Substitution of inorganic fertilizers with organic manure reduces nitrate accumulation and improves quality of purslane

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Abstract

Growers often apply high amounts of chemical fertilizers for vegetable production and this application contributes to concerns about nitrate levels in food. An experiment was conducted to investigate soil N amendment effects for reducing the nitrate accumulation and improving the quality of fresh purslane (*Portulaca oleracea* L.). Treatments included four levels of animal manure as follows, N-based broiler litter (T_1), P-based broiler litter (T_2), N-based cattle manure (T_3), P-based cattle manure + urea (T_4), four levels of chemical fertilizer equivalent to organic treatments, and a T_0 (no fertilizer and manure). Results showed that nitrate levels of broiler litter and cattle manure treatments were significantly lower than those of chemical fertilizer treatments. The highest level of nitrate in the first harvest was recorded in the T_7 treatment and higher levels were recorded in T_5 and T_7 treatments in the second harvest. In the first harvest, T_1 and T_2 treatments had higher appearance quality but in the second harvest, appearance quality of T_2 and T_5 treatments showed a significant increase compared to the other treatments. It is concluded that organic manure application results in higher quality as compared with the inorganic fertilizer and reduces nitrate accumulation in fresh purslane.

Keywords: broiler litter; fresh vegetable; nitrate; *Portulaca oleracea* L.

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Introduction

Green leafy vegetables are an important part of the human diet worldwide, so consideration of their shelf life is a major concern for all consumers (Ghate et al., 2013; Sawe et al., 2014). In addition, vegetable consumption has health benefits and high nutritional value that can help to prevent the onset of diseases. Vegetables are important for their supplement nutritive value so consumption can be beneficial for increasing the human body's energy supply and preventing nutritional deficiency (Robinson, 1990). Vegetables are also a rich source of vitamins (A, C, pyridoxine, thiamine, and niacin), minerals (potassium, calcium, magnesium, sodium, and iron) and dietary fiber (Wargovich, 2000). It is well known that such vitamins and minerals are an important part of the human diet (Duncan, 1996). The use of vegetables in the daily diet certainly reduces risks of a variety of conditions such as cancers, heart diseases, strokes, and other chronic health problems.

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Although nitrogen is an important element in providing carbon skeleton and producing metabolites and enzymes (Noorani Azad et al., 2016), one factor that affects the quality of vegetables is that they lack nitrate accumulation. Nitrate is often the main source of available nitrogen to most plants, especially vegetables (Marschner, 1995). High nitrate concentration in vegetables causes a variety of poisoning that ranges from death to anemia in children and nitrosamines, which is carcinogenic for adults (Ishivata et al., 2002).

Chemical fertilizers are used in vegetable production in order to gain maximum yield (Stewart et al., 2005). But fertilizer application can have harmful effects on the environment and on human health (Aisha et al., 2007); it can also affect the quality of such products (Balogh et al., 2006). Organic fertilizers however, do not have the aforementioned harmful effects but create sustainable soil fertility and higher quality products (Sharpley et al., 2004).

When using organic fertilizers in vegetable production, a little nitrate is stored in plants' usable organs, for this reason organic cultivation based on the use of organic manure is commonly preferred to inorganic cultivation. Bonasia et al. (2013) observed that more nitrate content (64.6 %) was in lettuce supplemented with N100 fertilizer treatment compared to that with no nitrogen, and N50 fertilizer treatment has shown 56.9% more nitrate compared to that with no nitrogen. It was reported that nitrogen is important for preservation of the green color of vegetable leaves. After 7 days' storage, the quality (loss of weight, reduced chlorophyll, and appearance quality) of lettuce was significantly reduced.

In a study on celery, tests were done on two nitrogen levels (80 and 120 kg ha^{-1}) of organic and chemical fertilizer sources and it was concluded that shelf life of celery in organic manure treatment was significantly higher than that in the control treatment (Rizzo et al., 2010).

Alpha-linolenic acid (ALA) is one of the two essential fatty acids in humans. Epidemiological studies and dietary trials in humans suggest that alpha-linolenic acid is a major cardio-protective nutrient. The optimal dietary intake of alpha-linolenic acid seems to be about 2 g per day or 0.6 to 1% of total energy intake. One of the most important sources of alpha-linolenic acid for the population is green leafy vegetables such as purslane (*Portulaca oleracea* L.) (Xiang et al., 2005). Purslane is used as a fresh vegetable in human diet as well as for its medicinal properties. Purslane shoots are used as a vegetable, as well as a diuretic, antipyretic, antiseptic, and antispasmodic medicine (Xiang et al., 2005). Also, this group of vegetables provides a rich source of omega-3 fatty acids, coenzyme Q_{10}, vitamins A, C, E, and potassium, selenium, and antioxidant compounds including tocopherol, ascorbic acid, and glutathione (Liu et al., 2000). Therefore, it is essential to improve the health quality of such vegetables through optimized use of fertilizers. The aim of the present study was to reduce adverse effects of chemical fertilizers on quality, nitrate accumulation, and shelf life of fresh purslane. In addition, effects of organic and inorganic nitrogen sources on vegetable quality were compared.

Materials and Methods

Study site description

A field experiment was conducted at the Agricultural Research Farm of Shahrekord University (latitude 320 21' N, 500 49' E, 2050 m above sea level), Iran in 2014. According to Emberger and Gossen classification, this area is arid and cold steppe climate. The mean annual rainfall is 334 mm and annual temperature is 10.8 °C. The annual rainfall is not synchronized with purslane growing season and the air temperature is relatively high.

Experimental setup

Treatments included no fertilizer (T_0), chemical fertilizers of N-based broiler litter (T_1), P-based broiler litter (T_2), N-based cattle manure (T_3), P-based cattle manure + urea (T_4), T_5, T_6, T_7 and T_8 (urea and triple super phosphate) equivalent to organic treatments of T_1, T_2, T_3, and T_4, respectively. Concentrations of purslane N and P were 120 and 50 kg ha^{-1}, respectively.

Table1
Chemical characteristics of the soil, broiler litter, and cattle manure in the study

Property	Unit	Soil	Broiler litter	Cattle manure
EC	dS m^{-1}	1.01	18.99	14.5
pH	-	7.96	8.21	8.1
OC	%	0.995	32.08	48.9
Ca	%	-	1.9	1.3
N	%	0.082	1.86	1.028
P*	mg kg^{-1}	10.8	3470	2900
K*	mg kg^{-1}	391	19000	8000

* P_2O_2 and K_2O forms are recorded for broiler litter and cattle manure.

Before preparation of the seedbed, a combined sample from the depths of zero to 30 cm of field soil was taken and the physicochemical properties were determined. Also, the properties of broiler litter and cattle manure were measured (Table 1).

Chemical fertilizer and manures treatments were applied after land preparation operations. Firstly, experimental plots were made and then manures, triple super phosphate fertilizer, and 50% urea fertilizer were added to the given plots according to the relevant treatments and then thoroughly mixed into the soil. The remaining amount of urea chemical fertilizer was added to each plot after the first harvest. The soil had adequate potassium (Table 1), so no potassium fertilizer was added.

Seeds of purslane were planted in rows, spaced 25 cm apart with high density at soil depth of 1 cm, on 5th July. Then, in order to achieve the desired density (80 plant m^2) thinning was done at the 4-6 leaf stage. The first irrigation was done after planting and the next irrigations were done according to environmental conditions and plant requirements once every 3 days. Weeding was done during the growth season. Plants were harvested after reaching an average height of about 20 cm, and so both harvests were done on July 27th and August 16th, respectively.

During harvest, plants were cut at the height of 5 cm above the soil level and then 10 plants were selected from each plot randomly to take measurements of parameters. After preparation of samples from selected plants, measurements were done for nitrate and calcium concentration. Evaluations were also done for appearance quality and physiological loss of weight; after determining appearance quality before storage, one sample (50 g) was taken from stems and leaves randomly which was weighed and rinsed with tap water for 2 minutes. The original weight was recorded (every samples weighted) and then the samples were placed in plastic bags and transferred to a refrigerator for storage at the temperature of 7 °C. After a week of storage, the secondary weight and appearance quality were recorded.

Nitrate concentration

Nitrate concentrations were measured by calorimetric method after reduction (Emami, 1996). Briefly, 0.5 g mixture powder was added to 10 ml of extract and shook for 30 seconds and then immediately the colored solution was passed through a piece of filter paper. After 10 minutes, the intensity of the solution color was read using a spectrophotometer (Pharmacia LKB Novaspec II) at 540 nm wave length.

Calcium concentration

For a measurement of calcium, the samples were analyzed using digestion method (Emami, 1996) and applying the Atomic Absorption Spectrophotometer (Farmsia- model LKB, England).

Quality assessment

Appearance quality of leaves before and after refrigerated storage (7 days) was investigated. The overall appearance was determined by scoring each leaf using a 1–5 hedonic scale with 5= excellent, no defects; 4 = very good, minor defects; 3= fair, moderate

Table 2
ANOVA for the effects of inorganic fertilizers and organic manure on nitrate concentration, calcium concentration, and appearance quality before storage of purslane

S.O.V	df	Nitrate concentration		Calcium concentration		Apearance quality before maintenance	
		First harvest	*Second harvest*	*First harvest*	*Second harvest*	*First harvest*	*Second harvest*
Replication	2	14.8^{ns}	16.61^{ns}	0.03^{ns}	0.02^{ns}	0.03^{ns}	0.111^{ns}
Nitrogen source	8	1745.7^{**}	2840^{**}	0.23^{**}	0.2^{**}	1.39^{**}	0.66^{**}
Error	16	25.5	3939	0.005	0.02	0.12	0.06
CV (%)		9.74	9.85	4.71	10.29	9.27	8.17

ns= Not significant, **= *P*< 0.01.

defects; 2= poor, major defects; 1= unusable. A score of 3 was considered as the limit of marketability and a score of 2 as the limit of edibility (Rinaldi et al., 2010).

Physiological loss of weight (PLW %)

Physiological loss of weight (PLW) was recorded during the storage as was suggested by Thakur et al. (2002) and Bonasia et al. (2013). Edible parts of purslane from each treatment were separated randomly and sample size was 50 g. The difference in weight was considered as the physiological loss of weight (PLW%) and results were expressed as percent loss as given below:

$$\text{Physio. Loss of Wt. (PLW\%)} = \frac{\text{Original edible parts Wt.} - \text{ edible parts Wt. after 7 days}}{\text{Original edible parts Wt.}} \times 100.$$

Statistical Analysis

Data were subjected to one-way analysis of variance (ANOVA) using SAS software package (v. 9. SAS, 2003). Mean comparison was performed using Fisher's least significant difference (LSD) method (P≤0.05).

Results

Nitrate concentration

The effect of fertilization in the first harvest on the nitrate concentration was significant at 1% probability level (Table 2). The comparison of means showed that in the first harvest nitrate concentration of T_7 treatment

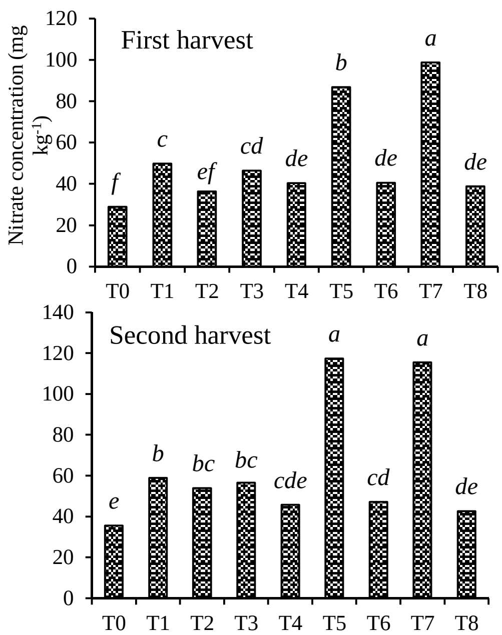

Fig. I. Effect of inorganic fertilizer and organic manure on nitrate concentration of purslane; Values followed by different letters show significant differences between the treatments at P≤0.05. T_0= no fertilizer and manure, T_1= broiler litter based on N requirement, T_2= broiler litter based on P requirement, T_3= cattle manure based on N requirement, T_4= cattle manure based on P requirement; T_5, T_6, T_7, and T_8= inorganic fertilizer equal to T_1, T_2, T_3, and T_4 treatments, respectably.

(98.77 mg kg^{-1}) significantly increase compared to the other fertilizer treatments and the T_0 (no fertilizer) treatment (Fig. I). Nitrate concentration of T_7 treatment showed significant difference compared to T_5 treatment, while T_2, T_4, T_6, and T_8 treatments showed no significant difference with each other (Fig. I).

Calcium concentration

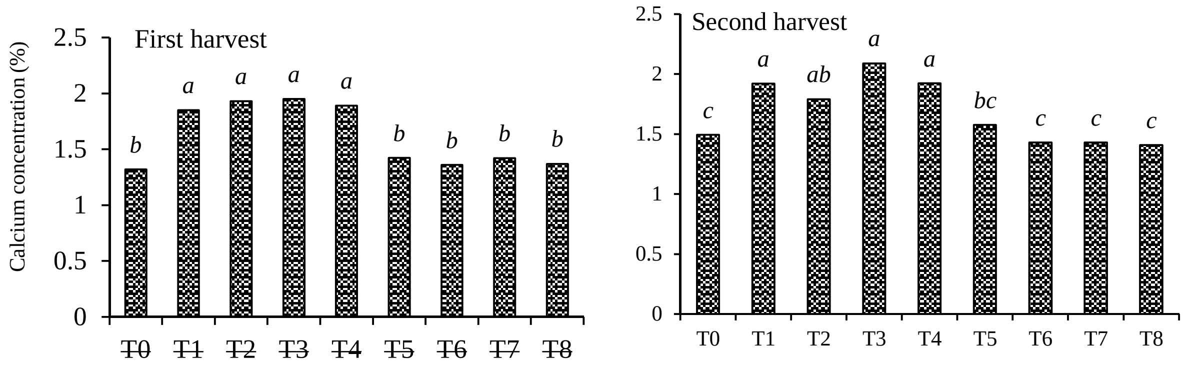

Fig. II. Effect of inorganic fertilizers and organic manure on calcium concentration of purslane; Values followed by different letters show significant differences between the treatments at P≤0.05. Treatment symbols are defined in the text and under Fig. I.

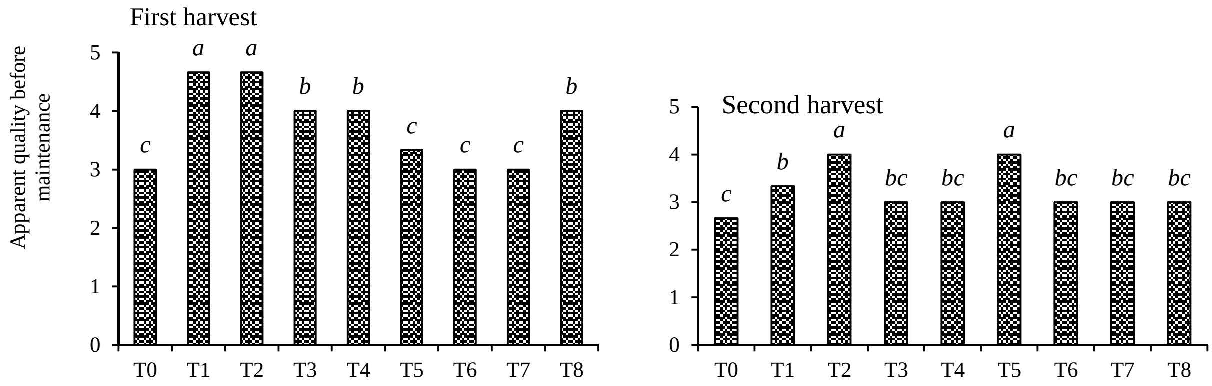

Fig. III. Effect of inorganic fertilizers and organic manure on apparent quality before storage of purslane; Values followed by different letters show significant differences between the treatments at P≤0.05. Treatment symbols are defined in the text and under Fig. I.

As seen in Table 2, the concentration of calcium in the first harvest was affected by fertilization. As Fig. II suggests, the first harvest organic fertilizer treatments showed the maximum calcium concentration compared with fertilizer treatments of a chemical source and T_0 (no fertilizer) treatment.

Results of analysis of variance indicated that the effect of fertilization on the concentration of calcium in the second harvest was significant at 1% probability (Table 2). T_1, T_3, T_4, and T_2 treatments showed no significant difference in terms of calcium concentration. Also T_5 treatment showed no significant difference with T_6, T_7, T_8, and unfertilized treatments in terms of calcium concentration (Fig. II).

Appearance quality

According to the results of variance analysis (Table 2) appearance quality before storage in the first harvest was affected by fertilization ($p \leq 0.01$). Results of mean comparison indicate that T_2 and T_1 treatments had the highest appearance quality. In the first harvest, appearance quality of cattle manure treatments was determined with no significant difference (Fig. III).

Results of variance analysis of the second harvest showed that the effect of fertilization on appearance quality before storage was significant at 1% probability level (Table 2). In the second harvest, appearance quality of T_2 and T_5 treatments had a significant increase compared to the other fertilizer treatments and the T_0. Also, appearance quality of fertilizer treatments of the N-based organic source, T_4 combined treatment with chemical equivalents of mentioned treatments, and T_6 showed similar results (Fig. III).

Results of variance analysis indicate that the effect of fertilization on appearance quality

Table 3
ANOVA for the effects of the inorganic fertilizer and organic manure on appearance quality after storage and physiological loss of weight in purslane

S.O.V	df	Apparent quality after maintenance		Physiological loss of weight	
		First harvest	*Second harvest*	*First harvest*	*Second harvest*
Replication	2	0.1^{ns}	0.03^{ns}	0.71^{ns}	1.8^{ns}
Nitrogen source	8	2.91^{**}	1.06^{**}	15.6^{**}	4.8^{**}
Error	16	0.19	0.12	0.37	1.25
CV (%)		13.68	14.4	9.2	20

ns= Not significant, **= *P*< 0.01.

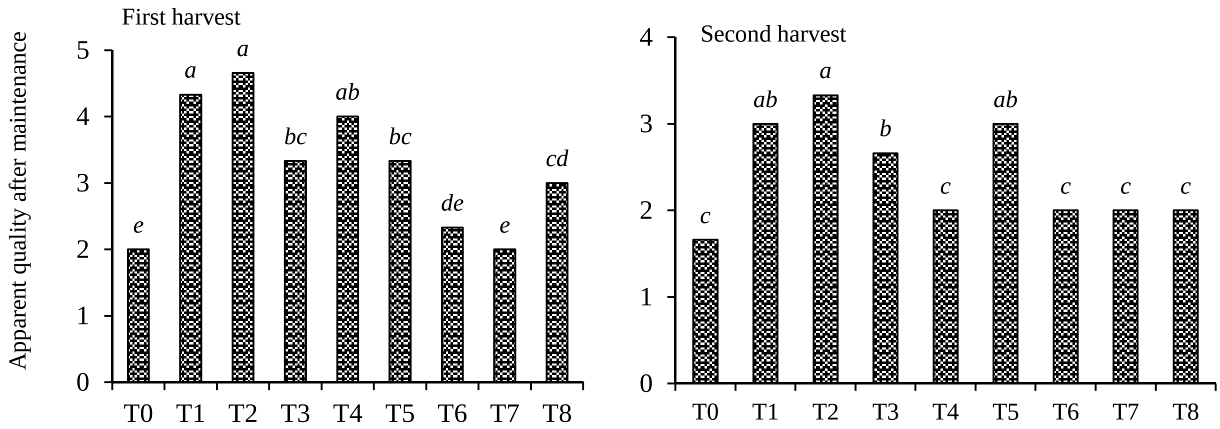

Fig. IV. Effects of inorganic fertilizers and organic manure on appearance quality after storage of purslane; Values followed by different letters show significant differences between the treatments at P≤ 0.05. Treatment symbols are defined in the text and under Fig. I.

after storage for the first harvest was significant at 1% probability level (Table 3). In the first , T_1 and T_2 treatments showed no significant difference with T_4 treatment, while the two treatments showed a significant difference with the other fertilizer treatments of organic and chemical sources and the T_0 (no fertilizer). The minimum appearance quality after storage was assigned to T_7, T_6 and the T_0 (no fertilizer) treatments (Fig. IV).

Results of variance analysis in the second harvest showed that the effect of fertilization on appearance quality after refrigerated storage was significant at 1% probability level (Table 3). According to Fig. IV, T_2 treatment had no significant difference with T_1 and T_5 treatments in the second harvest in terms of appearance quality after storage, in T_4, T_6, T_7, T_8, and the T_0 (no fertilizer) treatments and the minimum score for the appearance quality was observed after refrigeration.

Physiological loss of weight

Results of ANOVA (Table 3) indicated that in the first harvest the effect of fertilization on physiological loss of weight was significant at 1% probability level. According to Fig. V, the first harvest physiological loss of weight in T_0 (no fertilizer) treatment showed a significant difference, in addition to T_1, T_2, T_3, and T_4 treatments, with T_6, T_5, T_7, and T_8 treatments. However, in T_6 treatment more physiological loss of weight was observed compared to T_5, T_7, and T_8 treatments.

The results of variance analysis of the second harvest showed that the effect of fertilizing on physiological loss of weight was significant at 1% probability level (Table 3). According to Figure 5, it can be said that in the second harvest T_8 treatment showed no significant

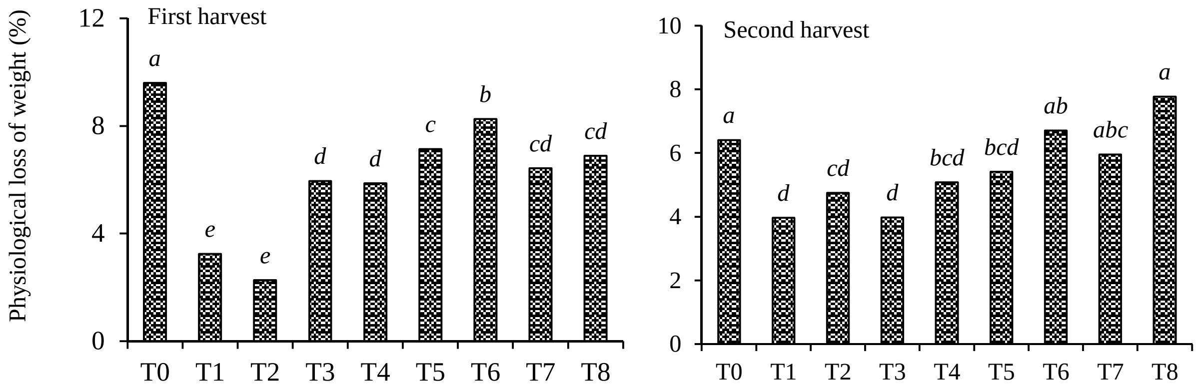

Fig. V. Effects of inorganic fertilizers and organic manure on physiological loss of weight in purslane; Values followed by different letters show significant differences between the treatments at P≤0.05. Treatment symbols are defined in the text and under Fig. I.

difference with T_6 and T_7 and T_0 (no fertilizer) treatments in terms of physiological loss of weight while a significant difference was determined between other treatments receiving organic fertilizer of organic and urea sources.

Discussion

According to results of variance analysis (Table 2) the concentration of nitrate in the second harvest was affected by fertilization ($P \leq 0.01$). In the second harvest, T_5 and T_7 treatments with averages of 117.4 and 115.5 mg kg^{-1}, respectively had higher level of nitrate than other fertilizer treatments. However, T_1, T_2, and T_3 treatments had no significant difference in terms of nitrate (Fig. I).

Chemical fertilizer application is the quickest method to compensate for a lack of nutrients needed by plants but it increases nitrate accumulations in plants (Wilkins, 2008). In the first harvest, the highest nitrate accumulation was observed in T_7 treatment with an average of 98.77 mg kg^{-1} and in the second harvest the highest nitrate accumulation was observed in chemical treatments of T_5 and T_7 (with an average of 117.4 and 115.5 mg kg^{-1}, respectively). This can be due to the reduced nitrogen absorption in the structure of plant organic compounds. In other words, chemical treatments have provided more and/or faster mineral nitrogen so that the amount is relatively high in plants, and lack of synchronization between nitrogen provided according to plants' needs has led to accumulation of excess nitrogen in the form of nitrate (El- Sayed et al., 2000). Ahmadi et al. (2010) reported that nitrate content in spinach leaves was significantly increased by nitrogen supplement in the form of urea fertilizer. Heeb et al. (2005) showed that organic nitrogen sources lead to nitrate reduction and increased levels of starch and organic acids compared to inorganic nitrogen sources to achieve the desired taste.

Calcium is needed for cell wall strengthening and provides protection against biotic and abiotic stress (Dini Torkamani and Samadi, 2014). Basically, chemical fertilizers only provide one or more of the elements required for plant growth, while organic fertilizers, in addition to providing lowly and highly used elements improve soil physical and chemical properties and create a proper environment for better quality plant growth (Fallah et al., 2013). It seems that organic treatments with calcium absorbed by the plant also have an effect on calcium absorption in soil (Toor, 2009) that increase the amount of calcium absorbed by plants and prolong shelf life of the produce. Reports indicate that by adding organic material (broiler litter) to the soil, secretion of organic acids in the environment of root rhizosphere reduces pH and creates desirable conditions for absorbing elements such as nitrogen, phosphorus, iron, and calcium that are involved in synthesis of photosynthetic pigments (Toor, 2009).

A significant increase in appearance quality before and after storage in broiler litter treatments can be attributed to the nutritive effects of fertilizer. As shown in Table 1, calcareous soil (pH=7.96) and bicarbonate in chemical treatments and unfertilized treatment may result in decreased absorption of nutrients by plants (Maschner, 1995). Increase of phosphorus in plants tissues could result in increased superoxide dismutase activity. This increase in antioxidant enzymes activities causes the control of hydrogen peroxide and malondialdehyde contents (Daei-hassani et al., 2016). By addition of organic materials (broiler litter) to the soil, secretion of organic acids in the rhizosphere creates desirable conditions for absorption of elements such as nitrogen, phosphorus, iron, and calcium that are involved in the synthesis of photosynthetic pigments (Toor, 2009), and this is effective on shoot chlorophyll of these treatments. So, it is probable that broiler litter, by providing nutrients at a plant's peak time, and supplying micronutrients through growing season, could affect photosynthetic pigments, chlorophyll, and finally improve its appearance quality. However, for shelf life in refrigerated storage for organic and chemical treatments it can be said that the broiler litter treatment showed appearance superiority in both harvests after storage. Broiler litter with nutrients (nitrogen, phosphorus, potassium and calcium), micronutrients (zinc, manganese, iron, and magnesium, etc.) and gradual mineralization of the elements in the essential steps of plant growth could increase the concentration of intracellular materials by increasing soluble solids and starch accumulation, which in turn, due to metabolism of stored carbohydrates, lipids, and proteins during shelf life can protect plant tissues (Kays, 1999). Low appearance quality before and after storage in chemical treatments probably was due to the use of artificial mineral fertilizers for plants that actually replace organic fertilizers to achieve higher yield in the shortest time. A study on organic production of tomatoes demonstrated that organic farming systems increase soluble solids and measurable acids and can affect appearance quality (Chassy et al., 2006; Barrett et al., 2007). In a study investigating nitrogen-rich fertilizers from organic and chemical sources on quality characteristics in celery, it was demonstrated that celery fertilized with organic nitrogen and packaged in an anti-fog polyolefin film had a 37-day shelf life while the control sample packaged in a micro-perforated polypropylene film after 20 days from packaging was no longer marketable (Rizzo et al., 2010).

In both harvests, the T_0 (no fertilizer) treatment during storage in a refrigerator for a week at a relatively cool temperature (7 °C) during processes such as transpiration, cell water loss and activation of defensive mechanisms including increasing soluble sugars increased the concentration of cell materials to prevent cell freezing and degradation during storage. The trend of relative weight reduction in chemical treatments compared to organic treatments is also consistent with the results of appearance quality after storage (Fig. IV). Since organic sources creating desirable conditions in soil environment caused better absorption of other nutrients by the plant (Toor, 2009), they had a desirable effect on the concentration of dissolved solids of the plant cell. According to the above process, it is probable that organic treatments had physiological loss of weight (Fig. V). Znidarcic et al. (2010) reported that weight loss after harvest in vegetables was usually due to water loss through transpiration. Weight loss can lead to a withered state that reduces market-friendly and consumer acceptance. Shafiee et al. (2010) reported similar results in tests on strawberry that determined weight loss during storage due to metabolic activity, transpiration, and respiration. De Castro et al. (2006) reported the effect of different storage temperatures on tomato and demonstrated that weight loss was proportional to storage temperature and duration.

Conclusions

A balanced nutrient supply is crucial to reach high yields. In the present study, it was determined that reduced nitrate accumulation of fresh purslane, by application of organic manure instead of chemical fertilizer played an important role in increasing health and appearance quality of fresh vegetable.

Superior appearance of quality before and after storage with low physiological loss of weight during storage can increase marketability and subsequently improve short-time storage capability, which will be effective on increasing amounts of fresh vegetables in peoples' daily food intake. In general, it can be concluded that application of organic nitrogen source for growth and production of fresh medicinal plant an effective strategy for fresh and healthy vegetable consumption with a positive effect on consumer's health.

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اثر جایگزینی کودهای غیرآلی با کودهای آلی بر کاهش تجمع نیترات و افزایش کیفیت خرفه

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چکیده فارسی

کشاورزان اغلب برای تولید سبزیجات مقدار زیادی کود شیمیایی مصرف می‌کنند و نحوه کاربرد باعث نگرانی در مورد سطوح نیترات موجود در محصولات غذایی می‌شود. به منظور بررسی اثرات کودهای نیتروژن دار برای کاهش تجمع نیترات و بهبود کیفیت خرفه (*Portulaca oleracea* L.) آزمایشی انجام شد. تیمارها شامل چهار سطح کود دامی به ترتیب زیر بودند: کود مرغی بر اساس نیتروژن (T1)، کود مرغی بر اساس فسفر (T2)، کود گاوی بر اساس نیتروژن (T3)، کود گاوی بر اساس فسفر + اوره (T4) و چهار سطح از کود شیمیایی معادل با تیمارهای آلی و T0 (بدون کود آلی و کود شیمیایی). نتایج نشان داد که سطوح نیترات در تیمارهای کود مرغی و کود گاوی به طور معنی‌داری کمتر از تیمارهای کود شیمیایی بود. بالاترین میزان نیترات در اولین برداشت در تیمار T7 ثبت شد و سطوح بالاتر در تیمارهای T5 و T7 در برداشت دوم ثبت شد. در برداشت اول، تیمارهای T1 و T2 دارای کیفیت ظاهری بالاتری بودند، اما در برداشت دوم، کیفیت ظاهری تیمار T2 و T5 افزایش معنی داری نسبت به تیمارهای دیگر نشان داد. به طور کلی نتیجه‌گیری می‌شود که کاربرد کود آلی کیفیت خرفه را در مقایسه با کود شیمیایی افزایش داده و تجمع نیترات این محصول را کاهش می‌دهد.

کلمات کلیدی: سبزی تازه، نیترات، کود مرغی، خرفه