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Change in the contents of fatty acids and antioxidant capacity of purslane in relation to fertilization

César Omar Montoya-García[a,b,*], Víctor Hugo Volke-Haller[a], Antonio Trinidad-Santos[a], Clemente Villanueva-Verduzco[c]

[a] Colegio de Postgraduados, Campus Montecillo, Km. 36.5, Carretera México-Texcoco, Montecillo, Texcoco, 56230, Estado de México, Mexico
[b] Campo Experimental Iguala-INIFAP, Carretera Iguala-Tuxpan, Km. 2.5, Iguala de la Independencia, A P. 29, Guerrero, CP 40000, Mexico
[c] Departamento de Fitotecnia, Universidad Autónoma Chapingo, Km. 38.5, Carretera México-Texcoco, 56230, Chapingo, Estado de México, Mexico

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ABSTRACT

Purslane (*Portulaca oleracea* L.) is considered as a vegetable rich in nutraceutical compounds (fatty acids and antioxidants); however, abiotic factors like nitrogen (N), phosphorus (P), and potassium (K) fertilization can influence the concentrations of these compounds. The objective of the study was to evaluate the effect of NPK fertilization on the nutraceutical content of purslane on two harvest dates (27 and 42 days after emergence). The doses were: N: 0, 100, 200 and 300 kg N ha^{-1}; P: 0 and 60 kg P_2O_5 ha^{-1}; K: 0 and 80 kg K_2O ha^{-1}. Nitrogen application at 100 kg N ha^{-1} decreased total phenolics (TPC), flavonoids (TFC), β-carotene (BC), and chlorophylls in foliage purslane; however, these contents increase at higher doses, up to 300 kg N ha^{-1}, without surpassing the initial contents with 0 kg N ha^{-1}; the ascorbic acid (AAC), palmitic and stearic acids, and antioxidant activity (DPPH scavenging capacity) decrease gradually; and linoleic and α-linolenic acids increased with the applied N. The application of P and K decreased TFC and AAC, but BC increased with the applied K. The TFC increased as well as linoleic and α-linolenic acids by the first harvest, while BC, chlorophylls, and palmitic and stearic acids increased by the second harvest. This study shows the possibility of maximizing the nutraceutical content and the antioxidant capacity of purslane through fertilization, besides providing an alimentary and medicinal panorama with these compounds.

1. Introduction

A nutraceutical foodstuff provides medicinal benefits like preventing and treating diseases. Some examples of nutraceutical compounds are dietary fiber, polyunsaturated fatty acids like α-linolenic acid and linoleic acid, and antioxidants like phenols, flavonoids, terpenoids, and vitamins (Barros et al., 2008).

One of the crops that has obtained high regard since the discovery of its content of linoleic (18:2ω6) and α-linolenic (18:3ω3) acids and antioxidant compounds is purslane (*Portulaca oleracea* L.). It has a wide range of pharmacological effects, among which are: antidiabetic (El-Sayed, 2011; Sharma et al., 2010), antiviral (Cai-Xia et al., 2010), and antitumoral (Shen et al., 2013; Zhao et al., 2013) activities, as well as decreasing the levels of triglycerides and cholesterol in blood (Zidan et al., 2014).

However, studies on the makeup of fatty acids and antioxidants of purslane have been carried out on wild, ornamental, or local individuals (Alam et al., 2014; Oliveira et al., 2009; Lim and Quah, 2007), and the concentrations of these compounds vary depending on the stage of maturation, climatic conditions, and harvest date (Uddin et al., 2012).

Purslane has been consumed in Mexico since pre-Hispanic times, especially in the Valley of Mexico (McClung de Tapia et al., 2013; Vázquez-Alonso et al., 2014). Nowadays, it is field grown in the states of Baja California, Morelos, and Mexico City, under different climatic and soil conditions (Montoya-García et al., 2017).

Although nitrogen (N), phosphorus (P), and potassium (K) are mineral elements that are required by plants in high amounts, since they play an important role assuring adequate crop growth and development (Hawkesford et al., 2012), under deficiency conditions, the plant increases the biosynthesis of secondary metabolites in its tissues as a response to the abiotic stress (Taiz and Zeiger, 2002). Particularly in the case of nitrogen deficiency, the accumulation of phenols and flavonoids increases (Verardo et al., 2013; Zhu et al., 2009). Contrastingly, increasing the application of nitrogen increases the production of polyunsaturated fatty acids (Verardo et al., 2013; Zheljazkov et al., 2012).

* Corresponding author at: Colegio de Postgraduados, Campus Montecillo, Km. 36.5, Carretera México-Texcoco, Montecillo, Texcoco, 56230, Estado de México, Mexico.
E-mail address: montoya.cesar@colpos.mx (C.O. Montoya-García).

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In Mexico, no studies have been done on the nutraceutical content of purslane and how fertilization influences these compounds. The objective of the present research was to determine the effect of NPK fertilization in purslane on its nutraceutical content, in terms of total fatty acids, phenols, and flavonoids, as well as ascorbic acid, β-carotene, *a* and *b* chlorophylls, and its antioxidant activity with the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical.

2. Materials and methods

The field experiment was carried out in The State of Mexico, Mexico 19° 28' 2.07'' N and 98° 54' 1.71'' O, 2215 masl. in July and August, 2014. The rainfall in the months of June, July, and August, 2014 was 135.2, 142.5, and 134.1 mm, respectively. The mean maximum daily temperatures varied between 19 and 28 °C, while the minimum varied between 6 and 11 °C in July and August. The soil of the experimental area was clay loam texture, the pH was 8.2; the EC 0.36 dS m^{-1}; 2.29–3.0% organic matter; 12–35 mg kg^{-1} P; 546–819 mg kg^{-1} K; 560–780 mg kg^{-1} Ca; 125–192 mg kg^{-1} Mg; 6.9 mg kg^{-1} Na; 9 mg kg^{-1} Fe; 1.3 mg kg^{-1} Cu; 24–29 mg kg^{-1} Zn; 1.5 mg kg^{-1} Mn (Montoya-García et al., 2017).

2.1. Plant material

The purslane cultivar used came from the town of Mixquic, Tlahuac, Mexico City. It is characterized by having a straight growing pattern with few branches, large leaves, and green stems.

2.2. Treatments and experimental design

The mean levels of each element were defined based on information from literature regarding the nutrient extraction of the plant, according to a given yield and the concentration of the element. The treatments were four N doses: 0, 100, 200, and 300 kg N ha^{-1}, and two doses of P and K: 0 and 60 kg P_2O_5 ha^{-1}, 0 and 80 kg K_2O ha^{-1}, respectively, distributed in a completely randomized block design, with four replicates. The base fertilization when the element was not variable was 200 kg ha^{-1} N, 60 kg P_2O_5 ha^{-1}, and 80 kg K_2O ha^{-1}. The experimental units consisted of 3 m long by 1.5 m wide rectangles, hand-formed, considering the useful parcel of 4.5 m^2. The sources of fertilizers were ammonium sulfate [$(NH_4)_2SO_4$, 21% N], mono-ammonium phosphate [$NH_4H_2PO_4$, 12% N and 61% P_2O_5], and potassium sulfate [K_2SO_4, 51% K_2O].

2.3. Management of the experiment

The preparation of the soil and sowing was done traditionally, following the procedures of the farmers from the town of Mixquic, which consists in plowing and harrowing, forming the irrigation canals and plots, and subsequently contouring the plots with 0.30 m-wide borders. Sowing was done on July 3rd, 2014, randomized seeding 1.86 g m^2 (4726 seeds g^{-1}). Then, 3.5 t ha^{-1} chicken manure (2.14% N) was applied to stimulate germination (personal communication with local producers). Emergence came about 7 days after sowing (das). Half of the nitrogen fertilizer and all of the P and K were applied at random 25 das, and the rest of the N at 32 das. Weed control was done manually. There was no need to apply insecticides or fungicides. The purslane was harvested twice: at vegetative maturity, 27 days after emergence (C_1), and at second flowering, 42 days after emergence (C_2).

2.4. Plant sampling and analysis

In the two harvests, leaf sampling was done with a 0.0625 m^2 wooden frame which was placed randomly in each experimental unit. The plant within the frame was cut at ground level and all the plant material was collected. The fresh aerial biomass of each replicate was placed in an oven at 70 °C for 72 h, ground, and sifted in a 1 mm sieve.

2.5. Fatty acids content

Fatty acids were determined according to procedures previously described by Barros et al. (2008), by gas-liquid chromatography with flame ionization detection (GLC-FID)/capillary column based on the following trans-esterification procedure: fatty acids were methylated with 5 mL of methanol:sulphuric acid:toluene 2:1:1 (v:v), during at least 12 h in a bath at 50 °C and 160 rpm; then 5 mL of deionised water were added, to obtain phase separation; the fatty acids methyl ester were recovered with 5 mL of diethyl ether by shaking in vortex, and the upper phase was passed through a micro-column of sodium sulphate anhydrous, in order to eliminate the water; the sample was recovered in a vial with Teflon, and before injection the sample was filtered with 0.2 lm nylon filter from Milipore. The fatty acid profile was analyzed with a DANI model GC 1000 instrument equipped with a split/splitless injector, a flame ionization detector (FID) and a Macherey-Nagel column (30 m * 0.32 mm ID * 0.25 µm d_f). The oven temperature program was as follows: the initial temperature of the column was 50 °C, held for 2 min, then a 10 °C/ min ramp to 240 °C and held for 11 min. The carrier gas (hydrogen) flow-rate was 4.0 mL/min (0.61 bar), measured at 50 °C. Split injection (1:40) was carried out at 250 °C. For each analysis 1 µL of the sample was injected in GC. Fatty acid identification was made by comparing the relative retention times from samples with FAME peaks (standards). The results were recorded and processed using CSW 1.7 software (DataApex 1.7) and expressed in relative percentage of each fatty acid.

2.6. Preparation of the purslane extract

To determine the phenols, flavonoids, and antioxidant activity, the dry and ground purslane leaves were homogenized with distilled water in a 1:25 ratio (v/v). The homogenized material was placed in boiling water for 5 min and filtered through filter paper.

2.7. Total phenol content

It was quantified using the Folín–Ciocalteu method (Waterman and Mole, 1994). 10 µL of the aqueous purslane extract of each treatment per replicate and harvest date (48 samples) were used, adding 490 µL distilled water, 25 µL of the Folín–Ciocalteu solution and water at the ratio 1:1 (v/v), and 975 µL of the Na_2CO_3 solution at 2.5%. This was agitated for 10 s in the vortex and incubated at room temperature for 1 h in darkness. Absorbance was measured at 740 nm. The concentration was calculated from a standard curve elaborated with Folín–Ciocalteu (Absorbance = 0.0614x; R^2 = 0.998, x = Folín–Ciocalteu concentration). The analysis was done four times per sample. Total phenolics contents were expressed as milligrams of gallic acid equivalent in dry weight (mg GAE g^{-1} DW).

2.8. Total flavonoid content

It was homogenized 20 µL of the aqueous purslane extract per treatment per replicate per harvest date (48 samples), 980 µL CH_3OH (80%), 2 mL CH_3CO_2K (1 M), and 2 mL $AlCl_3$ (10%). The mixture was agitated for 10 s in the vortex and incubated for 30 min in darkness. Absorbance was measured at 415 nm. The concentration was calculated from a pattern curve elaborated with quercetin (Absorbance = 0.1067x; R^2 = 0.997, x = quercetin concentration) (Chang et al., 2002). The analysis was done four times per sample. Total flavonoid content were expressed as milligrams of quercetin equivalent (mg QE g^{-1} DW).

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