The influence of nitrogen fertilization on growth, yield, nitrate and oxalic acid concentration in purslane (*Portulaca oleracea*)

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Abstract

Purslane (*Portulaca oleracea*) is widely used for culinary purposes throughout Mediterranean region, and the interest in this plant increased due to it being a source of bio-protective compounds, such as fatty acids and antioxidants. However, the use of purslane could be limited by accumulation of high levels of compounds harmful to human health, such as nitrate and oxalic acid. The main objective of present study was to evaluate the influence of nitrogen fertilization on growth and yield parameters and on nitrate and oxalic acid concentrations in leaves and stems. Plants of golden-leafed purslane of *sativa* subspecies were grown in styro-foam boxes with substrate and fertilized two times per week during four weeks with ammonium-nitrate solution (16.9% NO_3^--N and 17.6% NH_4^+-N), for testing of four nitrogen levels (0, 30, 60 and 90 kg N ha^{-1}). Plant growth, yield, nitrate and oxalic acid concentrations were significantly affected by nitrogen application. The best quantity/quality ratio was achieved at fertilization level of 60 kg N ha^{-1}, which gave a yield of 5.1 kg m^{-2} FW, while nitrate concentration was 48.98 and 43.90 mg g^{-1} DW in leaf and stem, respectively, and oxalic acid concentration was 1.27 and 0.55 mg g^{-1} DW, in leaf and stem, respectively: values which are not harmful for consumer health.

Keywords: *Portulaca oleracea* subsp. *sativa*, golden-leafed purslane, ammonium-nitrate fertilizer, nitrate concentration, oxalic acid concentration

INTRODUCTION

Interest in purslane (*Portulaca oleracea* L.) has increased in recent years due to recognition of the potential of this plant as source of bio-protective compounds, such as fatty acids (Cros et al., 2007), anti-oxidants (Fernández et al., 2007), vitamins (Liu et al., 2000), flavonoids and phenolic acids (Erkan, 2012). The alpha-linolenic acid, an omega-3 fatty acid is the main beneficial compound for human health found on purslane, contributing to reduction the incidence of coronary heart diseases (Cros et al., 2007). However, the consumption of purslane may lead an accumulation of compounds harmful to human health, such as nitrate and oxalic acid.

Nitrate accumulation in plants is result of nutritional, environmental and physiological factors (Anjana and Iqbal, 2007), but nitrogen fertilization, especially with nitrogen in nitrate form, has been reported as one of main factors for this (Luo et al., 1993). Nitrogen uptake in an amount not fully usable by plant metabolism, originate a nitrate accumulation on shoots, and it may be transformed on nitrite (Anjana and Iqbal, 2007; Santos, 2012), which can lead the formation of methaemoglobin (Santamaria, 2006).

Oxalic acid synthesis by plants has been associated with metabolic reduction of nitrate nitrogen as a mode of balancing the cytoplasmic pH (Proietti et al., 2009), and is higher when plants are fertilized with nitrogen in nitrate form (Ugrinović et al., 2012). Oxalic acid may combine with some minerals, such as calcium and iron, creating insoluble salts, commonly known as oxalates, hindering the availability of these minerals to humans, which can cause kidney stones and anemia (Palaniswamy et al., 2004). Oxalic acid has been reported as the main barrier for increase of purslane consumption (Palaniswamy et al., 2004).

Thus, the objective of this study was to evaluate the influence of nitrogen fertilization on purslane growth and yield, and on nitrate and oxalic acid concentrations in leaves and stems.

MATERIALS AND METHODS

Plant material and growing conditions

The experiment was conducted in a thermal polyethylene-covered greenhouse, under natural irradiance conditions, at the "Herdade Experimental da Mitra" (38°31'52"N, 8°01'05"W), University of Évora, Portugal. Air temperature and air humidity during the experiment ranged from 16.7 to 33.7°C, and from 12.2 to 49.7%, respectively, measured using the weather station Helios Mini V400 (Skye Instruments).

Plants of golden-leafed purslane (*Portulaca oleracea* subsp. *sativa*) were grown in styro-foam boxes (45 cm length, 25 cm wide and 12 cm height, with an area of 0.11 m²) perforated in the center for allow the flow of excess water, and filled with 8 L of organic substrate obtained commercially (Agriloja Hortícolas, Agridistribuição), formed by forest residue, composted grape husk and white peat. Physical and chemical characteristics of substrate are shown in Table 1. Seeds were acquired from a local producer (with a germination rate of 91.7% in growing conditions), and manually sowed on August 19, 2013, with a density of 2,200 plants m^{-2}, with seeds placed on surface of substrate.

Table 1. Physical and chemical characteristics of substrate (according to manufacturer).

Particle size (mm)	<15
Moisture content (%)	40-60
Organic matter content (%)	60
Nitrogen (mg L^{-1})	200-400
Phosphorus (mg L^{-1})	100-200
Potassium (mg L^{-1})	150-300
pH	5.5-6.5
EC (mS cm^{-1})	1-3
C/N	<20

A completely randomized design with four replications was adopted, and nitrogen levels of 0, 30, 60 and 90 kg N ha^{-1} were tested, with an ammonium-nitrate fertilizer, dosing 16.9% in nitrate form (NO_3^--N) and 17.6% in ammonium form (NH_4^+-N). Two applications were realized weekly during four weeks, with 40% of total nitrogen applied in first two weeks and the remaining 60% in last two weeks. The fertilizer for each box was dissolved in 0.5 L of water, and the solution obtained was administered using a watering-can. Each styro-foam box received approximately 28.8 L (262 L m^{-2}) of water (24.8 L of irrigation water by micro-aspersion system + 4 L from fertilizer solution) during the experiment.

Plant growth and yield

Four representative plants for each styro-foam box were harvested 31 days after sowing and were determined the plant height and numbers of true-leaves, stems and flowers per plant. In order to evaluate the yield, one randomized sample of shoots contained in 0.01 m² was harvested per each styro-foam box. Shoots were weighed (fresh weight, FW) and oven-dried at 65°C for 48 h and weighed again (dry weight, DW).

Samples of purslane plants from each styro-foam box were harvested, washed and separated in leaves and stems, and stored in ultra-freezing conditions at -80°C, for nitrate and oxalic acid determinations.

Nitrate determination

Nitrate determination in leaves and stems was conducted according to Lastra (2003).

Samples were oven dried at 65°C for 48 h, weighed (0.1000 g), macerated in a mortar, and homogenized in a test tube with 10 mL of distilled water, followed by agitation in a vortex and incubated for 1 h at 45°C in a water-shaking bath in order to obtain an extract rich in nitrate. The filtrate extract was mixed with salicylic acid in 5% (m v^{-1}) in sulfuric acid (1:4), and incubated for 20 min at room temperature, followed by addition of 9.5 mL of 2 M sodium hydroxide. Nitrate content was determined by reading the absorbance level on a UV-VIS spectrophotometer (Thermo Scientific, Genesys 10S) at 338 and 440 nm. Nitrate concentration in leaves and stems was obtained by interpolation in a standard curve previously prepared with potassium nitrate in range from 0 to 100 mg L^{-1}.

Oxalic acid determination

Oxalic acid determination in leaves and stems was conducted according to Palaniswamy et al. (2004). Samples were oven dried at 65°C for 48 h, weighed (0.0100 g), macerated in a mortar, and homogenized in a test tube with 5 mL of distilled water, followed by agitation in a vortex, and then was added 5 mL of 10 mM EDTA, followed by another agitation in vortex in order to extract oxalic acid. Oxalic acid concentration in filtrate extract was determined using the Oxaloacetate Assay Kit (Sigma-Aldrich) and were followed the manufacturer's instructions.

Statistical analysis

Data were analyzed by analysis of variance, using IBM SPSS Statistics 21 (Chicago, Illinois, USA) software, licensed to University of Évora, and means were separated at 5% level using Duncan's test.

RESULTS AND DISCUSSION

Growth and yield

Plant height increased with nitrogen fertilization level until 60 kg N ha^{-1} (Table 2). Plant height at 60 and 90 kg N ha^{-1} was higher than 14.7 cm reported by Cros et al. (2007), although, the duration of experiment (18 days) differed from the present study (31 days), which may explain the greatest height.

Table 2. Influence of nitrogen fertilization on growth and yield of purslane.

Fertilization level (kg N ha^{-1})	Plant height[1] (cm)	True-leaf[1]	Stem[1]	Flower[1]	Shoot FW[2]	Shoot DW[2]
		(no. $plant^{-1}$)			($g\ m^{-2}$)	
0	10.2 c	7.3 c	1.0 c	4.1 c	2,376 b	196 a
30	14.5 b	10.5 b	2.5 b	5.3 bc	4,008 ab	262 a
60	17.8 a	12.2 ab	2.7 ab	6.9 b	5,057 ab	367 a
90	20.4 a	15.0 a	3.5 a	9.0 a	5,300 a	372 a

Different letters in a same column symbolize significant differences between nitrogen fertilization levels at $p<0.05$ according to Duncan's test.

[1]Data represent means of 12 observations.

[2]Data represent means of four observations.

Numbers of true-leaves and stems were significantly higher in purslane plants subjected to nitrogen application than in control plants (0 kg N ha^{-1}) (Table 2). Cros et al. (2007) reported 16 true-leaves per plant, grown for 18 days. In the present study a similar number of true-leaves was obtained, although the duration of experiment was longer. Reasons for this may be related to plant cultivar used, which is unknown in both studies. Purslane plants subjected to a fertilization level of 0 kg N ha^{-1} showed yellowing and senescence of older leaves, and only one stem, which could be due to nitrogen deficiency.

Number of flowers was significantly higher at a fertilization level of 90 kg N ha^{-1} than at all others fertilization levels tested (Table 2).

Shoot fresh weight (yield) increased with nitrogen application, but was not affected by nitrogen level applied (Table 2). Levels of 30, 60 and 90 kg N ha^{-1} did not differ, and fresh weight ranged from 4,008 to 5,300 g m^{-2}. With the same plant density, in peat, Cros et al. (2007) obtained 2,241 g m^{-2}, a lower value than obtained in present study, which may be related to the duration of their experiment (18 days), and different plant cultivars and culture systems. Shoot dry weight increased with nitrogen application, but not significantly (Table 2).

Nitrate concentration

Nitrogen application increased significantly a nitrate concentration in leaf, while in stem only a fertilization level of 60 kg N ha^{-1} was significantly higher than control plants (0 kg N ha^{-1}) (Figure 1). Nitrate concentration in leaf was 36.82 and 56.61 mg g^{-1} DW in control plants and fertilized plants with 90 kg N ha^{-1}, respectively. Nitrate accumulation in leaf was higher than in stem with nitrogen fertilization. Purslane plants subjected to fertilization levels of 90 and 0 kg N ha^{-1} presented, respectively, the biggest differences (56.6-42.4 mg g^{-1} DW) and the smallest differences (36.8-33.5 mg g^{-1} DW) between nitrate concentration in leaf and stem. In consideration to nitrate concentration found in leaf and stem and their respective fresh weights, the amount of nitrate obtained in shoots was about 3900 mg kg^{-1} FW, considerably higher than 900 mg reported by Wang et al. (2009) for spinach. According to the classification of Santamaria (2006), purslane can be classified as a plant with a very high nitrate concentration. In 1997, the European Commission published guidelines established that the admissible daily intake for nitrates in humans is 0-3.65 mg kg^{-1} of body weight (Scientific Committee on Food, 1997), thus, a person with a body weight of 60 kg can consume daily about 50 g FW of purslane plants produced in this study, with no adverse health effects.

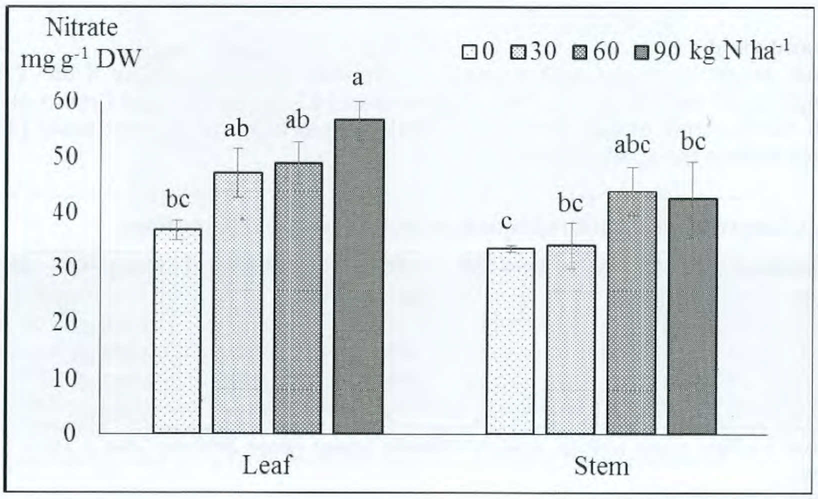

Figure 1. Nitrate concentration in leaf and stem of purslane. Different letters above bars symbolize significant differences between nitrogen fertilization levels at $p<0.05$ according to Duncan's test. Data represent means of four observations.

Oxalic acid concentration

Oxalic acid concentration in leaf increased linearly ($r=0.98946$, $p<0.01$) according to level of nitrogen applied, presenting 0.55 and 1.46 mg g^{-1} DW in treatments of 60 and 90 kg N ha^{-1}, respectively (Figure 2). Oxalic acid concentration in stem only increased significantly with a fertilization level of 90 kg N ha^{-1}, being ≈170% higher than in others treatments (Figure 2). Oxalic acid concentration in leaf and stem presented the highest difference in 60 kg N ha^{-1}, which content in leaf was ≈130% greater than stem. Purslane plants subjected to nitrogen fertilization level of 90 kg N ha^{-1} showed, respectively, a concentration of oxalic acid in leaf and stem, 95 and 91% lower than those reported by Palaniswamy et al. (2004), whose plants were subjected to nitrogen fertilization with a nutrient solution containing NO_3^--N/NH_4^+-N (50:50) and harvested at 16 true-leaf stage. In spinach, Noonan and Savage (1999) reported 890 mg 100 g^{-1} FW of oxalic acid concentration, a value significantly higher than 12 mg 100 g^{-1} FW found in purslane shoots subjected to a level of 90 kg N ha^{-1} in present study. According to Dolan et al. (2010), a dose of 22 g of oxalic acid may be lethal in the case of a human with 59 kg body weight. Thus, the daily consumption of 1 kg FW of purslane does not affect the health of consumer.

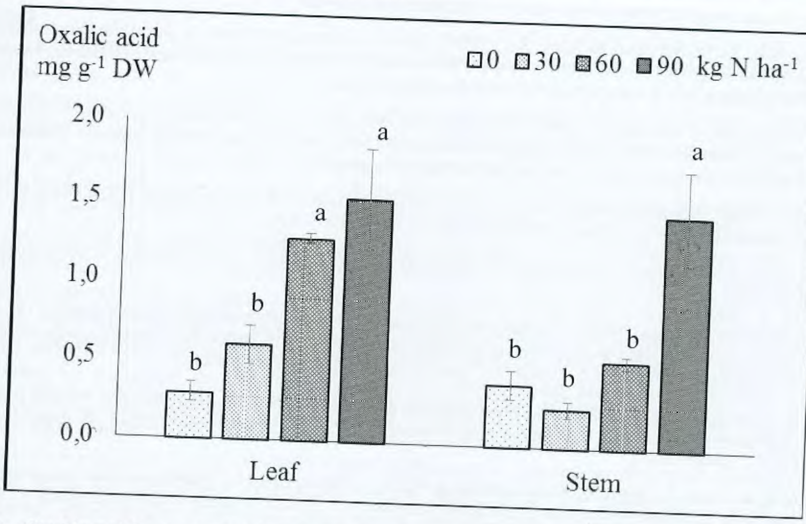

Figure 2. Oxalic acid concentration in leaf and stem of purslane. Different letters above bars symbolize significant differences between the nitrogen fertilization levels at $p<0.05$ according to Duncan's test. Data represent means of four observations.

CONCLUSIONS

The golden-leafed purslane showed a positive response to nitrogen fertilization. Plant height, numbers of true-leaves, stems and flowers, shoot fresh weight, nitrate and oxalic acid concentrations all increased as a result of nitrogen application. Shoot dry weight was not affected by nitrogen application. The best quantity/quality ratio was achieved with a fertilization level of 60 kg N ha^{-1}, resulting a yield of 5.1 kg m^{-2} FW and concentrations of nitrate (48.98 and 43.90 mg g^{-1} DW in leaf and stem, respectively) and oxalic acid (1.27 and 0.55 mg g^{-1} DW, in leaf and stem, respectively) which are not harmful to consumer health.

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