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

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ABSTRACT

Overmuch 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 determining optimum usage of each macronutrients for obtaining safe with maximum growth and yield in order to extension among farmers. Treatments were chemical fertilizers including ammonium sulfate, super phosphate triple 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, but no in length and wide of leaves.

Keywords: Mineral Nutrition; Macronutrients; Nitrate; Spinach

1. Introduction

Spinach (Spinacia oleracea L.) belongs to chenopodiaceae is a leafy vegetable known as a winter and alternative crop eaten raw (conte et al., 2008). People believed that it is useful for anemia in Iran. It is rich of nutrients and vitamins with low energy (Sies, 1997). Spinach composed of highly content of β-karotene, lutein, zeaxanthine and phenolic (Bergquist, 2006). High bioactive compounds in spinach can be decreased risk of some diseases associated with ageing, such as cardiovascular disease (Christen, 2000; Manach et al. 2005; Khaw et al. 2001; and Arts & Hollman, 2005) Alzheimer’s disease (Commenges et al. 2000), cataracts (Brown et al. 1999), and several forms of cancer (Williamson, 1996; Liu et al. 2000; Gandini et al. 2000; Liu et al. 2001; Joshipura et al. 2001; & Kang et al. 2005). 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 (Ahmadi et al., 2016). Mineral fertilizers supplied abundant by petrochemical companies as well as low prices resulted in overmuch usage of them by farmers. In addition to, 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 (Kader, 2008). Fertilization is one of the most practical and effective pre-harvest agronomic practices could improve the yield and nutritional quality of crops for human consumption (Arts & Hollman, 2005).

The objectives of the study were determining optimum application rates of macronutrients such as N, P, and K and interaction among them for safe production and increased yield in order to promulgate 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 1545 m at winter 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 PakanBazrt™ (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, super phosphate triple (46%, P2O5) as P source and potassium sulfate (50%, K2O) 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 (by Dumas method), P and K (by Mudau et al., 2005). Data analysis were done by SAS v. 8.

3. Results

Nitrogen effects: results showed that nitrogen usage in the experiment could be increased 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>
<thead>
<tr>
<th>N(kg/h)</th>
<th>Fresh weight</th>
<th>Dry weight</th>
<th>Leaf nitrate (%)</th>
<th>Total nitrogen (%)</th>
<th>Chlorophyll content (nm)</th>
<th>Leaf No.</th>
<th>Leaf length(cm)</th>
<th>Leaf width(cm)</th>
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<tr>
<td>0</td>
<td>43.08d</td>
<td>12.05d</td>
<td>0.02c</td>
<td>1.34b</td>
<td>158.1d</td>
<td>10.5d</td>
<td>6.8a</td>
<td>5.3a</td>
</tr>
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<td>50</td>
<td>52.88c</td>
<td>18.35c</td>
<td>0.03bc</td>
<td>4.05a</td>
<td>357.23c</td>
<td>13.65c</td>
<td>7.2a</td>
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<td>22.85bc</td>
<td>0.04bc</td>
<td>4.31a</td>
<td>443.35a</td>
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<tr>
<td>150</td>
<td>63.15b</td>
<td>24.32b</td>
<td>0.12b</td>
<td>4.38a</td>
<td>430.88ab</td>
<td>18.03ab</td>
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<td>200</td>
<td>66.84a</td>
<td>26.88a</td>
<td>0.56a</td>
<td>4.69a</td>
<td>386.15b</td>
<td>19.67a</td>
<td>7.8a</td>
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</table>

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

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

Phosphorus effects: results obtained from different levels of P on spinach showed that super phosphate triple could be increased fresh weight; as elevation of P up to 200 kg/h, fresh weight increased, but this is significant up to 100 kg/h and no for above it. This means that optimum usage of super phosphate triple 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).

<table>
<thead>
<tr>
<th>P(kg/h)</th>
<th>Fresh weight</th>
<th>Dry weight</th>
<th>Leaf nitrate (%)</th>
<th>Total Phosphorus (%)</th>
<th>Chlorophyll content (nm)</th>
<th>Leaf No.</th>
<th>Leaf length(cm)</th>
<th>Leaf width(cm)</th>
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</thead>
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<td>0.94a</td>
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<td>0.99a</td>
<td>89.41ab</td>
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Numbers followed by the same letter are not significantly different (P<0.05)

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

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).
The dry could result from ammonium tissue experiments. Results showed that the same nitrogen (Sadeghipour et al., 2002) were increased by ammonium in leaf tissues. By applying nitrogen fertilizers, the maximum for fresh weight observed for 200 kg/h ammonium nitrate was higher than that of fresh weight (Kang et al., 2001). The results reported by authors (Luo et al., 2006; Hammad et al., 2007; Cooke et al., 2003) showed our results that nitrogen nutrition of spinach by ammonium nitrate had no effects on leaf size, but had significant effect on leaf number. In addition to this, this fertilizer had significant effects on leaf chlorophyll content at the level 0.01. The same results reported in spinach nitrogen nutrition (Sadeghipour Marvi, 2011); although urea used as nitrogen source and reported that 250 kg/h urea could be the best treatment for high yield. Koohkan and Maftoon (2011) emphasized that chlorophyll content of leaf increased linked to higher nutrients. There are findings about the relationship between nitrogen applications by micronutrients in yield (Dhillon et al., 1987; Reif et al., 2012).

It is documented that source of nitrogen used can effect plant tissue components and nutrient levels because they have interactions with nutrient uptakes directly or indirectly, as replacement nitrate by ammonium is suitable way for anion and cation uptakes (Assimakopoulou, 2006). This argumentation caused using farmers ammonium nitrate 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 nitrate, dry matter increments. Dry weight in control plants were low significantly, whereas plants treated by nitrogen it showed elevation continuously up to 200 kg/h. Findings confirmed that spinach was strictly sensitive to nitrogen nutrition lead to vigor vegetative growth (Cantliiffe, 1992; Magnifico et al., 1992).

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 nitrate. Some studies stated that less or up extreme usage of nitrogen could resulted in leaf chlorophyll content fall due to low content of nitrogen in leaves (James and Van Lerser, 2001; Kang and Van Lerser, 2002; Van Lerser, 1999). Chlorosis in plants resulted from low N is fundamental reason for chlorophyll destruction. Extra application of nitrogen in our experiment lead to lower chlorophyll content in leaves. This fact could be due to increasing osmosis stress of over dose of nitrogen resulted in lower uptake of nitrogen that affects metabolite biosynthesis including chlorophyll. These findings were opposite with past reports (Hortensteiner, 2006; Ni et al., 2001) pointed out bio and non-bio stresses were critical reason for chlorophyll destroy.

Data analysis in our experiment showed application of nitrogen had no significant effect on leaf indices including

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\begin{array}{|c|c|c|c|c|c|c|c|}
\hline
K2O(kg/h) & Fresh weight & Dry weight & Leaf nitrate (%) & Total potassium (%) & Chlorophyll content (nm) & Leaf No. & Leaf length(cm) & Leaf width(cm) \\
\hline
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 \\
\hline
\end{array}
\]

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

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; whereas had no effects on total content of each elements 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 of vegetables and leafy plants for suitable vegetative growth and obtaining arbitrary yield is necessary. Nielsen and Halvorson (1991) 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 reported by authors (Luo et al., 2006; Hammad et al., 2007; Cooke et al., 2003). 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 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, this fertilizer had significant effects on leaf chlorophyll content at the level 0.01. The same results reported in spinach nitrogen nutrition (Sadeghipour Marvi, 2011); although urea used as nitrogen source and reported that 250 kg/h urea could be the best treatment for high yield. Koohkan and Maftoon (2011) emphasized that chlorophyll content of leaf increased linked to higher nutrients. There are findings about the relationship between nitrogen applications by micronutrients in yield (Dhillon et al., 1987; Reif et al., 2012).

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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 (Taylor et al., 1993; Gastal and Lemaire, 2002); since nitrogen nutrition in our study could not be resulted significant increase in leaf area. Nitrogen nutrition mostly stimulates young leaf growth rate. In according to table 1, increased yield (fresh and dry weight) in spinach had been rather due to enhancement of leaf numbers nor leaf area.

Dry weight, total nitrogen content and nitrate increased by more usage of nitrogen (Biemond et al., 1996). Etman (1993) 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 (Aworh et al., 1980). Tei and colleagues (2000) emphasized that spinach tended accumulation of nitrate (NO3- ) more than other vegetables, because of efficient uptake but insufficient reducing systems. More nitrogen application resulted in more leaf spinach nitrate (Takebe et al., 1995). 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 lead to 293% increase compared to control. In addition to, increasing nitrogen caused higher leaf chlorophyll contents (Ronaghi et al., 2001).

Our findings showed that phosphorus had significant effect of growth and yield in spinach. Increasing P in form of super phosphate triple up to 200 kg/h improved yield. Jia and colleagues stated that growth and yield of vegetables enhanced by increment of P, but overmuch usage of this element could be negative effects on quality and yield of vegetables. Linear and positive increasing of yield by more supply of P, slightly could be due to high concentration level of minerals and electrical conductivity (EC) in water irrigation that adjusted by phosphate ions (Hu, and Schmidhalter, 2005). Some authors believed that increased growth following supplying phosphorus was due to increasing glutamine synthetized and protein production in plant body (Azcon et al., 1996).

Dry weight of spinach in our experiment affected by phosphorus supply. As the maximum dry weight obtained in treatment with 200 kg/h super phosphate triple. In maize, adding phosphorus to soil resulted in up to 60% increasing in yield (Plenets et al., 2000). 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 decreased chlorophyll and protein contents up to 40% (Xu et al., 2007). In addition to, application of phosphorus enhanced chlorophyll content about 21% resulted from increasing biosynthesis of chlorophyll a and b in sufficient humidity (Nyborg et al., 1999).

Phosphorus treatments could not significant effects on leaf length and width in this study. Fredeen and colleagues pointed out decreasing leaf expansion, leaf number and leaf area were common indices of phosphorus deficiency in plants often resulted in yield fall. As our experiment showed there were not low supply in phosphorus at all P treatments, and excess supply of phosphorus didn't take place.

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 findings confirmed Soundy and colleagues (2001) studies that reported application of potassium had not significant effect on fresh and dry weight and leaf area and other growth parameters on lettuce. Some believed that availability of potassium lead to limited growth in plants (Tiwari et al., 1998). On the other hand, some stated that potassium fertilizers could resulted in enhanced chlorophyll content, root length, dry matter and yield in cabbage (Singh and Blanke, 2000).

It is documented that potassium mostly used for reproductive growth, flowering and fruiting improvement in plants (Kumar et al., 2017), therefore it's clear the element has not any effect on vegetative organs and leafy plants. Our results showed increasing potassium had not any 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 super phosphate triple at the level of 200 kg/h lead to enhancing yield and
growth characters in spinach. Potassium in form of potassium sulfate had no significant effects on spinach growth parameters.

References


