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EFFECT OF NITROGEN FORMS ON GROWTH, YIELD AND NITRATE ACCUMULATION OF CULTIVATED PURSLANE (*PORTULACA OLERACEA* L.)

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Abstract

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The present investigation was carried out to determine not only the most appropriate nitrogen form but also the effect of these forms on growth, yield, element content and nitrate accumulation of cultivated purslane (*Portulaca oleracea* L.) when the usual N dose was applied. The experiment was conducted under field conditions during 2009 and 2010. The nitrogen forms used in this work were ammonium nitrate, urea, ammonium sulfate, calcium ammonium nitrate. While the highest yield was being determined in urea (2889 g $plot^{-1}$) in 2009, in ammonium nitrate (3022.5 g $plot^{-1}$) in 2010. The macro and microelements in purslane are N, Ca, K, Mg, Na, P, S and B, Cu, Fe, Mn, Zn, respectively. In spite of the fact that the lowest nitrate accumulation was determined in control and ammonium sulfate application as 1247.75 mg kg^{-1} and 1232.25 mg kg^{-1} respectively, the highest nitrate accumulation was in calcium ammonium nitrate (1435.00 mg kg^{-1}). Considering the nitrate accumulation in leafy vegetables is harmful for human health, therefore, the usual dose of ammonium sulfate is firstly suggested that it should be applied to not only have better yield and agronomic traits but also produce healthy crops for human nutrition in cultivated purslane. However, when the highest yield was taken into consideration, ammonium nitrate was also suggested as a fertilizer for purslane.

Key words: element content, nitrogen, nitrate accumulation, *Portulaca oleracea* L., yield

Introduction

Purslane is belonging to family Portulacaceae and the genus *Portulaca*. Purslane (*Portulaca oleraceae* L.) is an important palatable vegetable crop can be eaten tender stems and leaves, raw or cooked with mild flavor in Turkey and other Mediterranean countries, southern Europe, and Asia (Palaniswamy et al., 2001). Although, wild and cultivated purslane plants have been spreading throughout Turkey with approximately 5000 tons annual production, the most commercial production area is East Marmara with 3171 tons annual production (TUIK, 2010).

Nitrogen is an essential mineral fertilizer for plant growth and development and is the world's largest agricultural chemical. Nitrogen forms are widely used in vegetable production in Turkey and other countries (Güvenç, 2002; Wang et al., 2008). It has important role as a basic element of protein, nucleic acids, chlorophyll and growth hormones (Barker et al., 1974) and is essential in periods of rapid growth. However, farmers have increased application of N fertilizers to their land year by without considering the response of different species to N rate and forms. A major drawback of fertilizer use, particularly in the case of N is excessive use beyond the crop's needs leads to negative implications for the environment, especially groundwater pollution and its associated health hazards (Korkmaz et al., 2008). Adequate supply of nitrogen (N) can promote plant growth and increase crop production, but under excessive application of nitrogen fertilizer, especially, vegetables can accumulate high levels of nitrate and, upon being consumed by living beings, pose serious health hazards (Hord et al., 2009). Nitrate accumulation in leafy vegetables such as rocket, spinach has a detrimental impact on human health (Ahmadi et al., 2010). These vegetable were shown to contain nitrates at varying levels ranging from 1 to 10 000 mg kg^{-1} (Gangolli et al., 1995). The level of nitrates varies according to the kind of vegetables. There are several factors affecting nitrate accumulation in vegetables, e.g. genetic, environmental (temperature, photoperiod) and agricultural factors (nitrogen doses and chemical forms), the most important factor is soil nitrogen content according as nitrogen fertilization (Güvenç, 2002; Santamaria, 2006). In-

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terest in the dietary intakes of nitrates and nitrites has arisen from the concern about their possible adverse effect on health. EU Scientific Committee has established an acceptable daily intake (ADI) of 0–3.7mgkg^{-1} body weight for nitrate and of 0–0.06 mg kg^{-1} body weight for nitrite for Food (EU Scientific Committee 1995). Vegetables (including potatoes) are one of the major sources of dietary exposure to nitrate, being responsible for 85% of intake (Gangolli et al., 1994).

The key measure for regulating nitrate accumulation in this type of crop and in others lies in applying reasonable agronomic techniques, particularly in N fertilizer management. The effect of cultivar on nitrate and nitrite accumulation in vegetables is controversial (Stagnari et al., 2007). Several investigations were reported on the effect of nitrogen forms and doses on nitrate accumulation in vegetables. For instance, Güvenç (2002) reported that yield, growth, total nitrogen and nitrate content of radish roots increased with making raise the ammonium nitrate doses. Similarly, Ahmadi et al. (2010) indicated that nitrate content in spinach leaves increased with enhancing by urea application. In addition, ammonium-N and urea can be transformed quickly into nitrate-N, therefore increasing nitrate accumulation in vegetables (Wang et al., 2008). Application of ammonium chloride, ammonium nitrate, sodium nitrate and urea significantly increased the yields and nitrate concentrations of Peking cabbage and spinach (Wang and Li, 2004). Therefore, effects of different nitrogen forms on vegetable growth and nitrate accumulation have attracted great attention in recent years (Santamaria et al., 1999; Stagnari et al., 2007; Wang et al., 2008; Ahmadi et al., 2010).

Otherwise, since its identification as a rich source of omega-3 fatty acids (ω3FA) and antioxidants has increased interest in cultivating purslane (Palaniswamy et al., 2001). Although there are a lot of reports about content of omega-3 fatty acids (ω3FA), concentration of oxalic acid and antioxidants (Simopoulos et al., 1992; Ezekwe et al., 1999; Liu et al., 2000; Palaniswamy et al., 2001; Palaniswamy et al., 2004; Fontona et al., 2006), most of the researches do not cover the agronomic characters such as yield, growth, element content and nitrate accumulation etc. in cultivated purslane.

In addition, it was known in mentioned studies that the more nitrate accumulation increased the more the nitrogen doses raised. Therefore, the aim of this work is to determine not only the most appropriate nitrogen forms but also the effect of these forms on growth, yield, element content and safety and nutritional features of purslane (*Portulaca oleracea* L.) when the usual N dose was applied.

Material and Methods

The experiment was conducted under field conditions during June – July of 2009 and 2010 in Erzurum, Turkey. Seeds for purslane (*Portulaca oleracea* L.) variety, which is the only known wild and cultivated species in Turkey, tested in this study were provided by the Manier Vegetable Seed Corporation (Adana, Turkey). The soil of the experimental area was sandy loam texture (clay 22.88%, silt 33.16%, sand 43.96%) Ustorthent great soil group with neutral pH (7.23) and EC 403 µmhos cm^{-1}. It had 1.16% organic matter, 12.55 cmol kg^{-1} Ca, 3.16 cmol kg^{-1} Mg, 2.78 cmol kg^{-1} K, 0.66 cmol kg^{-1} Na, 22.87 mg kg^{-1} P and 0.06% total N. Seeds were sown on plots of 1.2 x 1.5 m, on 5 and 2 July in 2009 and 2010, respectively, in rows, at a separation between rows of 20 cm. Irrigation was on a need basis, about twice a week. Experimental plots were kept weed-free using hand weeding.

The plots were fertilized with different forms of nitrogen. The nitrogen forms were ammonium nitrate [(NH_4NO_3), (33% N)], urea [($CO(NH_2)_2$) (46% N)], ammonium sulfate [($(NH_4)_2SO_4$), (21% N)], calcium ammonium nitrate [($Ca(NH_4NO_3)_2$), (26% N)]. The same N dose (150 kg ha^{-1}) was accepted usual (normal) dose according to Vural et al. (2000) and Günay (2005) reports were applied all fertilized plots. Nitrogen fertilizers were applied uniformly prior to planting onto soil surface by hand and incorporated before sowing. Plots not exposed to nitrogen fertilizer served as control.

Purslane plots were harvested before blossoming 35 days after sowing according to the preliminary study results conducted out in 2008 in both experiment years despite the fact that when purslane was blossoming, its quality was decreasing for fresh consumption (Vural et al., 2000). Afterwards, growth-promoting effects of nitrogen forms were evaluated by determining yield (g plot^{-1}), plant height (cm) and dry matter (%), root length (cm) and dry matter (%). In addition, the effect of the nitrogen forms on the element content and nitrate accumulation of purslane were evaluated under optimal N fertilization.

In order to determine the mineral contents of purslane, plants samples were oven-dried at 68^{o}C for 48 h and then grounded to pass 1 mm sieve. Macro- (P, K, Ca, Mg, S and Na) and microelements (B, Fe, Mn, Zn, and Cu) were determined after wet digestion of dried and ground subsamples using a HNO_3–H_2O_2 acid mixture (2:3, v/v) with three steps (first step: 145 ^{o}C, 75% RF, 5 min; second step: 180 ^{o}C, 90% RF, 10 min; third step: 100 ^{o}C, 40% RF, 10 min) in a microwave oven (Bergof Speedwave Microwave Digestion Equipment MWS-2) (Mertens, 2005a). Tissue P, K, Ca, Mg, S, Na B, Fe, Mn, Zn and Cu were determined by using an Inductively Couple Plasma spectrophotometer (Perkin-Elmer, Optima 2100 DV, ICP/OES, Shelton, CT, USA) (Mertens, 2005b). The Kjeldahl method and a Vapodest 10 Rapid Kjeldahl Distillation Unit (Gerhardt, Konigswinter, Germany) were used to determine total N (Bremner, 1996). In addition, reflectoctoquant nitrate test (Merckoquant nitrate test 1.16995.0001) was used to determine NO_3-N in plant by reflectometer (Coltman, 1989).

The field experiments were conducted as randomized complete block designs, with four replicates. Data obtained were subjected to ANOVA and differences between means were compared by using Duncan's multiple range test. There were no statistical differences between years for elemental analyses; therefore, the data were pooled for only element contents and nitrate accumulation.

Results and Discussion

The effect of nitrogen forms on plant growth in purslane was presented in Table 1. It was seen that nitrogen forms had plant height in purslane increased significantly in both experiments years statistically. While the lowest plant height values were determined in control (15.6 and 11.5 cm) in 2009 and 2010, the highest plant height value was obtained from ammonium sulfate (28 cm). However; when nitrogen forms were evaluated among themselves, according to experiment years' means it was determined that ammonium sulfate and calcium ammonium nitrate had shown better performance than urea and ammonium nitrate. When the effect of nitrogen forms on plant dry matter content was examined, the highest dry matter content was determined in control (5.4%) in 2009; in ammonium sulfate application (5.6%) in 2010. Yet, the effect of nitrogen forms on dry matter content in plant was not statistically significant. In addition, the lowest root length values were determined in control 8.0 cm and 5.9 cm in both 2009 and 2010, respectively. The highest root length values were in urea (10.3 cm) and ammonium sulfate application (10.2 cm) in 2009. Nevertheless; as nitrogen forms were evaluated among themselves, calcium ammonium nitrate application's positive effect on root development was less than other nitrogen forms. Likewise, among nitrogen forms the lowest root length values were obtained in calcium ammonium nitrate application as 9.2 cm and 7.4 cm respectively in both 2009 and 2010. There were no significant effects on dry matter content of purslane root by nitrogen forms. Although the highest dry matter content was determined in ammonium nitrate application (11.0%) and control (11.7%) in 2009 and 2010 respectively, the lowest dry matter content in root was in urea application (9.9%) in 2010.

Table 1
Effects of different nitrogen forms on growth of purslane

Treatments	2009	2010	Mean
	Plant height, cm		
Control	15.6 c*	11.5 b*	13.6 C*
Urea	23.6 b	20.4 a	22.0 B
Calcium ammonium nitrate	27.7 a	21.7 a	24.7 A
Ammonium sulfate	28.0 a	20.0 a	24.0 A
Ammonium nitrate	24.0 b	20.2 a	22.1 B
	Plant dry matter, %		Mean
Control	5.4 NS	5.4 NS	5.4 NS
Urea	5.2	5.1	5.2
Calcium ammonium nitrate	4.8	5.3	5.0
Ammonium sulfate	5.1	5.6	5.3
Ammonium nitrate	5.0	5.2	5.1
	Root length, cm		Mean
Control	8.0 b*	5.9 b*	6.9 C*
Urea	10.3 a	8.0 a	9.1 AB
Calcium ammonium nitrate	9.2 ab	7.4 ab	8.3 B
Ammonium sulfate	10.2 a	8.1 a	9.1 AB
Ammonium nitrate	9.9 a	8.8 a	9.3 A
	Root dry matter, %		Mean
Control	10.9 NS	11.7 NS	11.3 NS
Urea	10.1	9.9	10.0
Calcium ammonium nitrate	10.5	10.1	10.3
Ammonium sulfate	10.8	10.2	10.5
Ammonium nitrate	11.0	10.3	10.7

* – indicated that 0.05 probability level ($P < 0.05$).
NS – non significant at the $P = 0.05$.

Responses of purslane plants to nitrogen fertilizers were consistent in both experimental years. The results indicated that there were significant differences among N forms in terms of the plant height and root length. Thus, Elmi et al., (1997) reported that plant height of purslane changed according to the varieties and average plant height ranged from 27.4 cm to 55.0 cm. Similarly, according to Vural et al., (2000) and Günay (2005) reports plant height changed between 20 and 30 cm. On the other hand, Palaniswamy et al., (2004) declared that plant height was affected by the stage of harvest and it was ranging between 27.4 cm and 44.3 cm. And also, dry matter content of plant affected by the stage of harvest as in plant height and dry weights of leaves and shoot were ranged between 0.5 g and 2.2 g, and 0.9 g and 4.2 g, respectively. For all that, although there were no detailed reports about the root length and root dry matter of cultivated purslane, Vural et al. (2000) and Günay (2005) reported that the biggest parts of cultivated purslane roots were spread under the 20 cm of soil. Results of our work were similar and verified agronomic traits those obtained by other researches.

The effect of nitrogen forms in purslane on yield was given in Table 2. Nitrogen forms affected significantly the yield in purslane statistically. While the highest yield was being determined in urea (2889 g plot^{-1}) in 2009, in ammonium nitrate (3022.5 g plot^{-1}) in 2010. Other growth properties with similar way to the lowest yield values were in control in both experiment years. However; as both experiment years' means values were taken into consideration, besides nitrogen forms showed close effect to each other on yield, it was seen that ammonium nitrate came to front as to other nitrogen forms (Table 2).

The results of study showed that yield in purslane changed according to the nitrogen forms. All the four applied N fertilizers significantly increased yield with respect to the untreated control. Amonium nitrate (NH_4NO_3) gave the highest yield compare to other treatments. Researches about the agronomic characters of purslane are very limited and there are a few researches about yield of cultivated purslane. For example, Elmi et al., (1997) report, yield of purslane varied to the years and variety, and the highest yields were obtained from *Portulaca sativa* (70003 kg ha^{-1}) and Egyptian (37130 kg ha^{-1}). Closely, Vural et al. (2000) notified that yield of cultivated purslane was ranging between 30 ton ha^{-1} and 50 ton ha^{-1}. In addition, the yield of cultivated purslane was affected by the seed quality; planting time, growing conditions and plant care practices (Vural et al., 2000) and the results from this study confirmed these researchers' findings.

Table 2
Effects of different nitrogen forms on yield of purslane

Treatments	Yield, g plot^{-1}		Mean
	2009	2010	
Control	1114.5 c*	1243.5 d*	1179.0 C*
Urea	2889.0 a	2097.0 c	2493.0 AB
Calcium ammonium nitrate	2449.5 ab	2268.0 c	2358.8 B
Ammonium sulfate	1983.0 b	2680.5 b	2331.8 B
Ammonium nitrate	2466.0 ab	3022.5 a	2744.3 A

* – indicated that 0.05 probability level ($P < 0.05$).

As it is shown in Tables 3 and 4, macro and micro elements were varied according to the nitrogen sources and differences between nitrogen sources were statistically significant (except for K, Na and S) at 0.05 and 0.01 probability levels in both years of experiment. The macro elements in purslane are N, Ca, K, Mg, Na, P and S. The amounts of N in purslane ranged from 2.09 mg kg^{-1} (control) to 2.42 mg kg^{-1} (NH_4NO_3 + $CaCO_3$) in experimental years. However; nitrogen forms had amount of Ca, Na, P and S increased in purslane according to control. While the highest content of Ca, K, Na and P was determined in calcium ammonium nitrate application, Mg in ammonium nitrate and S in ammonium sulfate.

Microelements were varied according to the nitrogen sources for both experiments. The differences between nitrogen sources were statistically significant (except for Mn) at 0.05 and 0.01 probability levels for both years (Table 3). Microelements among all samples were B, Cu, Fe, Mn and Zn. As in macro elements, the lowest values in microelements were obtained in control. With another words, the content of B, Cu, Fe and Zn was much in nitrogen forms according to the control. Also, while the highest content of B and Fe was determined in ammonium sulfate, Cu in urea, Zn in ammonium nitrate.

The element composition of plant leaves fertilized by different N forms may provide important information about the

Table 3
Effects of different nitrogen forms on element content of purslane, mg kg^{-1}

		Means of 2009 and 2010					P Values
		Control	Urea	Calcium ammonium nitrate	Ammonium sulfate	Ammonium nitrate	
Macro Elements	Ca	12886.50	14604.00	15861.50	14528.50	14471.50	0.000
	K	22668.50	22500.25	24045.25	23172.75	22908.50	0.147
	Mg	1412.25	1435.75	1429.75	1396.50	1580.00	0.020
	Na	155.50	224.75	241.75	194.25	217.50	0.118
	P	2330.50	2770.75	2960.00	2451.75	2523.00	0.001
	S	467.50	502.25	544.50	547.75	515.50	0.388
Micro Elements	B	8.96	9.46	9.65	9.71	9.07	0.000
	Cu	8.32	11.48	10.97	8.84	8.99	0.000
	Fe	184.00	210.25	205.25	255.25	213.25	0.010
	Mn	14.75	15.71	15.67	15.75	15.73	0.698
	Zn	16.01	17.66	17.10	18.35	20.26	0.001

Table 4
Effects of different nitrogen forms on N and NO_3^- content of purslane

Treatments	Means of 2009 and 2010	
	N, %	NO_3^-, mg kg^{-1}
Control	2.09	1247.75
Urea	2.40	1301.00
Calcium ammonium nitrate	2.42	1435.00
Ammonium sulfate	2.28	1232.25
Ammonium nitrate	2.23	1309.50
P Values	0.041	0.021

effect of N fertilization in plant nutrient element uptake. In this study it was found that N forms significantly effected element contents of purslane compared with the control except for K, Na, S, Mn. In spite of the limited data about element content of cultivated purslane, Teixeira and Carvalho (2009) reported that element content of purslane varied according to the harvest time and sowing season. For instance, according to their reports, purslane had 813 mmol kg^{-1} Ca, 1170 mmol kg^{-1} Mg, 618 mmol kg^{-1} Na, 1173 mmol kg^{-1} K, 151 mmol kg^{-1} Fe, 135 mmol kg^{-1} Zn and 151 mmol kg^{-1} Cl in summer production and late harvest. On the other hand, although the data about the macro and microelement contents of cultivated purslane were limited, our present results on elements were obtained from purslane in accordance with mentioned study.

The nitrate accumulation in purslane showed difference according to the nitrogen forms (Table 4). The effect of nitrogen forms on nitrate accumulation was found statistically significant ($P < 0.05$). Although the lowest nitrate accumulation was determined in control and ammonium sulfate application as 1247.75 mg kg^{-1} and 1232.25 mg kg^{-1} respectively, the highest nitrate accumulation was in calcium ammonium nitrate (1435.00 mg kg^{-1}) application.

In this research, nitrogen content of the purslane was significantly affected by nitrogen fertilization with different N forms. There was a much greater increase in nitrate N as well as total nitrogen in purslane compared with control. Furthermore, the data from this study strongly indicated that nitrogen fertilization with different N forms was more responsive for nitrate accumulation of purslane. Purslane absorbs nitrate very quickly and nitrate concentration in leaves can be much higher than in the growth medium. Accumulation is genetically controlled and is modified by environment, fertilizer management, and crop production practices. Indeed, nitrate content can vary also species, cultivars and even genotypes (Radzevičius et al., 2009; Santamaria 2006).

Nitrate accumulation in vegetables often depends on the amounts and kinds of nutrients present in the soil and is especially closely related to the amounts, time of application, and composition of the fertilizers applied. Besides, light intensity have been identified as the major factors that influence nitrate content in vegetables, in particular, light intensity and nitrate content in soil before or at harvest are known to be critical factors in determining nitrate levels in spinach or other leafy vegetables (Santamaria, 2006). These experiments have shown that purslane preferred nitrate N, and that the application of nitrate fertilizer usually promoted their growth. In these experiment, NO_3^- content in purslane showed a significant increase when the doses of $Ca(NH_4NO_3)_2$, NH_4NO_3 and $CO(NH_2)_2$ were increased while reduced with $(NH_4)_2SO_4$ (Table 4). The experimental area soil was in neutral pH was taken into consideration, as calcium in calcium ammonium nitrate increased soil pH and availability of nitrate for plant, it might have increased nitrate accumulation in purslane especially macro elements (Tables 3 and 4). Similarly, it could be said that as sulfate decreased soil pH it decreased nitrate accumulation (Yildiz, 2008). These results showed it is accumulated a considerably higher amount of NO_3^-, with the nitrogen fertilizers applications. $Ca(NH_4NO_3)_2$ and NH_4NO_3 showed to induce the highest NO_3^- accumulation in purslane leaves while $(NH_4)_2SO_4$ the lowest. The fertilizers containing nitrate ($Ca(NH_4NO_3)_2$ and NH_4NO_3), thanks to its fast N-nitrate release, proved to be a more suitable match to the N-uptake of a short life cycle crop such as purslane allowing the highest crop yields, but it also increased particularly nitrate. In addition, imbalance between nitrate absorption and reduction by plants caused nitrate accumulation in vegetables and when nitrate answer the supply in soil, plants usually absorb much more nitrate than they can reduce (Wang et al., 2008). According to results of this research, near to imbalance between nitrate absorption and reduction by plants, increasing doses of nitrogen, genetic and environmental (temperature, photoperiod) factors or lack of water (Güvenç, 2002; Wang and Li, 2004; Santamaria, 2006; Wang et al., 2008; Radzevičius et al., 2009; Ahmadi et al., 2010), usual doses of nitrogen forms can also cause and affect nitrate accumulation in varied ratios. In addition, our present results on nitrate accumulation reported here are similar to those reported for mentioned previous studies.

Conclusion

Consequently, the results of this study suggest that cultivated purslane had increased the growth characteristics such as yield with the application of different N forms. On the other hand, the lowest nitrate accumulation was determined in ammonium sulfate application when compared with other N forms. When considering the nitrate accumulation in leafy vegetables is harmful for human health, therefore, the usual dose of ammonium sulfate is firstly suggested that it should be applied to not only have better yield and agronomic traits but also produce healthy crops for human nutrition in cultivated purslane (*Portulaca oleracea* L.). Morover, it can be said that nitrate accumulation on purslane was low and under the critical values across N forms. With this reason, although ammonium sulfate was firstly suggested, when the highest yield was taken into consideration, ammonium nitrate was also suggested as a fertilizer for purslane. The levels of nitrates in purslane were similar to or even lower than the values obtained in other vegetables. Therefore, nitrates consumed from purslane are concluded to be harmless to human health.

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