Australian Journal of Crop Science AJCS

AJCS 9(4):303-308 (2015) ISSN:1835-2707

Research Note

Influence of organic and conventional farming on seed yield, fatty acid composition and tocopherols of Perilla

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Abstract

Consumer's interest in organic farming is increasing due to the high request of healthier and more nutritious foods in national and international markets. Seed of perilla (*Perilla frutescens* L. Britt.) is an important source of unsaturated lipids and tocopherols. During 2005 growing season, perilla plants were grown under conventional and organic farming in a typical Mediterranean area such as Southern Italy, aiming to evaluate the biomass production and partitioning, seed yield, tocopherol content and fatty acid composition. Organic farming caused a 27% seed yield reduction when compared to conventional farming. The seeds of perilla produced 37% oil on average. Irrespective of the farming systems, the C 18 family, in particular in α-linolenic acid (C 18:3), had the predominant fatty acid in seeds, representing 62% of the total fatty acids in the lipid fraction. No differences were recorded among treatments for the content of plamitic C16:0 (avg. 6.0%), stearic C18:0 (avg. 1.0%), and oleic C18:1 (avg. 14.0%), and linoleic acid (avg. 15.0%). No significant differences were observed between conventional and organic farming on α-tocopherol (avg. 9.2 mg kg^{-1}), and β + γ-tocopherol (avg. 565.9 mg kg^{-1}), whereas the δ-tocopherol was not detected. In summary, it has been possible to produce high quality seeds of perilla under both conventional and organic farming. Improvement in management techniques and cultivation factors are highly needed to fill the gap between organic and conventional yields and also to enhance and standardize the quality of organic products.

Keywords: Alpha-linolenic acid (C 18:3), linoleic acid (C 18:2), β+γ tocopherol, oil content, oil seed crops, organic products.

Abbreviations: α-linoleic_Alpha-linoleic; α-tocopherol_Alpha-tocopherol; β+γ-tocopherol_Beta plus gamma tocopherol; δ-tocopherol_Delta-tocopherol; ANOVA_Analysis of variance; d_Diameter; DHA_Docosahexaenoic; EC_European Comission; EPA_Acid eicosapentaenoic acid; ET_o_Reference evapotranspiration; EU_European Union; FAMEs_ Fatty acid methyl esters; FID_Flame ionization detector; GC_Gas chromatography; h_Height; ha_Hectare; HPLC_ High-performance liquid chromatography; K_Potassium; LA_Leaf area; LAI_Leaf area index; N_Nitrogen; P_Phosphorus; SLA_Specific leaf area; v_Volume

Introduction

Perilla frutescens (L.) Britton, commonly referred as perilla, is an annual plant of the mint family *Lamiaceae*, native to East Asia and widely cultivated in India, China, Japan, Hong Kong, South and North Korea (Omer et al., 1998). Perilla, has been traditionally cultivated for leaves and seeds (Kim, 1995). Perilla leaves are edible and commonly used in the preparation of raw fish dishes (e.g. sushi and sashimi) in Japan (Omer et al., 1998) and are also used as spice cooked or fried and combined with rice and soups in many Korean and Chinese dishes (Park et al., 2013). The perilla leaves has been used in Chinese traditional medicine as a diaphoretic, antipyretic, antiseptic, anti-inflammatory, antibiotic, sedative and laxative (Omer et al., 1998). Its health benefits have been mainly attributed to its content of bioactive flavonoids and phenolic compounds (Lee et al., 2013; Guan et al., 2014). Moreover, perilla seed oil has also been demonstrated to be a rich source of saturated (e.g. plamitic and stearic) and unsaturated fatty acids (e.g. oleic, linoleic and especially omega-3 linolenic acid) (Guan et al., 2014). Linoleic and α-linolenic are essential polyunsaturated fatty acids which cannot be produced in the human body and; therefore, must be integrated in the diets of animals and humans (Jorgensen et al., 2012). Within the body, these two polyunsaturated fatty acids can be converted to other polyunsaturated fatty acids such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (Heuer et al., 2002). The α-linolenic, EPA, and DHA known as omega-3 fatty acids are linked to a variety of potential therapeutic uses, primarily as part of heart-protecting diets by lowering the risk of cardiovascular heart diseases (Yaniv et al., 1999).

Cultivation factors, particularly type and concentration of fertilizer, climatic conditions or soil type may significantly influence the chemical composition of crops (Jorgensen et al., 2012). Organic foods as defined in the EU regulation (EC) (No 834/2007) "are products produced under controlled cultivation conditions characterized by the absence of synthetic fertilizers and very restricted use of pesticides".

Organic food is one of the fastest growing sectors of the food and agriculture industry worldwide (Ballester-Costa et al., 2013) since they are perceived by consumers to be healthier, having better quality and more nutritious than conventionally produced foods (Magnusson et al., 2001). Nevertheless, there is little information about nutritional and sensorial quality and food safety of organic versus conventional crops (Gennaro and Quaglia, 2003). It seems that scientific research on organic products is still contradictory (Kahl et al., 2012). As a consequence, elucidating the relationship between cultivation system (organic *vs.* conventional) and nutritional value of agricultural products, in particular oil seed crops, is still missing.

Organic agriculture is well implemented in Italy, which is the European country with the largest organic area and the highest number of organic farms (Dorais, 2008). Recently, there has been a growing interest in the use of organic perilla seeds in the diet (e.g. as dietary supplement) for their health-benefit properties.

Therefore, the aim of the current work was to assess the influence of organic versus conventional cultivation systems on biomass, seed production, tocopherols and fatty acid composition of perilla grown under open-field conditions.

Results

Biomass production, partitioning and grain yield

The leaves fresh weight was significantly affected by the farming system, with the highest values recorded in conventional compared to organic farming (Table 2). No significant differences were observed among treatments for the leaves (avg. 74.8 g plant^{-1}), stems (avg. 136.9 g plant^{-1}), flowers quantity (avg. 88.3 g plant^{-1}) and total dry weight (avg. 300.5 g plant^{-1}) (Table 2). Similarly, no significant differences between the conventional and organic farming were recorded for the following: number of leaves (avg. 518.1), stems (avg. 8.5) and flowers (avg. 262.6). Organic farming significantly reduced the final leaf area (LA) and the specific leaf area (SLA) by 27.7% and 25.5%, respectively compared to those recorded in the conventional farming system (Table 3).

The organic system induced a significant reduction in seed yield, compared to conventional system (319.6 versus 437.6 g m^{-2}) (Table 3). The lowest seed yield reduction observed in organic farming was due to a reduction in the number of seeds per plant (data not shown) and not to the seed mean weight, since no significant differences among treatments were observed for the 100-seed weight (avg. 0.37 g).

Oil content, fatty acid composition and tocopherols

In this study, the fatty acid composition was studied by GC with flame ionization detector (FID) (Fig. 1). Irrespective of the farming systems, the C 18 family, particularly the α-linolenic acid, was the predominant fatty acid in seeds, representing 62% of the total fatty acids in the lipid fraction, followed by linoleic (15%), oleic (14%), palmitic (6%) stearic (1%), and vaccenic (ascelepic) acid (1%). No significant differences were recorded among treatments for the content of palmitic C16:0 (avg. 6.3%), stearic C18:0 (avg. 1.9%), oleic C18:1 (avg. 13.7%), vaccenic C18:1 (avg. 1.1%), linoleic C18:2 (avg. 14.6%), and linolenic 18:3 (avg. 61.7%) (Table 4). Finally, no significant differences among treatments (conventional *vs.* organic) were recorded on α-tocopherol (avg. 9.2 mg kg^{-1}), and $\beta+\gamma$-tocopherol (avg. 565.9 mg kg^{-1}), content in *Perilla frutescens*, whereas the δ-tocopherol was not detected (data not shown).

Discussion

It is well-known that yields in organic farming are typically lower than conventional methods. But these yield differences depend on system and farm characteristics. In a recent meta-analysis study, Seufert et al. (2012) observed that the performance of organic systems varies substantially across crop types and species. For instance, the yield reduction in organic farming was not significant for fruits (-3%) and oil seeds (-11%), when compared to conventional farming, while organic cereals and vegetables have significantly lower yields than conventional crops (-26% and -33%, respectively). In the current study, the organic farming reduced the leaves fresh weight and seed yield of *Perilla frutescens* by 8% and 27%, respectively, compared to the conventional system. Our results are in agreement with many open field experiments carried out on potato (Fiorillo et al., 2005), wheat (Fagnano et al., 2012), cauliflower, endive and zucchini squash (Maggio et al., 2013) in similar growth conditions (e.g. Southern Italy). The lowest seed yield under organic farming could be attributed to a reduced availability of nutrients, particularly nitrogen, at the right moment with the crop nutrient demand. Zarabi and Jalai (2012) reported that the nitrate and ammonium release patterns from organic residues may not optimally fulfill plant nutrient requirements. Similarly, Berry et al. (2002) showed that the productivity of organic farming is restricted by the supply of available nitrogen. The release of plant available mineral nitrogen from organic sources (e.g. animal manure, blood meal, and cover crops) behave as a slow-release source of nitrogen; thus, does not match the high crop nitrogen demand during the peak growing period, leading to a reduction crop performance (Seufert et al., 2012). The seeds of perilla produced on average 37% oil (Table 4), which is in the range (30-40%) reported by Asif (2012). The fatty acid composition of perilla seeds, recorded in the present experiment, was also in a range reported by Asif, (2012) (alpha-linolenic acid 52-64%, linoleic acid 13-20%, oleic acid 12-22%, palmitic acid 5-7%, and stearic acid 1.15%). It is well known that alpha-linolenic acid (omega-3) and linoleic acid (omega-6) are essential fatty acids because they cannot be synthesized in the human body and must be supplied in the diet (Sargi et al., 2013). The proper intake of *n*-3 long-chain (omega-3) fatty acids has positive health implications related to heart diseases, cancer, inflammatory bowel disease, and mental health (Simopoulos, 2011).

Tocopherols are frequently used in foods as antioxidants, since dietary tocopherol, especially α-tocopherol, can prevent excessive stress *in vivo* (Adhikari et al., 2006). The α-tocopherol levels detected in the perilla seeds (avg. 9.2 mg kg^{-1}) were in the range (2.7-13.3 mg kg^{-1}) reported by Um et al. (2013) on 54 selected perilla accessions, and also in the range observed by Kim et al. (2012) on seven perilla cultivars (6.2-10.1 mg kg^{-1}). Moreover, The $\beta+\gamma$ tocopherol content (avg. 565.9 mg kg^{-1}) recorded in perilla seeds was higher than those observed by Kim et al. (2012) (116.5-154.4 mg kg^{-1}), Um et al. (2013) (107.0-198.8 mg kg^{-1}), and similar to the values found by Wang et al. (2010). Explanations for these findings could be the different environments, in which the plants were cultivated, and variations between the perilla genetic materials. Based on the total tocopherol content observed in the current experiment, perilla seeds could be considered as a rich source of tocopherol (Kamal-Eldin and Andersson, 1997), when compared with other seed oil crops, such as mustard (69.0 mg kg^{-1}), linseed (83.0 mg kg^{-1}), and sesame (100.0 mg kg^{-1}) (Ryan et al., 2007).

Organic farming seems to not promote accumulation of tocopherols and fatty acids, indicating that improved nutritional value of organic versus conventional crops is not always proved (Gennaro and Quaglia, 2003). In a recent review, Benbrook (2009) observed that the majority of articles comparing the quality and nutritional value of vegetables and fruits coming from organic and conventional farming show modest to moderate increase of most nutrients.

Table 1. Climatic parameters prevailed during the 2005 growing season compared to the long-run data.

Climatic parameters	Min. air temp. (°C)	Max. air temp. (°C)	Rainfall (mm)	ETo (mm)[1]
		Long-run data (1950-1990)		
April	8.6	17.5	61.0	91.0
May	12.2	21.9	41.1	127.2
June	15.3	25.3	25.2	144.2
July	17.6	27.3	18.2	150.4
August	17.8	28.4	32.3	144.0
September	15.5	26.1	64.5	108.6
		2005		
April	8.8	19.4	80.0	100.0
May	13.7	26.1	12.6	130.25
June	16.7	30.1	5.0	145.2
July	18.5	32.1	0.6	160.9
August	18.2	31.0	44.8	150.5
September	16.9	29.8	63.6	120.6

[1]ET_o reference evapotranspiration calculated according to Penman-Monteith equation.

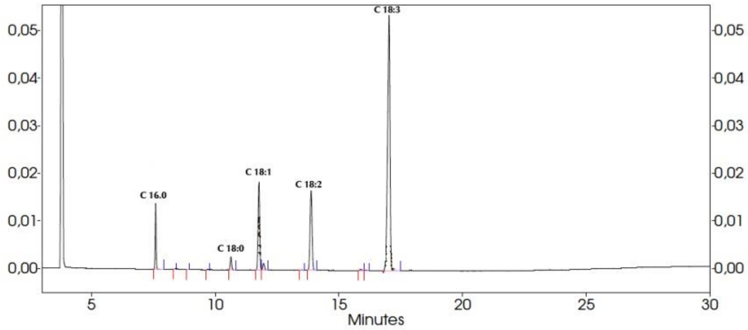

Fig 1. GC chromatogram of fatty acid as ester derivatives extracted from *Perilla frutescens* seeds. The peaks correspond to the following: C 16:0, palmitic acid; C 18:0 stearic acid; C 18:1, oleic acid (18:1,n-9) and asclepic acid (18:1, n-7); C 18: 2, linoleic acid; C 18: 3, linolenic acid.

Similarly, Maggio et al. (2013), reported that organic farming *per se* does not necessarily indicate an improvement in quality of vegetables (e.g. cauliflower, endive and zucchini squash). The authors concluded that understanding the functional links between cultural factors and physiological responses is an important requisite to enhance the quality of organic products.

Materials and Methods

Experimental site and climatic conditions

An experiment was conducted during the 2005 growing season in the experimental station of the Department of Agriculture of the University of Naples Federico II located in Portici, Naples, Italy (40°46´N, 14°21´E, 70 m a.s.l.). The soil was a sandy loam soil with the following characteristics: bulk density 1.1 g cm^{-3}, pH 7.2, organic matter 1.8%, 0.1% total N, available P 31 mg kg^{-1}, exchangeable K 287 mg kg^{-1}, with a textural analysis of 75% sand, 15% silt, and 10% clay). The measured field capacity (–0.33 bar) and permanent wilting point (–15 bar) averaged 0.245 cm^3 cm^{-3} and 0.146 cm^3 cm^{-3}, respectively. The soil had not been cultivated for five years before the current trial started, which is the requested period to stabilize soil properties following conversion from conventional to organic farming (Scow et al., 1994).

Annual precipitation and reference evapotranspiration (ET_o) were 1119.4 and 1100 mm, respectively, in 2005. This corresponds to 23% and 5% more rain and ET_o in 2005 than the long run average (908 and 1053 mm, respectively(Table 1). The highest air temperature recorded during the growing season (April-September) was observed in July and August, while the lowest values of air temperature were recorded in April (Table 1). Overall, the climatic conditions during the experiment were consistent with the 40-year mean temperatures, rainfall, and ET_o for the experimental region and they were representative of a typical growth season for *Perilla frutescens* production in South Italy.

Plant material, crop management, and treatments

Perilla seeds (*Perilla frutescens* L. Britt.) were sown in styrofoam trays containing commercial peat moss on February 18. On March 10, the perilla seedlings were transplanted into pots (d 8 cm, h 8 cm) containing peat moss, and placed on benches in a greenhouse tunnel. Plants were transplanted on April 11, in rows 0.6 m apart, with an intra-row spacing of 0.4 m, giving a plant density of 4.1 plants m^{-2}. A randomized complete-block design with four replicates was used to compare two farming systems: organic and conventional. Each experimental unit consisted of a 22 m^2 plot area. The organic farming was conducted following the EU regulations (EC Reg. 2092/91), whereas the conventional farming system was done according to the standard farming management practices. In organic farming system, 100 kg N ha^{-1} was applied before transplanting using organic N source (a mix of manure, feathers and torrefied bone-meat, 6% N). The organic nitrogen fertilizer used in the current experiment, is listed in the Italian document of the Ministry of Agricultural,

Table 2. Effect of farming system on fresh and dry weight biomass production and partitioning of *Perilla frutescens*.

Farming system	Fresh weight (g plant^{-1})				Dry weight (g plant^{-1})			
	Leaves	Stems	Flowers	Total	Leaves	Stems	Flowers	Total
Conventional	413.3 a	491.6	284.3	1189.6	76.1	134.6	86.6	297.7
Organic	378.0 b	512.6	291.6	1182.3	73.6	139.3	90.0	303.4
Significance[1]	*	NS	NS	NS	NS	NS	NS	NS

[1]ns, *, non-significant or significant at $P \leq 0.05$ respectively.

Table 3. Effect of farming system on number of leaves, stems, flowers, final leaf area (LA), specific leaf weight (SLW), specific leaf area (SLA), and seed yield of *Perilla frutescens*.

Farming system	Number (no. plant^{-1})			LA	SLA	Seed yield
	Leaves	Stems	Flowers	(cm^2 plant^{-1})	(cm^2 g^{-1})	(g m^{-2})
Conventional	530.0	8.6	266.0	8969	117.6	437.6
Organic	506.3	8.5	259.3	6480	87.6	319.6
Significance[1]	NS	NS	NS	*	*	*

[1]ns, *, non-significant or significant at $P \leq 0.05$ respectively.

Table 4. Effect of farming system on oil content (g 100 g^{-1}) and fatty acid composition (%): palmitic C16:0, stearic C18:0, oleic C18:1 *n*-9, vaccenic (ascelepic) 18:1 *n*-7, linoleic C18:2, and linolenic C18:3 of *Perilla frutescens*.

Farming system	Oil content (%)	Fatty acid composition (%)					
		Palmitic	Stearic	Oleic	Vaccenic	Linoleic	Linolenic
Conventional	37.1	6.3	1.9	13.9	1.1	14.6	61.6
Organic	37.0	6.3	1.9	13.5	1.1	14.7	61.9
Significance[1]	NS	NS	NS	NS	NS	NS	NS

[1]ns, *, non-significant or significant at $P \leq 0.05$ respectively. Values are the means of four replicate samples.

Food and Forestry Policies (MiPAAF) 13/09/99 n◦8 (Registro dei fertilizzanti per l'Agricoltura Biologica-ISNP). In the conventional system, ammonium nitrate (34%) was used to apply equal quantity of nitrogen. In addition, both systems received 80 kg ha^{-1} P_2O_5 and 80 kg ha^{-1} K_2O as organic or chemical fertilizers.

Drip lines, with in-line emitters located 0.30 m apart and an emitter flow rate of 3.4 L h^{-1}, were placed 10 cm away from the plants and were spaced with a 0.6-m distance between each lateral. All plots were grown at the 100% irrigation level. Plants were irrigated when soil water content reached to 2/3 of the soil available water (determined by weighting soil samples).

Biomass and yield measurements

At final harvest, on September 27th, plants were separated in leaves, stems, and flowers, and their tissues were dried in a forced-air oven at 80 °C for 72 h for biomass determination. Leaf area (LA) was measured with an electronic area meter (Delta-T Devices Ltd., Cambridge, UK). Leaf area index (LAI) was calculated as the accumulated leaf area divided per unit of ground area (m^2 m^{-2}). The number of leaves, stems and flowers was also recorded. Specific leaf area (SLA) was calculated by dividing leaf area by leaf dry weight of each plant and expressed in cm^2 g^{-1} (Rouphael and Colla, 2005). At maturity, seeds from all experimental plots were harvested and weighed and 100-seed weight was calculated.

Soxhlet extraction

The perilla seeds were dried, crushed and weighed, and then 10 g of each sample was extracted in a Soxhlet apparatus (FALC, modWBS2) with 100 ml of petroleum ether at 70 °C. Extraction was carried out for 8 h. The solvent was then evaporated in a rota-evaporator and the oil fraction residues were weighed (AOAC, 2005).

HPLC analysis of tocopherol content

The amount of tocopherols (α, $\beta + \gamma$, δ) was determined by reversed-phase high-performance liquid chromatography (HPLC) analysis on a Shimadzu HPLC mod. LC-10ADVP (Shimadzu Italia, Milan, Italy) with a Shimadzu UV-Vis Diode Array Detector mod. SPD-M10AVP (Shimadzu Italia, Milan, Italy). A reversed phase Spherisorb S5 ODS3 column was used (250 x 4.6 mm i.d.). Column flow: 1.8 mL min^{-1}. The wavelength for detector was set on 290 nm. Results were expressed as α-tocopherol, $\beta + \gamma$-tocopherol, and δ-tocopherol concentration (mg kg^{-1} oil), and pure α, β , γ and δ- tocopherols were used as external standard for the calibration curve. The elution conditions were those previously reported by Tonolo and Marzo (1989) with slight modifications. An isocratic elution was performed for 19 minutes with 100% eluent A (methanol/water/acetonitrile 73.2:1.8:25 v%), then to 100% eluent B (ethyl acetate) within 1 min. Analysis of tocopherols content were performed in triplicate.

Gas chromatography analysis of fatty acids composition

For the determination of fatty acid composition, oils were subjected to cold transmethylation in KOH/methanol according to the method of Christie (1982). Analyses of fatty acid methyl esters (FAMEs) of the perilla oil were carried out by gas chromatography using a GC17A chromatograph (Shimadzu Italia, Milan, Italy) equipped with a flame ionization detector (FID). A FAME capillary column, 60 m, 0.25 mm i.d. with 0.25 mm 50% cyanopropyl-methyl phenyl silicone was used (Quadrex Corporation, New Haven, USA). Helium was used as the carrier gas at flow rate of 2 mL min^{-1} and a split ratio of 1:60. The injector and FID temperature was 250°C. The oven temperature was programmed at 170°C for 20 min and increased to 220°C using a rate of 10°C min^{-1}, hold at the final temperature for 10 min. Peaks identification was performed by comparing retention times of fatty acids with those of a mixture of pure methyl esters of fatty acids (Larodan, Malmoe, Sweden) injected in the same conditions. All determinations were carried out in triplicates.

Statistical analysis

All data were statistically analyzed by ANOVA using the SPSS software package (SPSS 10 for Windows, 2001).

Conclusions

To summarize, it has been possible to produce high-quality seeds in Southern Italy from *Perilla frutescens* grown under both conventional and organic farming. Since consumer interest for organic products is increasing, the organic extract of *P. frutescens* may be suitable for use in the food matrix to help achieve potential health benefits. The results also indicated that organic farming caused a 27% seed yield reduction in comparison to the conventional system. For these reasons, a better understanding of the specific cultural conditions and improvement in management techniques that address factors limiting yields in organic systems are highly requested to close the gap between organic and conventional yields.

Acknowledgements

The authors are grateful to Dr. Rosanna Caputo for her technical support.

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