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ORIGINAL RESEARCH ARTICLE

Assessment effect of macronutrients on growth and phytochemical contents of Iranian native spinach

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ABSTRACT

Excessive usage of chemicals in crops, especially in leafy vegetables, caused people exposed to health and environmental risks. In Iran, spinach used as a winter vegetable that believed has high Iron and is useful for anemia. The objective of the experiment was to determine the optimum use of each macronutrients to obtain safe maximum growth and yield for scaling up among farmers. Treatments were chemical fertilizers including ammonium sulfate, triple superphosphate and potassium sulfate at 50, 100, 150 and 200 kg/h against control in a randomized complete block design. Results showed that nitrogen caused elevation of fresh and dry weight in spinach as the maximum obtained in 200 kg/h ammonium sulfate. Results obtained from effect of phosphorus showed that super phosphate increased fresh and dry weight of spinach; but potassium sulfate had no effect on its growth and yield. Analysis of variance on cross effect of data showed significant differences in fresh and dry weight, number of leaves, chlorophyll content and nitrate, and non-significant differences in length and wide of leaves.

Keywords: Mineral Nutrition; Macronutrients; Nitrate; Spinach

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1. Introduction

Spinach (*Spinacia oleracea* L.), belongs to Chenopodiaceae, is a leafy vegetable known as a winter and alternative crop eaten raw People believed that it is useful for anemia in Iran. It is rich in nutrients and vitamins with low energy[1]. Spinach composed of ample content of β-karotene, lutein, zeaxanthine and phenolic[2]. High bioactive compounds in spinach can decreased risk of some diseases associated with ageing, such as cardiovascular disease, Alzheimer's disease, cataracts, and several forms of cancer[3–8]. By the way, demand and consumption of its raw eating has increased. Despite of the importance, cultivation areas, yielding and national trading statistics is unknown in Iran, because most farmers cultivated it in orchards at winter[9]. Mineral fertilizers supplied abundantly by petrochemical companies and the low prices resulted in overmuch usage of them by farmers. Additionally, there is insufficient data on the effects of agronomic practices, such as providing mineral nutrition for native spinach in Iran.

The nutritional quality of vegetables including spinach can be affected by many pre- and post-harvest factors. Fertilization is one of the most practical and effective pre-harvest agronomic practices which could improve the yield and nutritional quality of crops for human consumption.

The objectives of the study were to determine the optimum application rates of macronutrients such as N, P, and K and interaction among them for safe production and increased yield for the promulgation among farmers.

2. Materials and methods

The experiment was done in Isfahan Agricultural Education Center farm at 51°51′ E and 32°31′ N in altitude of 1,545 m at winter of 2013. The area has laid in dry climate with less than 100 mm annual precipitation.

Cultivation practices: seeds of native spinach (Isfahan accession) purchased from PakanBazr™ (99%). Seeds were sown in special trays with 102 cavities, filled by same ratio mixture of perlite and cocopeat at greenhouse. Seedlings emerged after 5–8 days. They transplanted to the field after 16 days, when the seedling had 4–5 cm tall. Soil preparation was accomplished before transplanting, as sown 18 plantlet in a block.

Treatments and design: treatments were set as a completely randomized block. Nutrient treatments were: ammonium sulfate (21% N) as nitrogen source, triple superphosphate (46%, P_2O_5) as P source and potassium sulfate (50%, K_2O) as K source. All treatments were used in 0, 50, 100, 150 and 200 kg/h.

Parameters: growth indices were measured by calculating biomass production including fresh and dry weight (gr), number of leaves, length and width of leaf (cm) as well chlorophyll content (nm), leaf nitrate and N contents (Dumas method), P and K[10]. Data analysis were done by SAS V.8.

3. Results

Nitrogen effects: results showed that nitrogen usage in the experiment could increase fresh weight at the level of 200 kg/h ammonium sulfate compared to control. The same results observed in dry weight. Leaf nitrate elevated by increasing usage of nitrogen fertilizers, as the results showed the highest leaf nitrate observed in 200 kg/h ammonium sulfate. There are no significant differences among application of 100 kg/h N source and less treatments. Number of leaves in each plant showed significant differences among treatments; but there are no differences for length and width of leaf (**Table 1**).

Table 1. Biomass production parameters in response to nitrogen nutrition by ammonium sulphate in spinach

N (kg/h)	Fresh weight	Dry weight	Leaf nitrate (%)	Total nitrogen (%)	Chlorophyll content (nm)	Leaf No.	Leaf length (cm)	Leaf width (cm)
0	43.08d	12.05d	0.02c	1.34b	158.1d	10.5d	6.8a	5.3a
50	52.88c	18.35c	0.03bc	4.05a	357.23c	13.65c	7.2a	5.9a
100	58.04bc	22.85bc	0.04bc	4.31a	443.35a	15.7b	7.5a	6.1a
150	63.15b	24.32b	0.12b	4.38a	430.88ab	18.03ab	7.6a	6.1a
200	66.84a	26.88a	0.56a	4.69a	386.15b	19.67a	7.8a	6.09a
Significance	**	**	**	**	**	**	ns	ns

Numbers followed by the same letter are not significantly differentns ($P < 0.05$)

Table 2. Biomass production parameters in response to phosphorus nutrition by triple superphosphate in spinach

P (kg/h)	Fresh weight	Dry weight	Leaf nitrate (%)	Total Phosphorus (%)	Chlorophyll content (nm)	Leaf No.	Leaf length (cm)	Leaf width (cm)
0	38.6c	11.66c	0.02a	0.53b	62.7c	11.2d	6.6a	5.1a
50	42.55b	17.84b	0.03a	0.97a	79.1b	12.85c	7.4a	5.6a
100	43.68ab	21.93ab	0.04a	0.95a	99.5a	14.3b	7.6a	6.2a
150	46.1ab	23.87ab	0.03a	0.94a	88.65ab	18.32ab	7.3a	6.1a
200	50.4a	25.09a	0.05a	0.99a	89.41ab	19.97a	7.5a	6.3a
Significance	**	**	ns	**	**	**	ns	ns

Numbers followed by the same letter are not significantly differents ($P < 0.05$)

Phosphorus effects: results obtained from different levels of P on spinach showed that triple superphosphate could increase fresh weight; as elevation of P up to 200 kg/h, fresh weight increased, but this is significant up to 100 kg/h and not above it. This means that optimum usage of triple superphosphate for spinach could be define in 100 kg/h. the same results obtained for the fertilizer in dry weight of spinach plants. Different level of P had no effects of leaf nitrate, length and width, whereas had significant effects on leaf number and chlorophyll contents (**Table 2**).

Potassium effects: data analysis showed that K fertilizers had no significant effects on fresh and dry weight, leaf number, length and width and leaf nitrate and chlorophyll contents in spinach. Total K had linear and positive relationship with increasing plant availability of potassium (**Table 3**).

Table 3. Biomass production parameters in response to potassium nutrition by potassium sulfate in spinach

K_2O (kg/h)	Fresh weight	Dry weight	Leaf nitrate (%)	Total potassium (%)	Chlorophyll content (nm)	Leaf No.	Leaf length (cm)	Leaf width (cm)
0	39.8a	18.2a	0.03a	1.74b	80.08a	10.8a	7.06a	4.95a
50	40.1a	17.9a	0.06a	2.30a	76.4a	11.23a	6.78a	4.83a
100	39.4a	17.3a	0.08a	2032a	80.9a	11.6a	6.59a	5.78a
150	40.5a	18.4a	0.05a	1.78b	74.6a	10.7a	7.31a	6.04a
200	40.4a	18.6a	0.04a	1.84b	78.45a	10.4a	8.06a	6.24a
Significance	ns	ns	ns	**	ns	ns	ns	ns

Numbers followed by the same letter are not significantly differents ($P < 0.05$)

Analysis of variance showed that cross effects of N, P and K on fresh and dry weight, leaf number, nitrate and chlorophyll content were significant, while there was no effect on total content of each element and leaf length and width. Cross effects of N by P, P by K and N by K were no significant.

4. Discussion

Nitrogen nutrition is necessary for suitable vegetative growth and arbitrary yield of vegetables and leafy plants. Nielsen and Halvorson[11] stated that application of nitrogen increased plant yields due to stimulation of growth in roots and aerial organs. Our findings showed that spinach fresh weight had positive linear increase linked by nitrogen access, as the maximum yield for fresh weight observed for 200 kg/h ammonium sulfate. This increasing relationship was reported by authors Luo *et al.*[12], Hammad *et al.*[13] and Cooke *et al.*[14]. Studies showed that nutrition of native spinach by nitrogen fertilizers at the level of 200 kg/h resulted in incrementing of photosynthesis rate and leaf chlorophyll content as well as larger leaves. Since our results showed that nitrogen nutrition of spinach by ammonium sulfate had no effects on leaf size, but had significant effect on leaf number. In addition to, this fertilizer had significant effects on leaf chlorophyll content at the level 0.01. The same results reported in spinach nitrogen nutrition[15]; although urea used as nitrogen source and reported that 250 kg/h urea could be the best treatment for high yield. Koohkan and Maftoon[16] emphasized that chlorophyll content of leaf increased linked to higher nutrients. There are findings about the relationship between nitrogen applications by micronutrients in yield[17].

It is documented that source of nitrogen used can affect plant tissue components and nutrient levels because they have interactions with nutrient uptakes directly or indirectly, as replacement nitrate by ammonium is a suitable way for anion and cation uptakes[18]. This argumentation caused using farmers ammonium sulfate instead of Urea for solving nitrate accumulation in leaf petiole and blades.

Our results pointed out positive effects of higher dry weight due to increasing nitrogen usage. It means that by increasing nitrogen source up to 200 kg/h ammonium sulfate, dry matter increments. Dry weight in control plants were low significantly, whereas plants treated by nitrogen showed elevation continuously up to 200 kg/h. Findings confirmed that spinach was strictly sensitive to nitrogen nutrition leading to vigor vegetative growth[19,20].

In plants, leaf chlorophyll content is an index of health. Our results showed that increasing nitrogen fertilizers, extended leaf chlorophyll content significantly, as maximum content of leaf chlorophyll obtained in application of 100 kg/h ammonium sulfate. Some studies stated that the reducing or excessive usage of nitrogen could result in the falling of leaf chlorophyll content due to low content of nitrogen in leaves[21–23]. Chlorosis in plants resulted from low N is a fundamental reason for chlorophyll destruction. Extra application of nitrogen in our experiment leads to lower chlorophyll content in leaves. This fact could be due to the increasing osmosis stress of over dose of nitrogen that resulted in lower uptake of nitrogen that affects metabolite biosynthesis including chlorophyll. These findings were opposite with past reports of Hortensteiner[24] and Ni *et al.*[25] who pointed out bio and non-bio stresses were critical reasons for chlorophyll destruction.

Data analysis in our experiment showed application of nitrogen had no significant effect on leaf indices including length and width. Some authors stated that nitrogen extended leaf area by affecting cell proliferation and expansion[26,27]; since nitrogen nutrition in our study could not promote significant increase in leaf area. Nitrogen nutrition mostly stimulates young leaf growth rate. According to **Table 1**, increased yield (fresh and dry weight) in spinach had been due to enhancement of leaf numbers rather than leaf area.

Dry weight, total nitrogen content and nitrate increased by more usage of nitrogen[28]. Etman[29] reported that urea, as source of nitrogen by foliar spraying or adding to soil resulted in higher yield in spinach. In another experiment, 300 kg/h nitrogen enhanced spinach yield from 20.4 (in control) to 41.2 t/h. On the other hand, accumulation of nitrate in spinach leaves treated by nitrogen at harvesting and post harvesting were higher than control[30]. Tei *et al.*[31] emphasized that spinach tended accumulation of nitrate (NO^{3-}) more than other vegetables, because of efficient uptake but insufficient reducing systems. More nitrogen application resulted in more leaf spinach nitrate[32]. Nitrogen is an essential macronutrient in plants and farmers tend to obtain higher yields. N fertilizers caused enhanced dry weight in spinach at the level of 200 mg nitrogen per kg soil leading to 293% increase compared to the control. In addition to, increasing nitrogen caused higher leaf chlorophyll contents[33].

Our findings showed that phosphorus had significant effect of growth and yield in spinach. Increasing P in form of triple superphosphate up to 200 kg/h improved yield. Jia *et al.*[34] stated that growth and yield of vegetables enhanced by increment of P, but overmuch usage of this element bears negative effects on quality and yield of vegetables. Linear and positive increasing of yield by more supply of P, could be due to high concentration level of minerals and electrical conductivity (EC) in water irrigation that adjusted by phosphate ions[35]. Some authors believed that increased growth following supplying phosphorus was due to the increasing glutamine synthetized and protein production in plant body[36].

Dry weight of spinach in our experiment affected by phosphorus supply. As the maximum dry weight obtained in treatment with 200 kg/h triple superphosphate. In maize, adding phosphorus to soil resulted in up to 60% increasing in yield[37]. In fact, phosphorus affects yield indices in spinach by effects on increasing area and efficiency in plant roots.

Our results showed that phosphorus had significant effect on leaf chlorophyll content in spinach. Phosphorus deficiency could decrease chlorophyll and protein contents up to 40%[38]. In addition to, application of phosphorus enhanced chlorophyll content about 21% resulted from increasing biosynthesis of chlorophyll a and b in sufficient humidity[39].

Phosphorus treatments could not significantly affect leaf length and width in this study. Fredeen *et al.*[40] pointed out decreasing leaf expansion, leaf number and leaf area were common indices of phosphorus deficiency in plants often resulted in yield fall. The experimental results show that there is no shortage of phosphorus supply or excess phosphorus supply in each phosphorus application treatment.

Potassium fertilizers usually used for improvement of flower and fruit quality. In this study, application of potassium could not affect biomass production in spinach significantly. This finding confirmed the studies of Soundy *et al.*[41] that reported application of potassium had no significant effect on fresh and dry weight and leaf area and other growth parameters on lettuce. Some believed that availability of potassium leading to limited growth in plants[42]. On the other hand, some stated that potassium fertilizers could resulted in enhanced chlorophyll content, root length, dry matter and yield in cabbage[43].

It is documented that potassium mostly used for reproductive growth, flowering and fruiting improvement in plants[44], therefore it's clear that the element has no effect on vegetative organs and leafy plants. Our results showed increasing potassium had no significant effects on growth parameters. Leaf potassium content is the only parameter affected by supplying different doses of fertilizers, as it had no effect on the plant growth.

5. Conclusion

Spinach growth and development depends on sufficient and commensurate N, P and K supply. Our results showed that application of ammonium sulfate and triple superphosphate at the level of 200 kg/h leading to enhancing yield and growth characters in spinach. Potassium in form of potassium sulfate had no significant effects on spinach growth parameters.

Conflict of interest

The authors declare that they have no conflict of interest.

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