays (41 cm × 65 cm, with 260 cells) and placed in a greenhouse located at Santomera (Murcia, Spain) until 17th of October. Then, plantlets were transplanted to a commercial parsley orchard located at Sucina (Murcia, Spain) with a total surface of 2.5 ha. Parsley plants were grown using a high-frequency drip irrigation system.

Irrigation was carried out according to 3 irrigation doses consisting on the following total water amounts: (i) control treatment, ID0, normal irrigation conditions, with 1300 m^3 ha^{-1}; (ii) treatment 1, ID1, with lower than normal irrigation conditions, 861 m^3 ha^{-1}; and, (iii) treatment 2, ID2, with higher than normal irrigation conditions, 1788 m^3 ha^{-1}. The plant densities of the 3 treatments was reached by using plant lines separated by 0.9 m and a distance of 0.20 m between plants of the same line, leading to a plant density of 5.56 plants m^{-2} (e.g. 56 plants in a surface of 10 m^2). The total volume of water was settled according to the irrigation time. The water contribution was carried out using polyethylene pipes of 16 mm of diameter. The drippers were adjusted at both different flows and drippers distance to fit the established volume of water. At ID0 irrigation conditions, 16 mm pipes, separated by 0.9 m, were used, with a distance between two consecutive drippers of 0.32 m; the flow was 1.6 L h^{-1} for each emitter with an irrigation surface of 0.29 m^2; the total number of drippers per ha was 34722, with a total volume of water of 53.87 m^3 ha^{-1} (~24 h of irrigation). ID1 was the lowest irrigation dose and at these conditions, 16 mm pipes, separated by 0.9 m, were used, with an emitter distance of 0.50 m; the flow was 1.6 L h^{-1} for each emitter with an irrigation surface of 0.45 m^2; the total number of drippers per ha was 22,222, with a total volume of water of 35.55 m^3 ha^{-1} (~24 h of irrigation). ID2 was the highest irrigation dose, and at these conditions, 16 mm pipes, separated by 0.9 m, were used, with emitter distance of 0.32 m; the flow was 2.2 L h^{-1} for each emitter with an irrigation surface of 0.29 m^2; the total number of drippers per ha were 34722, with a total volume of water of 74.07 m^3 ha^{-1} (~24 h of irrigation).

Regarding plant density, 3 treatments were assayed. The water contribution was carried out using polyethylene pipes of 16 mm of diameter, with a distance between drippers of 0.33 m. The flow was 1.6 L ha^{-1} for each emitter, making a total volume of water applied of 1290 m^3 ha^{-1}, according to the irrigation time. Plant densities under study were as following: (i) control treatment, PD0 (5.56 plants m^{-2}), or normal plant density, with plant lines separated by 0.9 m, a distance of 0.20 m between plants of the same line; (ii) treatment 1, PD1 (4.44 plants m^{-2}), with plant lines separated by 0.9 m, a distance of 0.25 m between root balls; and, (iii) treatment 2, PD2 (7.41 plants m^{-2}), with lines separated by 0.9 m, 0.15 m between root balls. Thirty-six plants of each treatment were assayed in the following surfaces: PD0 required a total surface of 6.48 m^2, PD1 surface was of 8.10 m^2, and finally PD2 needed a total surface of 4.86 m^2. All the field treatments were run in triplicate.

The irrigation water was of good quality, highlighting its slightly basic pH (7.91), and its proper electrical conductivity (1.26 mS cm^{-1}), which is suitable for growing aromatic herbs crops. Soil was uniformly silty-loam in texture, with a low content in organic matter (1.22%), medium salinity conditions (3.35 mS cm^{-1}) and appropriate levels of sulfates (37.83 meq L^{-1}) for parsley development. Along the development of parsley plants, fertilization was carried out with a total amount of N of 130 kg ha^{-1}, P (P_2O_5) of 60 kg ha^{-1}, and K (K_2O) of 160 kg ha^{-1}.

2.2. Extraction of essential oil

Hydrodistillation (HD), using a Deryng system (the Polish version of the Clevenger apparatus), was used for isolating the essential oil in fresh parsley. About 15.0 g of fresh chopped parsley shoots (aerial part of the plant, including stems and leaves) were put in a 500 mL round bottom flask, together with 1.0 g sodium chloride (NaCl), 150 mL of distilled water, and 50 μL of benzyl acetate as internal standard. After the mixture started boiling, heating was maintained for 1 h. A cold refrigerant was used to condense the vapors, and 1 mL of cyclohexane was added to the Deryng apparatus at the beginning of the hydrodistillation process to retain the essential oil distilled from the samples of parsley shoots. After 60 min of extraction, the solvent, enriched with the volatile compounds, was transferred into a 2.5 mL vial, after drying it over anhydrous sodium sulfate (Na_2SO_4), and kept at −15 °C until the GC–MS and GC-FID analyses were conducted. The extractions were conducted in triplicate.

2.3. Chromatographic analyses

Isolation and identification of the volatile compounds were performed using a Shimadzu GC-17A gas chromatograph coupled with a Shimadzu QP-5050A mass spectrometer detector (Shimadzu Corporation, Kyoto, Japan). The GC–MS system was equipped with a TRACSIL Meta.X5 (95% dimethylpolysiloxane and 5% diphenylpolysiloxane) column (60 m × 0.25 mm, 0.25 μm film thickness; Teknokroma S. Coop. C. Ltd, Barcelona, Spain). Analyses were carried out using helium as carrier gas at a flow rate of 0.3 mL min^{-1} in a split ratio of 1:11 and the following program: (a) 80 °C for 0 min; (b) increase by 3 °C min^{-1} from 80 to 210 °C,