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Original article

Nitrogen-molybdenum-manganese co-fertilization reduces nitrate accumulation and enhances spinach (*Spinacia oleracea* L.) yield and its quality

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ABSTRACT

Spinach (*Spinacia oleracea* L.) is considered a nitrogen (N) intensive plant with high nitrate (NO_3^-) accumulation in its leaves. The current study via a two-year field trial introduced an approach by combining N fertilization from different sources (e.g., ammonium nitrate; 33.5 % N, and urea; 48 % N) at different rates (180, and 360 kg N ha^{-1}) with the foliar spraying of molybdenum (Mo) as sodium molybdate, and/or manganese (Mn) as manganese sulphate at rates of 50 and 100 mgL^{-1} of each or with a mixture of Mo and Mn at rates of 50 and 50 mg L^{-1}, respectively on growth, chemical constituents, and NO_3^- accumulation in spinach leaves. Our findings revealed that the highest rate of N fertilization (360 kg N ha^{-1}) significantly increased most of the measured parameters e.g., plant length, fresh and dry weight $plant^{-1}$, number of leaves $plant^{-1}$, leaf area $plant^{-1}$, leaf pigments (chlorophyll *a*, *b* and carotenoids), nutrients (N, P, K, Fe, Mn, Zn), total soluble carbohydrates, protein content, net assimilation rate, and NO_3^- accumulation, but decreased leaf area ratio and relative growth rate. Moreover, plants received urea-N fertilizer gave the highest values of all previous attributes when compared with ammonium nitrate –N fertilizers, and the lowest values of NO_3^- accumulation. The co-fertilization of N-Mo-Mn gave the highest values in all studied attributes and the lowest NO_3^- accumulation. The best treatment was recorded under the treatment of 360 kg N-urea ha^{-1} in parallel with the combined foliar application of Mo and Mn (50 + 50 mg L^{-1}). Our findings proposed that the co-fertilization of N-Mo-Mn could enhance spinach yield and its quality, while reducing NO_3^- accumulation in leaves, resulting agronomical, environmental and economic benefits.

1. Introduction

Spinach (*Spinacia oleracea* L.) is one of the popular leafy vegetables crops grown especially in Egypt. It is used fresh, canned, or frozen product. It is low in calories and a good source of water-soluble and lipid soluble vitamins (A, B, and C), and minerals especially iron (Fe) (Toledo et al., 2003). Spinach is a vegetable with a high biological value, extremely rich in antioxidants especially when fresh, steamed, or quickly boiled (Cho et al., 2008). Moreover, spinach leaves are low in fat but rich in fiber and phytochemicals (Alvino and Barbieri, 2016) and has high antioxidant activity (Ismail et al., 2004).

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Fertilization is the key factor controlling plant production. However, balanced fertilization with macro and micro nutrients is necessary to obtain optimal plant growth and high quality products. Spinach is a heavy feeder and needs high rates of nitrogen (N) for healthy growth and development (Felix et al, 2016). An insufficient supply of N to plants can reduce plant N content, which limits photosynthetic production, thus reducing plant growth and its yield quantity and quality (Boussadia et al., 2010; Mikkelsen and Hartz, 2008). Previous studies reported that N fertilization improves plant growth parameters and production of most crops (Najm et al., 2013). Urea represents 71 % N fertilizers in Egypt (Elrys et al., 2019). Farmers that used high rates of N fertilizers in order to elevate crop yield, ruined the equilibrium balance of soil nutrient elements, thus decreasing crop quality (Zeka et al., 2014).

Spinach is very sensitive to N fertilization and it is one of the highest nitrate (NO_3^-) accumulator's (Alessa et al., 2017; Canali et al., 2014; Cantliffe, 1992; Zeka et al., 2014). NO_3^- can be adversely altered to NO_2^-, which can react with amines and amides to produce nitrous compounds (Santamaria, 2006) (Choi et al., 2007). Excess NO_3^- can potentially compete for iodide absorption by the sodium iodide symporter (Tonacchera et al., 2004). It is revealed that acceptance daily intake of NO_3^- is 0–3.7 mg kg^{-1} body weight as recorded by food and agriculture organizations (Santamaria, 2006). The U.S. Environmental Protection Agency had set an acceptable daily intake for NO_3^- of 7.0 mg kg^{-1} body weight (Mensinga et al., 2003). As a result, NO_3^- in vegetables including spinach has gotten a lot of attention lately. Many studies had been undertaken in recent decades to reduce NO_3^- accumulation in plants. However, NO_3^- accumulation is a complicated process, with numerous internal and external variables influencing plant NO_3^- concentration (Márquez-Quiroz et al., 2014). There are different factors influencing NO_3^- uptake and accumulation in vegetable tissues such as environmental factors, genetic actors, and agricultural factors (e.g., N dose and form) (Santamaria et al., 2001). Previous studies revealed that NO_3^- accumulation in leafy vegetables was positively associated with inorganic N fertilizer rates, which are the controlling factor of NO_3^- accumulation in vegetables (Jun-liang et al., 2003; Krezel and Kolota, 2003; Wang and Li, 2003; Zeka et al., 2014). The different N sources and the nitrates accumulation in vegetable tissues have attracted attention in recent years. For example, higher weight of fresh leaves and vitamin C content of spinach was observed when ammonium nitrate fertilizer was used compared to urea and calcium nitrate, but the highest rate of NO_3^- accumulation was recorded when calcium nitrate was added (Zeka et al., 2014). Moreover, NO_3^- accumulation in spinach leaves was reduced by 79–98% when ammonium or urea-based fertilizers was used compared to nitrate-based fertilizer. Accordingly, the rational application of N fertilizers in spinach to achieve the highest yield and the least accumulation of NO_3^- is critical.

Leafy vegetables contain a high level of NO_3^- (Prasad and Chetty, 2008), and the consumption of large amounts of NO_3^- causes serious diseases to humans (Mensinga et al., 2003). Molybdenum (Mo) is an essential micronutrient for plants and animals. It participates in N metabolism in plants, as it involves in synthesis of nitrate reductase, which reduces NO_3^- to NO_2^- and this is the first step to integrate N into proteins (Bambara and Ndakidemi, 2010). Its deficiency affects N content in plants (Bullock et al., 2002). Molybdenum is highly mobile in the plant bark; therefore, its amount available to the plant is as low as 0.1–0.25 mg kg^{-1} (Mengel and Kirkby, 2001). Foliar spraying of Mo is more effective on early stages plants (25 days) grown in acidic soils (Valenciano et al., 2011). It is easily absorbed by the leaves. Foliar application of Mo (40 g ha^{-1}) significantly improved NO_3^- reductase and nitrogenase's activities, which increases the total N accumulated in common bean sprouts (Vieira et al., 1998). Molybdenum is an essential component in many enzymes such as NO_3^- reductase, nitrogenase, and these enzymes are vital for the uptake of NO_3^- in soil. Apart from its role in NO_3^- reductase, its function in higher plants is not well known (Cecílio-Filho et al., 2019). Foliar spraying of Mo can effectively increase the availability of Mo and improve the performance of molybdic enzymes (Kaiser et al., 2005). Elrys et al. (2018) found that using Mo as a foliar spay with N fertilizers reduced NO_3^- accumulation in potato tubers while increasing NO_3^- reductase enzyme level. Moreover, Chen et al. (2009) reported that the activity NO_3^- reductase was significantly enhanced by Mo addition, which caused significant reduction of NO_3^- accumulation in *Brassica campestris* ssp. *chinensis*. They also found that manganese (Mn) reduced NO_3^- accumulation. These important roles of Mn were clearly demonstrated through its significant role in increasing the plant growth attributes, yield, leaf pigments (chlorophyll *a* and *b*, and carotenoids), protein concentration, macronutrients (N, phosphorus (P), and potassium (K)), and micronutrients (Mn, Fe, and zinc (Zn)) of spinach. Manganese deficiency reduced photosynthesis and crop yield quality and quantity as Mg is active part in enzymes involved in carbohydrate metabolism (Diedrick, 2010; Malakouti and Tehrani, 1999). Malakouti and Tehrani (1999) reported that potato yield and storage dry matter improved when Mn was applied. Spraying plants with Mo and Mn combined together at any N fertilizers levels increased chlorophyll and nutrient content in leaves, thus increasing vegetative growth and increasing spinach yield. The combination between micronutrients foliar and N fertilizer enhanced chickpea yield quality and quantity as reported by Rahman et al. (2017).

Here, we provide an approach by combining N fertilization from different sources (e.g., ammonium nitrate and urea) at different rates with the foliar spraying of Mo and/or Mn on growth, chemical constituents, and NO_3^- accumulation in spinach leaves. The current study hypothesizes that use of both Mo and Mn as a foliar application in parallel with N fertilization would reduce NO_3^- accumulation and improve spinach leaves quality. We also hypothesized that the role of Mo in controlling NO_3^- accumulation and plant attributes would be higher than that of Mn, but the combined addition of both would be preferable. The agricultural, environmental, and economic benefits of N-Mo-Mn co-fertilization could contribute to the higher sustainability of spinach cropping system.

2. Materials and methods

2.1. Experimental design

In a 2-year field trial, spinach seeds (*Spinacia oleracea* L.) cv. Balady were sown on 12th November 2019/2020 and 2020/202021 seasons in a clay soil located at Fayoum, Egypt (29° 17′N; 30° 53′E). Before planting, the selected soil was analyzed. The chemical analyzes of soil were shown in Table 1. In two equal applications at 28 and 42 days after planting, the plants were supplied with 180 and 360 kg N ha^{-1} as urea [$CO(NH_2)_2$; 46 % N] or ammonium nitrate (NH_4NO_3; 33.5 % N). In addition, plants were sprayed with:

Table 1
Soil properties of the investigated soil.

Property	2019/2020	2020/2021
pH (1:2.5)	7.51	7.43
ECe (dS m^{-1})	4.40	5.00
Organic matter (g kg^{-1})	11.6	12.3
Total N (g kg^{-1})	4.4	5.9
$CaCO_3$ (g kg^{-1})	56.0	53.4
Fe (mg kg^{-1})	3.50	3.33
Mn (mg kg^{-1})	8.15	7.68
Zn (mg kg^{-1})	0.78	0.71
Cu (mg kg^{-1})	3.00	3.07

tap water (control), Mo (as sodium molybdate), or Mn (as manganese sulphate) at rates of 50 and 100 mg L^{-1} of each or with a mixture of Mo and Mn at rates of 50 and 50 mg L^{-1}, respectively. However, few drops of Tween-20 were added as a surfactant. Recommended cultural practices for spinach production were followed. The different treatments were applied in split-split plots in randomized complete block design (RCBD) with three replications. N-sources consisted the main plots, N-rates were assigned randomly to the sub-plots. While, micronutrients foliar sprays comprised the sub-sub-plots. Plots (10.5 m^2; 3 × 3.5 m of each) were seeded in excess and the plants were thinned to the desired stand after emergence.

2.2. Morphological and quality parameters

2.2.1. Growth parameters

At harvest time (56 days from sowing), ten plants were randomly chosen from each replication and were subjected to determine the following parameters; plant length (cm), plant fresh and dry weights (g $plant^{-1}$, without roots), and number of leaves $plant^{-1}$. Moreover, leaf area (cm^2), leaf area $plant^{-1}$ (dm^2), net assimilation rate (mg dm^2 day^{-1}); is an index of the productive efficiency of plants, calculated in relation to total leaf area (Hunt, 1982; Desoky et al., 2020), Some measurements were begun 35 days after planting and stopped when the plants began to bloom (56 days old; harvest time); leaf area ratio (dm^2 g^{-1}). The ratio between total leaf area $plant^{-1}$ and total dry weight $plant^{-1}$ determined the morphological index (Hunt, 1982; El-Saadony et al., 2021a), while relative growth rate (g g^{-1} $week^{-1}$) measures the increment rate in size. The previous indexes were calculated every week according to El-Saadony et al., (2021b)

2.2.2. Chemical measurements

At harvest time (56 days from sowing), fresh leaves were extracted in acetone 80%, the pigments in leaf extract; chlorophyll and carotenoids (mg g^{-1} fresh weight of leaf) were determined using colorimetric method as described by (El-Saadony et al., 2021a). In dried leaves (dried at 70 °C till constant weight then well ground for chemical analysis), total soluble carbohydrates were determined using phenol–sulphuric acid method as per (Saad et al., 2021a). Nitrogen was determined by using kejldahl method multiply in factor of 6.25% to calculate % protein (Saad et al., 2020). The mineral contents of P, K, Fe, Mn and Zn were determined as follows; 100 mg of powdered leaves were digested in sulphuric and perchloric acids as described by Piper (1947), then P was estimated by using chloro-stannous molybdophosphoric blue colour method in sulphuric acid system as described by JACKSON et al. (1978). Potassium was estimated by flame photometer (Perkin-Elmer,USA) (Page and Keeney, 1982). while Fe, Mn and Zn were estimated by atomic absorption spectrophotometer (Perkin-Elmer, Model 3300, USA) according to Chapman (1961). The NO_3^- content was determined as follow, 500 mg of leaves powder was homomgnized in 20 ml distilled water for 30 min., then filtered (Bar-Akiva, 1975). The nitrate was determined in the extract using phenol disulfonic acid method (Bremner, 1965). The average of both seasons was tabulated, statistically analyzed and discussed.

2.3. Statistical analysis

Treatment effects were determined by analysis of variance and error variances homogeneity using COSTAT software. Combined data analysis with the least significant difference (LSD) was carried out at a probability level of 99% ($p \leq 0.01$) for each treatment by Duncan's multiple range test (Steel, 1997).

3. Results

3.1. Effect of N-Mo-Mn co-fertilization on plant attributes of spinach at harvest time

Plant length, fresh weight, dry weight, leaves number $plant^{-1}$, leaf area, and leaf area $plant^{-1}$ were significantly ($p \leq 0.01$; except for leaf area) influenced by N and micronutrients (Mo, and/or Mn) management treatments (Table 2). These characteristics were significantly greater in urea treatment than in ammonium nitrate treatment, where these characteristics increased in case of N- urea treatment compared with ammonium nitrate-N treatment. All these characteristics were significantly increased when plants were treated with 360 kg N ha^{-1} compared to the treatment of 180 kg N ha^{-1}. The influence of Mo application was higher than that of Mn, whereas the highest values of these characteristics were observed when the mixture of both Mo and Mn was applied, where this treatment increased all previous characteristics compared to the control treatment (without micronutrients).

Significant (all $p < 0.05$) interaction influences were noted under the effect of N source × N level × micronutrients for all previous attributes. The highest values of plant length, fresh weight, dry weight, leaves number $plant^{-1}$, leaf area, and leaf area $plant^{-1}$ were recorded under the treatment of 360 kg N-urea ha^{-1} in parallel with the combined foliar application of Mo and Mn.

3.2. Effect of N-Mo-Mn co-fertilization on leaf area ratio and net assimilation rate of spinach leaves at various sampling times

Differences in leaf area ratio, net assimilation rate, and relative growth rate among all treatments were significant at various sampling times shown in Table 3. Across all treatments, leaf area ratio decreased with sampling time and the increase in N-level. Ammonium nitrate-N treatment applied caused a remarkable reduction in leaf area ratio compared to urea-N treatment. Differences in leaf area ratio among micronutrients and N application were significant for all N-level and N-source at all sampling times. The highest leaf area ratio was noted under the treatment of 180 kg N- ammonium nitrate ha^{-1} with 100 mg L^{-1} Mo as a foliar application at the time of 35–42 days, whereas the lowest leaf area ratio was recorded when plants were treated with 360 kg N- ammonium nitrate ha^{-1} in parallel with the combined foliar application of Mo and Mn. Moreover, urea-N gave the highest values of net assimilation rate. Net assimilation rate increased as N-level increased during the period of growth. Mo and/or Mn with N-application significantly increased net assimilation rate compared to N-application alone. Across all sampling times, application of micronutrients in parallel with N-application significantly increased net assimilation rate, and the highest values were observed when plants were treated with 360 N-urea ha^{-1} and the combined foliar application of Mo and Mn. Additionally, relative growth rate of all treatments increased with time. However, increasing the level of N, significantly decreased the relative growth rate. The treated plants with ammonium nitrate N appeared a remarkable decrease in relative growth rate as compared to urea-N treated ones. Similarly, relative growth rate was affected by the interaction of micronutrients and N application, and the highest values were recorded when plants were treated with 360 kg N- urea ha^{-1} in parallel with the combined foliar application of Mo and Mn.

3.3. The effect of N-Mo-Mn co-fertilization on leaves pigments, protein, total soluble carbohydrate, and NO_3^- accumulation of spinach leaves

Chlorophyll *a*, chlorophyll *b*, total chlorophyll, carotenoids, NO_3^- accumulation in spinach leaves were significantly ($p \leq 0.01$) influenced by N and micronutrients (Mo, and/or Mn) management

Table 2
The interactive effect of foliar application of micronutrients (Mo and/or Mn) with soil-applied N fertilizers on plant attributes at harvest time of spinach. (The means of both seasons).

N-source	N-level (kg N ha^{-1})	Micronutrient (mg L^{-1})	Plant length (cm)	Plant weight (g $plant^{-1}$) **Fresh**	**Dry**	Leaves no. $plant^{-1}$	Leaf area (cm^2)	Leaf area $plant^{-1}$ (cm^2)
Urea	180	Control	29.7 ± 1.4i	25.2 ± 2.3hi	2.30 ± 0.3kl	9.00 ± 1.0i	6.44 ± 0.3a	58.0 ± 3.7n
		Mo_{50}	32.2 ± 1.8gh	27.5 ± 1.4gh	3.00 ± 0.1hij	10.0 ± 0.8g	6.00 ± 0.2a	60.0 ± 7.1lm
		Mo_{100}	39.2 ± 2.7d	30.3 ± 0.8fgh	3.65 ± 0.1fgh	12.0 ± 2.1d	5.16 ± 1.2a	61.9 ± 5.3jk
		Mn_{50}	31.8 ± 2.2h	25.8 ± 0.4h	3.05 ± 0.2hij	10.0 ± 1.4g	5.90 ± 0.7a	59.0 ± 4.2mn
		Mn_{100}	34.8 ± 1.4ef	34.2 ± 1.1efg	3.50 ± 0.1ghi	11.0 ± 0.7e	5.45 ± 0.3a	59.9 ± 1.8lm
		Mo + Mn	44.1 ± 3.1c	81.9 ± 6.2ab	6.95 ± 0.6c	12.5 ± 0.7c	7.89 ± 1.4a	98.4 ± 8.3c
	360	Control	33.7 ± 2.5fgh	27.5 ± 2.3gh	2.55 ± 0.3jkl	10.0 ± 0.4g	6.10 ± 0.7a	61.0 ± 7.2kl
		Mo_{50}	38.5 ± 4.1d	43.6 ± 3.6cde	4.00 ± 0.2efg	12.0 ± 1.3d	5.83 ± 0.6a	69.9 ± 4.9h
		Mo_{100}	44.7 ± 2.3c	45.7 ± 3.1c	5.35 ± 0.2d	13.0 ± 0.8b	6.15 ± 0.9a	80.0 ± 6.2e
		Mn_{50}	44.1 ± 1.6c	36.7 ± 1.6def	3.90 ± 0.1fg	10.0 ± 1.0g	7.25 ± 1.0a	72.5 ± 2.8g
		Mn_{100}	36.2 ± 0.9e	42.1 ± 1.4cde	4.80 ± 0.7de	11.0 ± 1.1e	7.34 ± 0.8a	80.7 ± 7.4e
		Mo + Mn	47.7 ± 3.4a	87.1 ± 4.6a	9.10 ± 0.6a	13.5 ± 0.8a	8.94 ± 0.6a	120 ± 14.2a
Ammonium nitrate	180	Control	27.2 ± 1.2j	17.8 ± 0.6i	2.05 ± 0.2l	8.50 ± 01.2j	5.38 ± 0.5a	45.5 ± 2.8q
		Mo50	32.0 ± 2.3gh	25.0 ± 1.2hi	2.80 ± 0.4jk	10.0 ± 0.7g	5.80 ± 0.4a	58.0 ± 6.9n
		Mo100	39.3 ± 1.1d	33.5 ± 1.4fg	3.00 ± 0.2hij	11.0 ± 0.6e	5.45 ± 0.2a	60.2 ± 5.4l
		Mn50	30.5 ± 1.9hi	25.1 ± 0.8i	2.45 ± 0.6jk	9.00 ± 0.4i	4.96 ± 0.9a	44.6 ± 6.1q
		Mn100	33.8 ± 0.8fgh	31.5 ± 2.7fgh	2.85 ± 0.4ijk	9.50 ± 0.9h	5.56 ± 0.8a	53.0 ± 2.5o
		Mo + Mn	44.1 ± 3.5c	76.5 ± 6.1b	6.55 ± 1.1c	12.0 ± 0.7d	7.25 ± 0.9a	87.0 ± 10d
	360	Control	30.0 ± 2.4hi	27.5 ± 2.4gh	2.75 ± 0.2jk	9.50 ± 0.5h	5.38 ± 1.1a	51.0 ± 2.1p
		Mo50	34.3 ± 1.8efg	36.5 ± 1.9def	3.55 ± 0.1ghi	11.0 ± 1.4e	6.30 ± 0.2a	66.0 ± 4.7i
		Mo100	45.0 ± 5.2bc	44.0 ± 3.0cd	4.90 ± 0.9de	12.0 ± 1.1d	6.50 ± 0.6a	78.0 ± 8.2f
		Mn50	33.6 ± 3.2fgh	35.0 ± 2.2efg	2.90 ± 0.3ijk	10.0 ± 0.6g	6.25 ± 0.5a	62.5 ± 4.1j
		Mn100	35.9 ± 1.6ef	37.2 ± 1.6def	4.30 ± 0.2ef	10.5 ± 0.4f	6.83 ± 0.5a	71.5 ± 1.2gh
		Mo + Mn	46.1 ± 2.7a	82.8 ± 3.4ab	7.70 ± 0.5b	13.0 ± 1.8b	8.88 ± 0.9a	115 ± 9.7b

Data are means (n = 3) ± SD. Different letters within the each column denote significant differences between the treatments according to Fisher's least-significant difference test ($p \leq 0.01$).

treatments (Table 4 and Fig. 1A, 1B and 1C). Averagely, NO_3^- accumulation was significantly greater in ammonium nitrate-N treatment than in N- urea treatment, where N- urea treatment decreased NO_3^- accumulation in spinach leaves by 25.2% compared to ammonium nitrate-N treatment. Moreover, NO_3^- accumulation was significantly increased by 62.9 % when plants were treated with 360 kg N ha^{-1} compared to the treatment of 180 kg N ha^{-1}. Both Mo and Mn application significantly decreased NO_3^- accumulation, where NO_3^- accumulation decreased by 44.7, 55.1, 54.1, 63.9, and 73.7% under the treatments of Mo_{50}, Mo_{100}, Mn_{50}, Mn_{100}, and Mo_{50} + Mn_{50}, respectively compared to the control treatment (without micronutrients). The highest NO_3^- accumulation (6615 mg kg^{-1}) was noted under the treatment of 360 kg N-ammonium nitrate ha^{-1} without micronutrients, whereas the lowest NO_3^- accumulation (974 mg kg^{-1}) was recorded when plants were treated with 180 kg N-urea ha^{-1} in parallel with the combined application of Mo and Mn.

3.4. Effect of N-Mo-Mn co-fertilization on nutrients contents of spinach leaves at harvest

Spinach leaves-macronutrients (N, P, and K), leaves-micronutrients (Fe, Mn, and Zn) were significantly ($p \leq 0.01$) influenced by N and micronutrients (Mo, and/or Mn) management treatments (Table 5). Averagely, these characteristics except P content were significantly greater in N- urea treatment than in ammonium nitrate-N treatment, where these characteristics increased in case of N-urea treatment compared with ammonium nitrate-N treatment, but P content decreased by 35.3%. All these characteristics were significantly increased when plants were treated with 360 kg N ha^{-1} compared to the treatment of 180 kg N ha^{-1}. The influence of Mo application was higher than that of Mn, whereas the highest values of these characteristics were observed when the mixture of both Mo and Mn was applied, where this treatment increased all previous characteristics compared to the control treatment (without micronutrients). Significant (all $p < 0.05$) interaction influences were noted under the effect of N source × N level × micronutrients for all previous attributes. The highest values of these former characteristics were recorded under the treatment of 360 kg N-urea ha^{-1} in parallel with the combined foliar application of Mo and Mn.

4. Discussion

4.1. The vital role of N-Mo-Mn co-fertilization in mitigating NO_3^- accumulation

Vegetables are the main source of NO_3^- intake by humans and constitute about 40%–92% of the mean daily intake (Ximenes et al., 2000). A person who consumes 400 g of leafy vegetables will receive 1.6–1.7 mg of nitrate/60 kg as recommended by the World Health Organization. Ingestion of Mo alone would be four times the EPA's reference dose for $NO3^-$. The accumulation of NO_3^- in plants is a natural phenomenon that occurs when the uptake of NO_3^- exceeds its reduction and subsequent metabolism within the plant (Blom-Zandstra, 1989; Hanafy, 1997; Hanafy Ahmed et al., 2002). Our findings show that spinach grown in soils treated with N fertilization from different sources (e.g., ammonium nitrate, and urea) at different rates (180, and 360 kg N ha^{-1}) had the highest NO_3^- accumulation in spinach leaves compared with plants that grew in soil treated with N fertilizers in parallel with Mo and/or Mn as a foliar spray at the rates of 50 and 100 mg L^{-1}. Likewise, Ahmadi et al. (2010) in spinach leaves noted that NO_3^- accumulation increased when inorganic N fertilizer was applied. In parallel, NO_3^- will accumulate as a result of the rapid transformation of inorganic N fertilizers in soils (Wang et al., 2008). In Peking cabbage and spinach, Wang and Li (2004) found that amending with urea significantly increased NO_3^- accumulation. The excessive N fertilizer use increases NO_3^- accumulation in vegetable leaves and thus reduce vegetable quality (Liu et al., 2014; Qiu et al., 2014). The excess are absorbed quickly into the plant leading to higher and accumulated NO_3^- levels and stored predominantly in the green leafy part of the plant (Anjana and Iqbal, 2007). Our findings conformed these studies where NO_3^- accumulation increased significantly to increasing N rates. We also found that NO_3^-

Table 3
The interactive effect of foliar application of micronutrients (Mo and/or Mn) with soil-applied N fertilizers on leaf area ratio, net assimilation rate, and relative growth rate of spinach leaves at various sampling times. (The means of both seasons).

N-source	N-level (kg N ha^{-1})	Micronutrient (mg L^{-1})	Sampling interval (days)								
			35–42	42–49	49–56	35–42	42–49	49–56	35–42	42–49	49–56
			Leaf area ratio (dm^2g^{-1})			Net assimilation rate (mg dm^{-2} day^{-1})			Relative growth rate (g g^{-1} $week^{-1}$)		
Urea	180	Control	2.78 ± 0.2b	2.38 ± 0.3b	2.24 ± 0.1b	1.16 ± 0.1l	2.23 ± 0.1k	4.64 ± 0.3k	0.38 ± 0.1c	0.68 ± 0.1b	0.99 ± 0.1b
		Mo_{50}	2.68 ± 0.1c	1.97 ± 0.1e	1.67 ± 0.1c	1.44 ± 0.2jkl	2.88 ± 0.4ij	5.67 ± 0.6gh	0.35 ± 0.1c	0.60 ± 0.1cd	0.88 ± 0.1c
		Mo_{100}	4.32 ± 0.5a	3.54 ± 0.2a	2.58 ± 0.4a	3.55 ± 0.4c	7.03 ± 0.6a	13.3 ± 2.1a	0.50 ± 0.1b	0.91 ± 0.2a	1.60 ± 0.3a
		Mn_{50}	2.57 ± 0.2d	1.23 ± 0.1j	1.17 ± 0.2d	1.47 ± 0.2jk	2.70 ± 0.2j	5.15 ± 0.8ij	0.33 ± 0.0cef	0.58 ± 0.1de	0.86 ± 0.1c
		Mn_{100}	1.66 ± 0.1l	0.88 ± 0.1m	0.78 ± 0.2f	1.62 ± 0.2ij	3.12 ± 0.1gh	6.37 ± 1.0f	0.23 ± 0.0gi	0.37 ± 0.0ghi	0.73 ± 0.1ef
		Mo + Mn	1.40 ± 0.1n	0.71 ± 0.2p	0.51 ± 0.1i	2.11 ± 0.3h	3.89 ± 0.6e	7.31 ± 1.1d	0.59 ± 0.1a	0.71 ± 0.1b	0.99 ± 0.2b
	360	Control	2.66 ± 0.1c	2.15 ± 0.4c	1.67 ± 0.1c	2.07 ± 0.1h	2.53 ± 0.4jk	5.71 ± 0.9gh	0.34 ± 0.1ce	0.54 ± 0.1e	0.84 ± 0.2cd
		Mo_{50}	2.05 ± 0.1h	1.84 ± 0.2f	0.87 ± 0.1e	2.83 ± 0.5ef	3.84 ± 0.3e	6.49 ± 0.5f	0.32 ± 0.1cef	0.47 ± 0.0f	0.72 ± 0.1f
		Mo_{100}	1.92 ± 0.1j	1.67 ± 0.1h	0.70 ± 0.2g	3.43 ± 0.6c	5.13 ± 0.7cd	7.69 ± 0.8bc	0.15 ± 0.0kmo	0.38 ± 0.1gh	0.39 ± 0.0j
		Mn_{50}	1.67 ± 0.1l	1.04 ± 0.1k	0.58 ± 0.0h	2.39 ± 0.3g	3.06 ± 0.2hi	5.83 ± 0.8gh	0.17 ± 0.0i-n	0.42 ± 0.1fg	0.65 ± 0.1g
		Mn_{100}	1.51 ± 0.0m	0.70 ± 0.0q	0.39 ± 0.01	3.12 ± 0.4de	5.01 ± 0.7d	7.57 ± 1.2cd	0.11 ± 0.0no	0.25 ± 0.0klm	0.36 ± 0.0j
		Mo + Mn	0.60 ± 0.1	0.50 ± 0.1s	0.13 ± 0.0q	4.03 ± 0.4a	6.67 ± 0.6b	8.95 ± 1.0b	0.49 ± 0.1b	0.59 ± 0.1de	0.90 ± 0.2c
Ammonium nitrate	180	Control	2.60 ± 0.1e	2.10 ± 0.2d	0.59 ± 0.2h	0.43 ± 0.1n	0.91 ± 0.2m	2.53 ± 0.3n	0.36 ± 0.1c	0.58 ± 0.1de	0.69 ± 0.1fg
		Mo_{50}	2.37 ± 0.2f	1.78 ± 0.3g	0.43 ± 0.1k	0.66 ± 0.2mn	1.82 ± 0.4l	3.62 ± 0.5	0.30 ± 0.1def	0.43 ± 0.1fg	0.55 ± 0.1hi
		Mo_{100}	2.07 ± 0.1h	1.66 ± 0.3h	0.19 ± 0.0o	1.30 ± 0.1kl	2.49 ± 0.5jk	4.92 ± 0.5jk	0.22 ± 0.0lghij	0.39 ± 0.1gh	0.49 ± 0.1i
		Mn_{50}	1.99 ± 0.2i	0.96 ± 0.1l	0.36 ± 0.1l	0.58 ± 0.0n	1.25 ± 0.2k	3.01 ± 0.2m	0.28 ± 0.0efg	0.35 ± 0.0hij	0.51 ± 0.1hi
		Mn_{100}	1.06 ± 0.2o	0.76 ± 0.1o	0.16 ± 0.0p	0.90 ± 0.1m	2.31 ± 0.3k	4.03 ± 0.6l	0.21 ± 0.0lhijk	0.21 ± 0.0m	0.41 ± 0.1j
		Mo + Mn	0.66 ± 0.1p	0.55 ± 0.1r	0.13 ± 0.0q	1.63 ± 0.2ij	3.18 ± 0.5fgh	5.64 ± 0.8gh	0.52 ± 0.1b	0.66 ± 0.1bc	0.88 ± 0.2c
	360	Control	2.23 ± 0.3g	1.98 ± 0.3e	0.49 ± 0.1j	1.26 ± 0.2kl	1.80 ± 0.4l	4.26 ± 0.1l	0.27 ± 0.0fgh	0.40 ± 0.0gh	0.57 ± 0.1h
		Mo_{50}	1.90 ± 0.2j	1.67 ± 0.2h	0.32 ± 0.0m	1.55 ± 0.3ijk	2.27 ± 0.6k	5.39 ± 0.7hi	0.18 ± 0.0ijklm	0.37 ± 0.0ghi	0.52 ± 0.1hi
		Mo_{100}	1.77 ± 0.1k	1.53 ± 0.2i	0.16 ± 0.0p	2.61 ± 0.4fg	3.40 ± 0.8fg	6.85 ± 0.4e	0.12 ± 0.0mno	0.29 ± 0.0jkl	0.39 ± 0. 1 j
		Mn_{50}	1.05 ± 0.2o	0.82 ± 0.1n	0.27 ± 0.0n	1.80 ± 0.2i	1.99 ± 0.3l	4.78 ± 0.4k	0.14 ± 0.0lmno	0.31 ± 0.0ijk	0.35 ± 0.0j
		Mn_{100}	0.66 ± 0.1p	0.45 ± 0.0t	0.13 ± 0.0q	2.60 ± 0.2fg	3.48 ± 0.8f	5.92 ± 0.9g	0.10 ± 0.0o	0.24 ± -0.0lm	0.21 ± 0.0k
		Mo + Mn	0.56 ± 0.0q	0.22 ± 0.0u	0.10 ± 0.0r	3.71 ± 0.4b	5.40 ± 0.9c	7.40 ± 0.7cd	0.30 ± 0.0def	0.58 ± 0.1de	0.79 ± 0.1de

Data are means (n = 3) ± SD. Different letters within the each column denote significant differences between the treatments according to Fisher's least-significant difference test ($p \leq 0.01$).

Table 4
Interactive effect of foliar application of micronutrients (Mo and/or Mn) with soil-applied N fertilizers on leaf pigments, protein, total soluble carbohydrate, and nitrate accumulation of spinach leaves at harvest time. (The means of both seasons).

N-source	N-level (kg N ha^{-1})	Micronutrient (mg L^{-1})	Leaf pigments (mg g-1 fresh weight of leaf)				Protein (%)	Total carbohydrates (%)	Nitrate accumulation (mg kg^{-1})
			Chl. a	Chl. b	Chl. a + b	Carotenoids			
Urea	180	Control	0.82 ± 0.06r	0.46 ± 0.04r	1.29 ± 0.1q	0.24 ± 0.01l	2.60t	3.35q	2339g
		Mo_{50}	1.00 ± 0.02m	0.58 ± 0.02n	1.59 ± 0.08n	0.31 ± 0.04i	2.90r	3.62o	1393o
		Mo_{100}	1.13 ± 0.01j	0.72 ± 0.10i	1.87 ± 0.21i	0.31 ± 0.02i	3.20o	4.05l	1301q
		Mn_{50}	1.18 ± 0.2i	0.74 ± 0.1b	1.94 ± 0.17g	0.38 ± 0.00de	3.40n	4.26k	1204s
		Mn_{100}	1.20 ± 0.1g	0.81 ± 0.07e	2.09 ± 0.09d	0.42 ± 0.07c	4.50g	5.93g	1120t
		Mo + Mn	1.28 ± 0.09d	0.93 ± 0.07b	2.77 ± 0.13a	0.49 ± 0.05b	6.60b	7.71b	974w
	360	Control	1.09 ± 0.07k	0.54 ± 0.00o	1.65 ± 0.06l	0.28 ± 0.00j	3.70l	3.85n	4240b
		Mo_{50}	1.19 ± 0.3h	0.67 ± 0.04k	1.87 ± 0.08i	0.36 ± 0.01fg	3.80k	3.96m	2630f
		Mo_{100}	1.21 ± 0.2f	0.78 ± 0.09g	2.00 ± 0.21f	0.35 ± 0.03g	4.30h	4.47i	1914i
		Mn_{50}	1.28 ± 0.3d	0.80 ± 0.06f	2.08 ± 0.17de	0.42 ± 0.06c	4.30h	4.54i	2098h
		Mn_{100}	1.30 ± 0.08c	0.88 ± 0.12c	2.18 ± 0.19c	0.48 ± 0.06b	5.30e	6.55d	1614m
		Mo + Mn	1.40 ± 0.07a	0.99 ± 0.08a	2.46 ± 0.17b	0.53 ± 0.02a	7.20a	8.02a	1042v
		Control	0.81 ± 0.01s	0.37 ± 0.01s	1.18 ± 0.03r	0.21 ± 0.01m	2.10u	2.96r	3377c
Ammonium nitrate	180	Mo_{50}	0.92 ± 0.00q	0.52 ± 0.01p	1.45 ± 0.04p	0.25 ± 0.02kl	2.60t	3.51p	1828k
		Mo_{100}	1.00 ± 0.02m	0.58 ± 0.03n	1.60 ± 0.012m	0.28 ± 0.06j	3.00q	3.94m	1720l
		Mn_{50}	0.94 ± 0.02p	0.70 ± 0.05j	1.65 ± 0.09l	0.33 ± 0.05h	2.80s	3.96m	1496n
		Mn_{100}	1.13 ± 0.01j	0.75 ± 0.02h	1.90 ± 0.08g	0.37 ± 0.03ef	4.20i	5.71h	1383p
		Mo + Mn	1.21 ± 0.11f	0.85 ± 0.08d	2.07 ± 0.15e	0.41 ± 0.04c	5.40d	6.03f	1238r
		Control	1.01 ± 0.05l	0.51 ± 0.00q	1.54 ± 0.07o	0.25 ± 0.03kl	3.10p	3.29q	6615a
	360	Mo_{50}	0.95 ± 0.04o	0.62 ± 0.06m	1.58 ± 0.11n	0.26 ± 0.00k	3.60m	3.89m	3318d
		Mo_{100}	1.13 ± 0.17j	0.66 ± 0.05l	1.81 ± 0.15j	0.32 ± 0.02hi	3.90j	4.39j	2502g
		Mn_{50}	0.99 ± 0.06n	0.72 ± 0.09i	1.72 ± 0.16k	0.36 ± 0.03fg	3.90j	4.29k	2802e
		Mn_{100}	1.24 ± 0.03e	0.81 ± 0.07e	2.07 ± 0.08e	0.39 ± 0.02d	4.60f	6.13e	1866j
		Mo + Mn	1.34 ± 0.08b	0.91 ± 0.10a	2.27 ± 0.16a	0.43 ± 0.05a	6.10c	6.80c	1104u

Data are means (n = 3) ± SD. Different letters within the each column denote significant differences between the treatments according to Fisher's least-significant difference test ($p \leq 0.01$).

accumulation was significantly greater in N-NH_4NO_3 treatment than in N- urea treatment.

In this study, the combination of N fertilizers and foliar spraying of Mo, and/or Mn provided a better spinach cultivation method. One of the agricultural practices to reduce the accumulation of nitrates in vegetable tissues is using Mo. It plays a valuable role in N metabolism in the plant through fixing N, transferring the N compounds in plants, and reducing NO_3^-. Therefore, the addition of Mo is expected to improve plants' N uptake and thus reduce nitrates in plant tissues and thus increase the quality and quantity of spinach yield. There was a decrease in the NO_3^- content in spinach leaves with Mo spray, as it absorbs nitrates taken by plants.

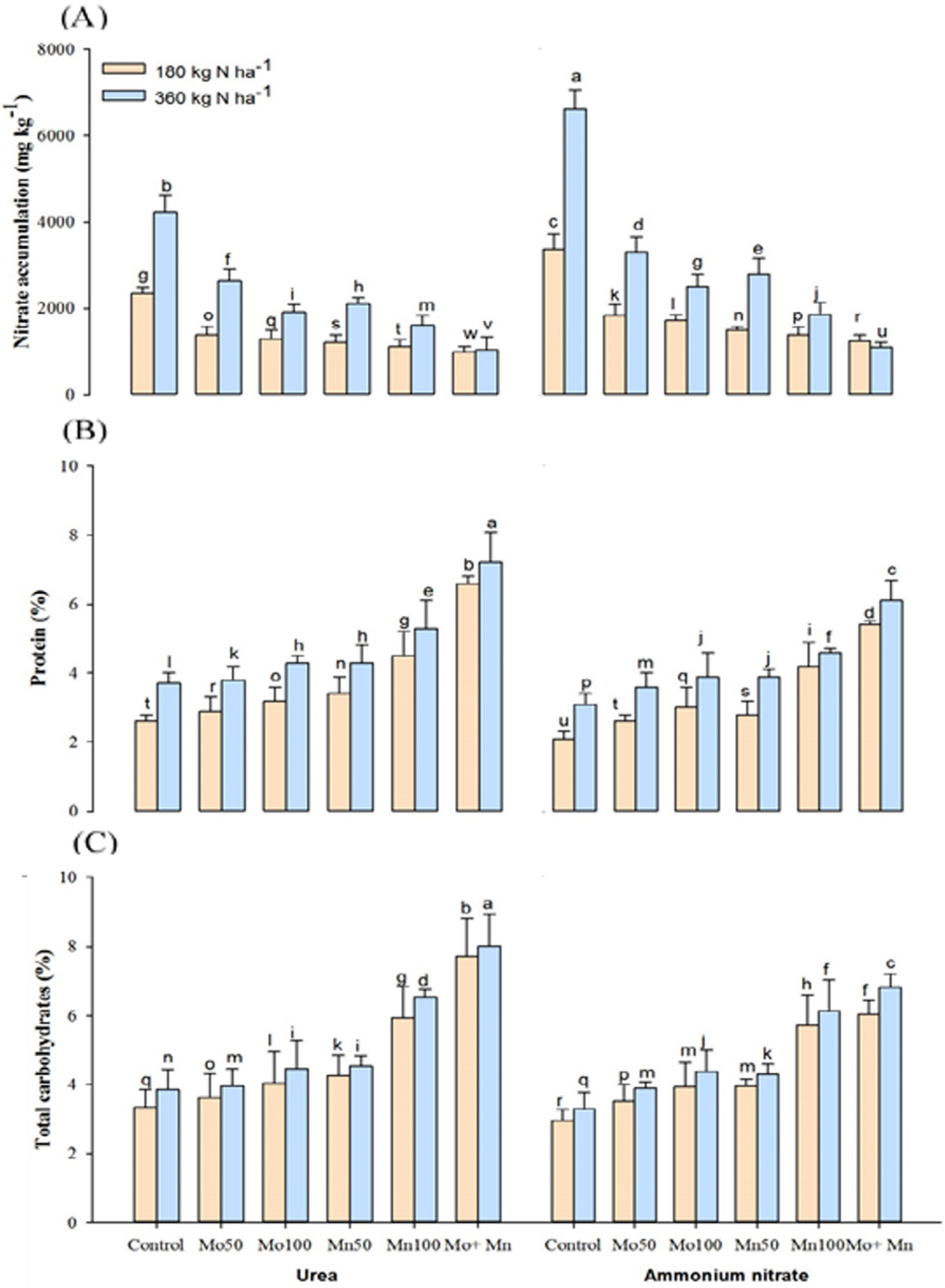

Fig. 1. The interactive effect of foliar application of micronutrients (Mo and/or Mn) with soil-applied N fertilizers on nitrate accumulation (A), protein (B), and total soluble carbohydrate (C) of spinach leaves at harvest. Data are means (n = 3) ± SD. Different letters above the columns denote significant differences between the treatments according to Fisher's least-significant difference test ($p \leq 0.01$).

In most cases, spraying of Mo on plant leaves results in easy recovery of NO_3^- reductase activity (Yaneva et al., 2000). The Mo is a main component of NO_3^- reductase enzyme, which plays a key role in NO_3^- reduction in plant (Bélanger et al., 2000). The overall NO_3^- reductase activity was higher in the Mo-treated plant (Elrys et al., 2018; Min et al., 2010). In lower concentrations of Mo, NO_3^- was accumulated in plant tissues (Zabihi-e-Mahmoodabad et al., 2010). The absence of Mo supply stimulated NO_3^- accumulation in common bean leaves, indicating the low efficiency of N assimilation by plants in the absence of Mo (Calonego et al., 2010). Due to the obvious role of Mn in NO_3^- reductase, the deficiency of Mn les to more NO_3^- accumulations in leaves (Tavakoli et al., 2014). Our study confirmed this view as NO_3^- accumulation significantly decreased when Mn was applied.

Table 5
The interactive effect of foliar application of micronutrients (Mo and/or Mn) with soil-applied N fertilizers on nutrients content (N, P, K, Fe, Mn, and Zn) of spinach leaves at harvest time. (The means of both seasons).

N-source	N-level (kg N ha^{-1})	Micronutrient (mg L^{-1})	Leaf-macronutrients (%)			Leaf-micronutrients (mg kg^{-1})		
			N	P	K	Fe	Mn	Zn
Urea	180	Control	0.42 ± 0.08p	0.27 ± 0.02r	0.51 ± 0.05j	2.61 ± 0.3q	72.0 ± 3.6p	48.1 ± 2.6t
		Mo_{50}	0.47 ± 0.10n	0.31 ± 0.02o	0.57 ± 0.10h	2.95 ± 0.4k	76.1d ± 7.8m	52.1 ± 3.2p
		Mo_{100}	0.51 ± 0.05m	0.38 ± 0.01k	0.61 ± 0.08f	2.11 ± 0.3t	78.4c ± 8.2l	55.3 ± 1.9m
		Mn_{50}	0.54 ± 0.04l	0.37 ± 0.06l	0.61 ± 0.03f	3.32 ± 0.5i	81.1b ± 6.8i	58.1 ± 3.7j
		Mn_{100}	0.72 ± 0.02f	0.44 ± 0.05g	0.68 ± 0.07d	3.82 ± 0.7f	85.2 ± 10.1g	61.0 ± 4.6f
		Mo + Mn	1.10 ± 0.06b	0.52 ± 0.08c	0.73 ± 0.08b	4.51 ± 0.3c	93.0 ± 9.4b	73.2 ± 2.6b
	360	Control	0.59 ± 0.05k	0.38 ± 0.02k	0.57 ± 0.04h	2.83 ± 0.8n	75.1 ± 2.8n	51.1 ± 4.4q
		Mo_{50}	0.61 ± 0.03j	0.39 ± 0.03j	0.61 ± 0.11f	3.17 ± 0.2j	79.2 ± 4.5j	56.2 ± 3.8l
		Mo_{100}	0.68 ± 0.06gh	0.43 ± 0.05g	0.65 ± 0.05e	3.45 ± 0.4h	83.1 ± 7.2h	59.0 ± 2.8i
		Mn_{50}	0.69 ± 0.06g	0.44 ± 0.09f	0.65 ± 0.06e	3.77 ± 0.5g	87.1b ± 6.9e	63.2 ± 5.8e
		Mn_{100}	0.85 ± 0.05d	0.51 ± 0.07d	0.71 ± 0.09c	4.15 ± 0.7d	90.4 ± 9.6c	68.3 ± 8.3c
		Mo + Mn	1.15 ± 0.2a	0.64 ± 0.03b	0.85 ± 0.08a	5.12 ± 0.9a	98.3 ± 11.3a	81.0 ± 7.7a
Ammonium nitrate	180	Control	0.34 ± 0.04q	0.23 ± 0.00s	0.27 ± 0.01s	1.80 ± 0.2v	63.2 ± 5.4t	43.1 ± 4.5v
		Mo_{50}	0.42 ± 0.03p	0.28 ± 0.02q	0.31 ± 0.04r	2.01 ± 0.3u	68.7 ± 4.6s	45.8 ± 5.4u
		Mo_{100}	0.48 ± 0.04n	0.33 ± 0.01n	0.36 ± 0.05o	2.37 ± 0.5s	71.2 ± 8.2q	48.8 ± 6.7st
		Mn_{50}	0.45 ± 0.04o	0.29 ± 0.05p	0.34 ± 0.04q	2.65 ± 0.5p	72.1 ± 2.5p	49.5 ± 8.0s
		Mn_{100}	0.67 ± 0.07h	0.33 ± 0.02n	0.43 ± 0.06m	3.15 ± 0.4j	79.0 ± 5.1k	57.2 ± 2.8k
		Mo + Mn	0.86 ± 0.06d	0.38 ± 0.04k	0.54 ± 0.05i	4.12 ± 0.6e	86.1 ± 4.9f	61.9 ± 3.3g
	360	Control	0.53 ± 0.07l	0.31 ± 0.03o	0.35 ± 0.06p	2.11 ± 0.3t	69.1 ± 6.4r	47.1 ± 3.6
		Mo_{50}	0.53 ± 0.04l	0.36 ± 0.04m	0.42 ± 0.07n	2.43 ± 0.4r	73.1d ± 6.8o	50.2 ± 5.1r
		Mo_{100}	0.62 ± 0.09ij	0.93 ± 0.06a	0.45 ± 0.03l	2.85 ± 0.7m	78.1 ± 3.7k	53.2 ± 1.8o
		Mn_{50}	0.63 ± 0.04i	0.42 ± 0.01i	0.47 ± 0.08k	2.90 ± 0.2l	76.0c ± 5.4m	54.1 ± 6.2n
		Mn_{100}	0.74 ± 0.11e	0.47 ± 0.07e	0.58 ± 0.05g	3.83 ± 0.8f	81.2 ± 8.3i	60.1 ± 5.5h
		Mo + Mn	0.98 ± 0.06c	0.51 ± 0.03d	0.71 ± 0.03c	4.80 ± 0.6b	90.0 ± 5.7d	67.1 ± 4.6d

Data are means (n = 3) ± SD. Different letters within the each column denote significant differences between the treatments according to Fisher's least-significant difference test ($p \leq 0.01$).

4.2. The yield and chemical constituents of spinach increased due to the co-fertilization of N-Mo-Mn

The improvement in the vegetative properties of spinach is attributed to the role of N in chlorophyll synthesis. It stimulates the absorption of carbon dioxide and photosynthesis (Leghari et al., 2016). Aisha et al. (2013) found that the increase of N fertilization led to the heaviest total leaves yield and its nutritional values, i.e., N, P, K, and protein content. Wilkinson et al. (2000) reported that P uptake increase with increasing N concentration as a result of increased root growth, and thus an increase in the ability of the roots to absorb and transport P, and also reduce soil pH as a result of the uptake of NH_4^+ increases the solubility of fertilizer P. Additionally, N levels in plant can be enriched with spraying plants with bioactive peptides (Saad et al., 2021b; El-Saadony et al, 2021c; El-Saadony et al, 2021d). On the contrary, (Smatanová et al., 2011) reported that higher doses of N slightly increased yield of spinach. Moreover, although some previous studies reported that N fertilization reduced the plant content of micronutrients (e.g., Mo, Fe, and Mn) because of the dilution effect (El-Ghamry, 2010; Gülser, 2005; Ronaghi et al., 2002), we found that the concentration of micronutrients in plant increased significantly as a results of increased N fertilizers rates (Table 5). Our findings are in the line with Ebed et al. (2009). In this respect, it may be suggested that, increasing N-level could enhance the physiological functions of plant cell and in turn the uptake of these micronutrients. Furthermore, we found that using urea fertilizer as a source of N was the best to give the higher values of previous characters of spinach plants. Also, lacking of Mo in plants suppress plant chloroplast, reducing chlorophyll contents. Molybdenum accelerates the activities of photosynthetic and products of photosynthesis (Nasar and Shah, 2017). An increase in Mo improves plants N uptake through nodule formation, enhancing protein content in plants (Kobraee, 2019). Molybdenum is a key element in the formation of enzymes that transform NO_3^- into nitrite and then into ammonia before they are transformed into amino acids within plants. Biscaro et al. (2011) demonstrated that N fertilization increased common bean yield only when combined with Mo as a foliar application. Nitrate reductase and nitrogenase activities are influenced by Mo status of plants as their activities in plants suppressed with Mo deficiency (Toledo et al., 2010). Whilst, Mo as a foliar spray significantly increased the activities of nitrate reductase and nitrogenase, increasing total N accumulated in plant (Biscaro et al., 2011). Molybdenum also utilized to convert inorganic P into organic P in plants. Consequently, the N and P contents of spinach were increased.

Manganese participates in enzymes and photosynthetic proteins structure. It also influences the water-splitting system of photosystem II (PSII), which supplies the important electrons for photosynthesis (Buchanan et al., 2000). Moreover, Mn plays a master role in plants as cofactor of many enzymes such as Mn-catalase, Mn-superoxide dismutase, phospho-enolpyruvate carboxykinase, and pyruvate carboxylase (Ducic and Polle, 2005). It is also necessary for the biosynthesis of chlorophyll, secondary products (e.g., lignin and flavonoids), and aromatic amino acids (Lidon et al., 2004). It also participates in the assimilation of NO_3^- (Ducic and Polle, 2005). These important roles of Mn were clearly demonstrated in our study through its significant role in increasing the plant growth attributes, yield, leaf pigments (chlorophyll *a* and *b*, and carotenoids), protein concentration, macronutrients (N, P, and K), and micronutrients (Mn, Fe, and Zn) of spinach. Manganese deficiency reduced photosynthesis and crop yield quality and quantity as Mg is active part in enzymes involved in carbohydrate metabolism (Diedrick, 2010; Malakouti and Tehrani, 1999). Malakouti and Tehrani (1999) reported that potato yield increased and storage dry matter improved when Mn was applied. Spraying plants with Mo and Mn combined together at any N fertilizers levels increased chlorophyll and nutrient content in leaves, thus increasing vegetative growth and increasing spinach yield. The combination between micronutrients foliar and N fertilizer enhanced chickpea yield quality and quantity as shown by Rahman et al. (2017).

5. Conclusions

Regarding environment and human health, it is very important to investigate the effect of foliar application on N fertilization reflected beneficial effects on leaf quality and NO_3^- accumulation in spinach leaves as the uptake of elements by plants can be associated with the N fertilization. Therefore, this study investigated the effect of foliar application of Mo and Mg on the quality of spinach leaves and the accumulation of nitrates in leaf tissues. Foliar spraying improved the leaf content of additives and decreased the nitrate content in spinach leaf tissues. There is need to investigate Mo-modulated molecular mechanisms regulating minerals uptake and accumulation in leaves tissues. In this respect, the chemical constituents of spinach could be improved and minimize the negative effects of NO_3^- on health by the application of urea-N at the rate of 360 kg N ha^{-1} and spraying with mixture of Mo and Mn at the rate of 50 and 50 mg L^{-1}, respectively.

Author contributions

Conceptualization: All authors have read and agreed to the published version of the manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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