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Essential oil content, yield, and components from the herb, leaf, and stem of curly-leafed parsley at three harvest days

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ABSTRACT

Parsley (*Petroselium crispum* L.) is commonly used for its flavor, desired nutritional contents, and other health benefits. However, since the profile of a recently introduced curly-leafed parsley cultivar in Egypt has not been studied, an experiment was conducted to compare three harvest dates in terms of the weight, essential oil (EO) content and yield, and the concentrations of major components in the whole herb, leaf, and stem parts. The results showed that the highest herb and leaf yields were obtained from the second harvest, but the first harvest gave the highest stem yield. The highest EO content and yield were obtained from the first harvest. The major EO components obtained from the three parts were β-phellandrene, α-terpinolene, 1,3,8-p-menthatriene, myristicin, and elemicin. The highest concentrations of α-terpinolene, myristicin, and elemicin were obtained from the whole herb; but the highest β-phellandrene and 1,3,8-p-menthatriene were obtained from the leaf and the stem. The findings revealed that the yield, EO content and yield, and concentration of the major components varied with harvest day and part of the plant. These results can be used to determine when and where to extract EO to maximize the desired content, yield, or component.

Keywords: *Petroselium crispum*, herb, harvest date, essential oil, β-phellandrene, 1,3,8-p-menthatriene, myristicin

INTRODUCTION

Parsley (*Petroselium crispum*) is a species of the Apiaceae (Umbelliferae) family, grown worldwide and native to the Mediterranean region (Alharbi et al., 2019). Parsley is gaining popularity since ancient times due to its ability to enhance flavor and aroma; and due to its composition of vitamins, minerals, and other bioactive compounds such as furanocoumarins, essentials oils, flavonoids, carotenoids, oleoresins, tannins, glycosides, and fatty acids (El-Sayed et al., 2018; Chauhan and Aishwarya, 2018; Dobricević et al., 2019; Petropoulos et al., 2010).

The detection of these bioactive compounds in parsley, led to its use in pharmaceutical, perfume, cosmetic industries, and as a flavoring and aromatic food additive in food industries (El-Sayed et al., 2018). Furthermore, it is included in many therapies such as for hepatoprotective, anti-diabetic, antibacterial, antifungal, analgesic, diuretic, gastroprotective, hypotensive, immunosuppressant, anaemia, inflammation, and diabetes, and in the relief of the symptoms of allergies, dyspepsia, chronic bronchitis, hypotension, thrombosis, and strokes (El-Sayed et al., 2018; Chauhan and Aishwarya, 2018).

Herb and biosynthesis of phyto-compounds may be affected by many factors such as the meteorological conditions, the harvesting time, the genotype, the irrigation regime, and the planting density and agronomic practices (Alharbi et al., 2019; El-Zaeddi et al., 2016a; Petropoulos et al., 2008). The accumulation of more polyphenols and pigment complexes (β-carotene) in parsley harvested in autumn compared to that harvested in spring show seasonal variations, and deficit in irrigation may increase the contents of vitamin C and anthocyanin in parsley leaves (Rowland et al., 2018; Najla et al., 2012).

The importance of harvest time and its effect on the quantity and quality of essential oil was studied by several researchers including Court et al. (1993), and Carvalho Filho et al. (2006). Previous studies showed that the time of harvest is an important factor that influences the quantity and quality of essential oil from herbal plants, and that the ideal harvest time varies with the plant and with what was harvested (Mahmoud et al., 2018; Sabra et al., 2018; Said-Al Ahl et al., 2019).

The goal of the present study is to evaluate the new parsley cultivar [*Petroselinum crispum* cv. Moss curled no. 2 (curly leaf type)] for its potential for mass production, its essential oil content, and its essential oil compound contents in the leaves, stems, and herb during three different harvest dates under growing conditions in Egypt, which represents the Mediterranean region.

MATERIALS AND METHODS

Plant material and growing conditions

A field experiment was done at the Experimental Farm of the Faculty of Agriculture, Cairo University, Giza, Egypt, during the 2017/2018 and 2018/2019 growing seasons (planted on November 17 and harvested on February 1, March 1, and April 1). *Petroselinum crispum* cv. Moss curled no. 2 (curly leaf type) seeds were obtained from the HEM ZADEN B.V- P.O. Box 4-1606 ZG Venhuizen-The Netherlands. The seeds were sown during the two seasons into 3 x 3.5 m plots on rows, with 60 cm apart and 5 cm between the seeds on both sides of the row. The climate of the study area is subtropical desert (BWh climate according to Köppen classification), with mild, wet winters and warm, dry summers. Mostafa et al. (2019) has more details of the weather and climatic data.

The soil texture was sandy loam, with the following physical composition: 45.5% sand, 27.6% silt, 26.9% clay, and 0.87% organic matter. Chemical analysis of the soil resulted in: pH = 8.05; EC = 0.80 dS/m; total nitrogen = 0.1%; available phosphorus and potassium were 2.11 and 0.021 mg/100g, respectively; sodium = 3.55 meq/100g soil, and chloride = 0.52 meq/100g soil. All soil properties were determined by using standard methods. The experimental layout was completely randomized blocks design with three blocks.

During each growing season, the plants were harvested 3 times (at vegetative stage) on 1st February (105 days after sowing), 1st March (135 days after sowing), and 1st April (165 days after sowing). On each harvest day, fresh herb was harvested at 5 cm above the soil and immediately transferred to the laboratory to extract the essential oil.

Fresh weights and volatile oil content were determined for whole herb, leaves, and stems separately at the three harvest dates. Representative plant samples were hydro distillated using a Clevenger-type apparatus for 3 h according to Guenther (1961). Essential oil content (%) was expressed as ml/100g fresh material, while essential oil yield was expressed as L/ha. The collected and dehydrated essential oils were kept in refrigerator until GC-MS analyses.

GC-MS analyses and identification of components

GC-MS analysis of the essential oil was performed using HP 5890 series II gas chromatograph and HP 5973 mass detector. A TRFAME (Thermo 260 M142 P) capillary column (30 m × 0.25 mm i.d., 0.25 µm film thickness) with helium carrier gas (at a flow rate of 1.5 ml/min) was used. The initial GC oven temperature was set at 40 °C for 5 minutes and then heated up to 140 °C at a rate of 5 °C/min and held at 140 °C for 5 min. Then the temperature was increased to 280 °C at a rate of 10 °C/min and held for 5 additional min. Injector and detector temperatures were 250 °C. Diluted samples (1/100, v/v in heptane) of 1.0 µl were injected automatically. Mass spectrometry was run in the electron impact mode (EI) at 70 eV. The components were identified based on their GC retention times. Mass spectra interpretation was confirmed by mass spectral library database of the National Institute of Standards and Technology (NIST) (Adams, 2007).

Statistical analyses

Analysis of Variance (ANOVA) of a Randomized Blocks Design (RBD) was completed to determine if there is a significant difference among 3 harvest dates. Since the experiment was designed as RBD with 3 blocks within each of the 2017/2018 and 2018/2019 growing seasons, the 6 combinations of the 3 original blocks and the 2 seasons were used as blocks. The 3 levels of harvest day are: 105 days after sowing, 135 after sowing, and 165 days after sowing. The analyzed 9 response variables are: herb weight, leaf weight, stem weight, oil content in herb, oil content in leaf, oil content in stem, oil yield in herb, oil yield in leaf, and oil yield in stem. The statistical analysis was completed using the Mixed Procedure of SAS (SAS, 2014). Since the effect of harvest day was highly significant ($P<0.01$) on all response variables, further multiple means comparison was completed using Tukey's multiple range test at 5% level of significance and letter groupings were generated. For each response variable, the validity of model assumptions was verified by examining the residuals as described in Montgomery (2020).

RESULTS AND DISCUSSIONS

The Analysis of Variance results showed significant differences among the means of the three harvest days in all nine response variables analysed. To determine the specific differences among the three harvest days, multiple means comparison was conducted, and the results shown in Table 1 reveal that the difference between the mean herb yield in the first (2275 kg/ha) and in the second (2356 kg/ha) harvests is not significant, but the mean herb yield in the third (1835 kg/ha) harvest is significantly different from that of the first and the second harvests. However, the mean weight of the leaves in the second (1683 kg/ha) was significantly larger than that in the first (1404 kg/ha) and the third (1385 kg/ha) harvests, which are not statistically different. But the mean stem weights in the first, second, and third harvests (871, 674, and 450 kg/ha, respectively) were significantly different from each other.

Alharbi et al. (2019) reported that the herb yield in three cultivars grown in Egypt ranged from 737 to 1482 kg/ha, which indicates that this recently released cultivar gives higher yield at each of the three harvests.

The pattern in the essential oil (EO) content was different from that of the weight. Accordingly, the mean EO content in the herb and in the leaf during the three harvests decreased significantly at each harvest, with the highest content being obtained during the first harvest (Table 1). However, the EO from the stem obtained at the first and the second harvests were not significantly different, but the third harvest gave significantly lower content (Table 1).

The EO yield from the herb and the stem decreased significantly at each harvest (Table 1) with the highest being obtained from the first harvest. However, the mean EO yields from the leaf during the first two harvests were not significantly different, and the third harvest gave significantly lower yield (Table 1).

Table 2 shows the volatile oil major compounds determined using GC/MS analysis of essential oil obtained from the herb, leaf, and stem during the second season of the experiment. Accordingly, there were five major compounds, namely, β-hellandrene, α-terpinolene, 1,3,8-p-menthatriene, myristicin, and elemicin. The GC/MS analysis also gave other compounds in the essential oil of herb, leaf, and stem of parsley such as α-pinene, sabinene, α-phellandrene, α-myrcene, α-sesquiphellandrene and α-elemene, but their concentrations were less than 1% or traces.

Comparison of the concentrations of these major compounds of the essential oil reveals that the essential oil extracted from the whole herb gave the highest average concentrations of α-terpinolene, myristicin, and elemicin. The leaf gave the next average concentrations of α-terpinolene and elemicin, but the stem gave the next average of myristicin. The concentrations of α-terpinolene and elemicin in all three parts of the plant increased with the harvest day after sowing, whereas that of myristicin decreased in the whole herb and stem with increasing harvest day and stayed similar in the leaf (Table 2).

Table 1. Mean herb weight (kg/ha), leaf weight (kg/ha), stem weight (kg/ha), herb essential oil (EO) content (%), leaf EO content (%), stem EO content (%), herb EO yield (L/ha), leaf EO yield (L/ha), and stem EO yield (L/ha) obtained during the 3 harvest days after sowing. The last row shows the overall mean values

Harvest day	Herb weight (kg/ha)	Leaf weight (kg/ha)	Stem weight (kg/ha)	Herb EO content (%)	Leaf EO content (%)	Stem EO content (%)	Herb EO yield (L/ha)	Leaf EO yield (L/ha)	Stem EO yield (L/ha)
105	2275 a[1]	1404 b	871 a	0.081 a	0.085 a	0.056 a	1.840 a	1.195 a	0.486 a
135	2356 a	1683 a	674 b	0.068 b	0.075 b	0.050 a	1.609 b	1.261 a	0.336 b
165	1835 b	1385 b	450 c	0.056 c	0.059 c	0.038 b	1.023 c	0.819 b	0.179 c
Mean	2155	1491	665	0.068	0.073	0.048	1.491	1.092	0.334

[1] within each column, means sharing the same letter are not significantly different at the 5% level of significance

Table 2. Concentrations (%) of major components (β-phellandrene, α-terpinolene,1,3,8-p-menthatriene, myristicin, and elemicin) obtained from *Petroselinum crispum* cv. Moss curled no. 2 essential oil during the three harvests of the second season

Plant part	Compound	Harvest 1	Harvest 2	Harvest 3	Mean
Whole herb	β-phellandrene	14.78	25.98	38.44	26.40
	α-terpinolene	0.16	4.78	13.16	6.00
	1,3,8-p-menthatriene	20.96	17.45	14.19	17.53
	Myristicin	62.02	48.56	30.03	46.87
	Elemicin	1.80	3.87	4.16	3.27
Leaf	β-phellandrene	35.37	30.85	19.13	28.45
	α-terpinolene	0.49	5.78	10.78	5.68
	1,3,8-p-menthatriene	37.90	38.43	39.08	38.43
	Myristicin	24.69	23.29	26.71	24.89
	Elemicin	1.01	1.64	2.49	1.71
Stem	β-phellandrene	13.25	18.72	19.34	17.10
	α-terpinolene	0.87	5.74	8.65	5.08
	1,3,8-p-menthatriene	44.11	38.99	44.42	42.50
	Myristicin	40.92	36.30	25.79	34.33
	Elemicin	0.58	0.99	1.13	0.90

The highest average concentration of β-phellandrene was obtained from the leaf, followed by the whole herb and the stem; and the highest average concentration of 1,3,8-p-menthatriene was obtained from the stem followed by the leaf and the whole herb (Table 2). The concentrations of β-phellandrene in the whole herb and the stem increased with harvest day after sowing, but that in the leaf decreased. On the other hand, the concentrations of 1,3,8-p-menthatriene decreased in the whole herb and the stem increased with harvest day, but that in the leaf decreased in the whole herb but stayed similar in the leaf and stem during the three harvests (Table 2).

In terms of the magnitude of the top three concentrations, in the whole herb oil, the order of the three compounds was myristicin, β-phellandrene, and 1,3,8-p-menthatriene; in the leaf, it was 1,3,8-p-menthatriene, β-phellandrene, and myristicin; and in the stem, it was 1,3,8-p-menthatriene, myristicin, and β-phellandrene (Table 2).

According to Alharbi et al. (2019), the essential oil content in three cultivars grown in Egypt range from 0.014 to 0.173%, and the contents from this new cultivar fall in this range. Also, Craft and Setzer (2017) reported that the essential oil contents from *P. crispum* var. crispum (curly leaf parsley) and *P. crispum* var. neapolitanum (flat leaf parsley) that were obtained by hydrodistillation were between 0.193 and 0.606%. However, Simon and Quinn (1988) reported that the essential oil % ranged from 0 to 0.16% (v/fresh weight) in different samples from curly and flat leaf and Hamburg types.

Alharbi et al. (2019) found that the essential oil yield in three cultivars grown in Egypt ranged from 0.296 to 1.886 L/ha, which indicates that the mean EO yields from the different parts of the plant of this new cultivar are at par with the other three cultivars grown in the same country.

In addition to agronomic practices that influence the various physiological parameters and the photosynthesis process, plant growth, yield and chemical composition are affected by the surrounding environment including temperature, humidity, and light intensity (Said Ah Ahl et al., 2016). The climatic condition at the study area, which is classified as subtropical desert based on the temperature and the annual cycle of precipitation, could be one of the reasons for the differences in the weight and essential oil at the different harvest dates. However, the magnitude of the differences can vary from plant to plant. The cause for the variation in herbage and oil production could also be the differences in the needs of the plants during the different growth stages. Kothari et al. (2004) reported larger biomass yield during the first harvest and then gradually decreased during the subsequent harvests of holy basil (*Ocimum tenuiflorum* L.). This was due to differences in temperature and its major role in plant growth, yield, and regulating changes of metabolic activities such as photosynthesis, respiration, and transpiration (Letchamo et al., 1995). Weiss (1997) reported that maximum leaf growth with high oil content of geranium was obtained under warm sunny conditions; however, growth of geranium increased under long-day photoperiods because of increased chlorophyll content. It was also reported that variability in shoot yield and essential oil of thyme was associated to photosynthetic activities (Letchamo et al., 1995).

Our study examined the effect of harvest time to find out the optimum harvest time for curly-leaf parsley under Egyptian agronomic condition based on the expected impact of harvest time, along with environmental conditions, and their effect on the essential oil and its components reported by Thompson et al. (2003). Several studies have revealed the great importance of the effect of harvest time on biomass production of medicinal plants and their active components such as essential oil and its components (e.g., of parsley (*Petroselinum crispum* L.) (Alharbi et al., 2019); of catmint (*Nepeta cataria*) and lemon catmint (*Nepeta cataria* var. citriodora) (Said-Al Ahl et al., 2018a; Mohamed et al., 2018); of thyme (*Thymus vulgaris*) (Said-Al Ahl et al., 2016); of lemon balm (*Melissa officinalis*) (Said-Al Ahl et al., 2018b); of cuban-oregano/Indian-borage (*Plectranthus amboinicus*) (Sabra et al., 2018); of white horehound (*Marrubium vulgare*) (Mahmoud et al., 2018); of moldavian dragonhead, moldavian balm (*Dracocephalum moldavica*), hyssop (*Hyssopus officinalis*) and sage (*Salvia officinalis*) (Hegazyet al., 2016).

The results of the current study reveal that the concentration of components vary both with the part of the plant and the harvest day after sowing. Previous studies also showed such variations, and variations with locations and other agronomic conditions. Accordingly, in USA, Craft and Setzer (2017) studied the leaf essential oils of two different samples of curly leaf parsley (*Petroselinum crispum* var. crispum) and two samples of flat leaf parsley (*P. crispum* var. neapolitanum) and found that, curly leaf parsley was rich in myristicin (between 39.4 and 43.2%), β-phellandrene (between 32.7 and 35%), and 1,3,8-p-menthatriene (between 7.9 and 14.9%); while flat leaf parsley was dominated by apiole (between 33 and 52.9%) and β-phellandrene (between 10 and 17%). Moreover, one sample of flat leaf parsley was rich in p-cymene (17.3%), while the other was rich in 1,3,8-p-menthatriene (23.4%). They added that, there are three different categories of parsley based on composition: 1) myristicin/β-phellandrene; 2) 1,3,8-p-menthatriene/β-phellandrene, and 3) apiole/β-phellandrene. The leaf oils of curly leaf parsley, and flat leaf parsley oil showed high concentrations of apiole, but there was no detectible myristicin. Concentrations of β-phellandrene were high in both samples (between 10 and 17%), but p-cymene was high in the sample from South Carolina (17.3%), while 1,3,8-p-menthatriene was high in the sample from another site in California (23.4%).

In Spain, the eight major compounds of the essential oil of parsley shoots were: 1,3,8-p-menthatriene (38.4 - 48.8%), β-phellandrene (22.2 - 29.5%), myristicin (6.2 - 11.1%), myrcene (5.8 - 6.5%), terpinolene (4.2 - 5%), limonene (2.7 - 3.3%), α-pinene (1.6 - 2.3%), and α-phellandrene (1.4 - 2.7%) (El-Zaeddi et al., 2016b). Also, the essential oil components identified in parsley leaves in Estonia were myristicin (30.7 - 42.7%), β-phellandrene (21.8 - 35.9%), *p*-1,3,8-menthatriene (5.4 - 10%), and β-myrcene (4.5 - 8.7%) (Vokk et al., 2011]). While 1,3,8-menthatriene (22.8 - 50.9%), myristicin (12.8 - 36.8%), β-phellandrene (14.1 - 29%), and β-myrcene (1.4 - 12.7%) were the major components of the essential oils obtained from parsley leaves in Serbia (Nemeš et al., 2018).

In Portugal, García-Díez et al. (2017) reported that the chemical constituents of parsley essential oil were myristicin (44.88%), limonene (11.72%), α-pinene (11.35%), cosmene (9.86%), β-pinene (5.38%), 1-butyin-3-one (3.49%), β-phellandrene (2%), elemicin (1.96%), α-terpinolene (1.71%), carvyl acetate (0.62%), and 1.3.8-p-menthatriene (0.36%). But myristicin (63.9%) and apiole (14.4%) were the major compounds of *Petroselinum crispum* Mill. herb oil in Cuba (Pino et al., 1997). However, Simon and Quinn (1988) showed that, the major compounds identified from 104 accessions including curly and flat leaf and Hamburg types were 1,3,8-p-menthatriene (68%), myristicin (60%), β-phellandrene (33%), apiol (22%), myrcene (16%), and terpinolene (13%).

In another study, Petropoulos et al. (2010) noticed that curly leaf parsley was affected by two different growth stages, and found that myristicin, β-phellandrene, and 1,3,8-p-menthatriene are the major compounds in the leaf oil in Greece, and observed that, seasonal variation had a profound effect on the chemistry of parsley leaf oils especially in myristicin and apiole. In Australia, Mangkoltriluk et al. (2005) reported 15 compounds found in fresh parsley leaves, with the major ones being 1,3,8-p-menthatriene, pinene, myrcene, phellandrene, and apiole. The major compounds in Mexico were 1,3,8-p-menthatriene, followed by β-phellandrene and apiole (López et al., 1999). Also, they observed a dramatic decrease in the concentrations of β-phellandrene (from 58.3 to 0%) and myristicin (from 12.0 to 0%) during the growth cycle, while the concentration of 1,3,8-p-menthatriene increased dramatically (from 0.4 to 79.9%). Hassanpouraghdam (2010) reported that the main components of parsley essential oil in Iran were myristicin (43.1%), apiole (13.9%), α-pinene (11.5%), β-pinene (9.6%), elemicin (7.6%) allyltetramethoxy benzene (6.1%), and sabinene (2.8%).

In Egypt, Alharbi et al. (2019) reported the components in three parsley cultivars to be α-pinene (0.25 - 5.48%), β-pinene (0.07 - 4.19%), β-myrcene (3.76 - 28.8%), α-phellandrene (0.06 - 5.23%), limonene (0.05 - 7.43%), β-phellandrene (13.81 - 32.52%), β-ocimene (0 - 2.56%), α-terpinolene (0.11 - 8.19%), p-cymene (0.09 - 1.3%), 1,3,8-p-menthatriene (15.58 - 28.33%), p-cymenene (0.03 - 3.38%), caryophyllene (0 - 1.44%), germacrene D (0 - 1.89%), β-sesquiphellandrene (0 - 1.73%), myristicin (6.98 - 30.07%), and apiol (1.23 - 9.34%). Furthermore, Said-Al Ahl et al. (2016) observed very little seasonal variation among the concentrations of the components of *Petroselinum crispum* cv. Moss curled no.2 in Egypt. These components were α-pinene (0 - 3.86%), β-myrcene (0.88 - 7.94%), α-phellandrene (0.14 - 3.81%), limonene (0.33 - 1.6%), β-phellandrene (6.09 - 32.19%), α-terpinolene (0.13 - 4.76%), p-cymene (0.44 - 1.28%), 1,3,8-p-menthatriene (7.81 - 20.96%), β-sesquiphellandrene (0.41 - 3.42%), myristicin (30 - 67.9%), elemicin (0.36 - 2.49), and apiol (0.4 - 3.56%). These results confirm that there are three cultivated varieties, among which, var. *latifolium* (broad-leaved) and var. *crispum* (curly-leaved) are grown for their leaves, and var. *tuberosum* is grown for its root. The curly leaf parsley tends to be richer in myristicin but contains much less essential oil than the other parsley.

Variations in the composition of essential oils were also reported to be influenced by the part of the plant, the stage of the development, and modifications due to the environment (Pirbalouti et al., 2013). These factors affect the plant's biosynthetic pathways and, consequently, the compounds' proportions. Harvesting time and environmental conditions are very important to obtain higher and better-quality essential oil content (Thompson et al., 2003; McGimpsey et al., 1994). In a study on geranium, Weiss (1997) reported that the maximum leaf growth and oil content were obtained from a warm sunny condition, which indicates that such photosynthetic activities create variations in shoot yield and essential oil.

CONCLUSIONS

The study investigated the presence of differences in the weight, essential oil content, and essential oil yield as well as the concentrations of the major essential oil compounds in the whole herb, the leaf, and the stem parts of a recently introduced curly-leafed parsley cultivar Moss no. 2 grown under Egyptian condition during three different harvest days after sowing. The results revealed variations among the different parts of the plant as well as among the different harvest dates. The findings of the study can help different stakeholders to optimize when and from which part of the plant to extract essential oil to maximize the desired compound.

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