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ABBREVIATIONS LIST

Abbreviations	Meaning
RD	Recommended dose
MASR	Ministry of Agriculture and Soil Reclamation
SP	Saturation percentage
EC	Electrical conductivity
FAO	Food and Agriculture Organization
IARC	International Agency for Research in Cancer
WHO	World Health Organization
OM	Organic matter
cv.	Cultivar
TSS	Total soluble solids
VC	Vitamin C
ATSDR	Agency for Toxic Research in Cancer

5-SUMMARY AND CONCLUSION

Oxalate and nitrate accumulation in spinach plant as affected by excess nitrogen applications under salinity stress

Nitrate accumulation in Egyptian vegetable showed considerable high values as compared to those found in vegetables grown in several foreign countries in spite of the high intensity and long duration of light in Egypt, which favor nitrate reduction in plants. This could be mainly due to intensive application of nitrogen fertilizers alone by Egyptian farmers which results in imbalanced nutritional status of the plants and consequent high nitrate accumulation.

Nitrate may harm the health of the consumer as it can be converted into nitrite in the human body. Nitrite inhibits oxygen transport by the blood or react with amines and be converted to nitrose amines which cause cancer. The current acceptable daily intake for nitrate for man according to the European scientific committee is set as 3.7 NO_3^-mg kg^{-1} body weight day^{-1}, while the same limit of NO_2 was recommended as 0.06 NO_2^-mg kg^{-1} body weight day^{-1}.

Oxalate is a common plant component which is considered an anti-nutrient as well as a toxin. Oxalate accumulates primarily as soluble oxalate salts such as potassium, sodium, and insoluble oxalate such as Ca oxalate and Mg oxalate. In general, it occurs as the combination of these two forms. Many researches have proposed critical levels of oxalate in dies. They reported that the lethal dose of oxalic acid for humans varies from 2 to 30 g depending on a variety of factors.

Two field experiments were conducted in the Experimental Farm of Tag Elez Station; Dakahlya Governorate, during the two successive winter seasons of 2014-2015 and 2015-2016 to find out mainly:

- The effect of different N doses including higher and lower rates than the recommended one by the Ministry of Agriculture and Soil Reclamation (MASR) on the nitrate accumulation and oxalate formation of spinach crop.
- The best N dose which produce vegetables with oxalate, nitrate and nitrite at the permissible limits.
- The effect of applying Fe and Mo in foliar way on decreasing oxalate, nitrate and nitrite content under the high dose of nitrogen due to its importance in the activity of nitrate reductase enzymes in plants.

Twenty four treatments were arranged in split plot design with 3 replicates, to evaluate the combination effect of:

- Six levels of N-fertilizer {0, 50, 75, 100, 125 and 150 %} from the recommended dose by the Ministry of Agric. and Soil Recl. (MASR) in the form of $NH_4 NO_3$ (33.5% N) as soil application (main plots).
- Micronutrients in foliar way were arranged in (sub plot) in four treatments including, control treatment, Fe (300 ppm), Mo (100 ppm) and the mixture of (Fe + Mo) at the same rates under study.

The obtained results could be summarized as follows:

1. Vegetative growth parameters:

- The average values of all the investigated growth parameters (plant height, number of leaves per plant, fresh weight and dry weight) for

spinach plant were significantly increased as the rate of N-fertilization was increased tell the rate of 100% N.

- The effect of Fe, Mo and Fe+Mo as foliar way on plant growth parameters under study, within the different treatments of micronutrients was studied. The best plant growth parameters were obtained by spinach plant treated with Fe+Mo followed in descending order by that supplied with Fe and lately Mo. The lowest levels for the previously mentioned traits were recorded for the untreated plants.
- At any level of N-fertilization and foliar application of Fe+Mo tended to increase the mean values of all vegetative growth parameters than those obtained from the single addition of Fe or Mo.

2. Chemical constituents:

A. (N, P and K %):

- The highest mean value of N % in the leaves of spinach plant was obtained from the plant treated with N-fertilization at the rate of 150% from the recommended dose by (MASR), while such effect for P and K % were realized at the rate of 100%N in single form. For all the investigated characteristics (N, P and K %); the lowest mean values were associated with the untreated plants.
- As for the effect of foliar application of macronutrient, a superiority effect on the average values of (N, P and K %) was happened due to the application of Fe+Mo in foliar way which recorded the highest mean values compared with the untreated plants.
- In spite of soil application of N-fertilizer at the level of 150% RD in combination with Fe+Mo has been realized the highest mean values of

N%, but the largest values of P and K% during both seasons of study were connected with the plants treated with 100%N RD+ (Fe+Mo). Increasing the rate of N-fertilization from 100% to 125 or 150% significantly decreased the average values of P and K% during both the experimental seasons.

B. Plant pigments:

- A stimulation effect was happened on the mean values of chlorophyll (a, b & total mg g^{-1}) and carotene mg $100g^{-1}$ FW due to an application of nitrogen fertilization as ammonium nitrate at 100% (RD) leading to record the highest mean values for both of them. More addition of N-fertilization levels tell the rate of 150% significantly decreased the average values of chlorophyll and carotene contents and this trend was true during both seasons of the experiment.
- The average values of chlorophyll and carotene contents for the plants treated with (Fe+Mo) were more than that obtained for the treatments of Fe, Mo as well as the untreated plants and this trend was significant during both seasons of the experiment.
- The highest mean values of both pigments were associated with the treatment of 100%N RD + (Fe+Mo) and such effect was more than that obtained from the other treatments under investigation.

C. (Fe and Mo ppm):

- Using the investigated levels of N- fertilizer significantly increased the mean values of Fe and Mo tells the rate of 100% RD. Increasing the rate

of N- fertilizer up to the level of 150% significantly decreased the average values of both elements.

- The highest values of Fe were connected with the plants treated with Fe as foliar addition, while such effect for Mo was realized for the treatment of Mo as foliar application.
- Interaction effect of nitrogen fertilization 100% with foliar application of Fe gave the highest value of Fe content. There was highly significant increase of Mo content as affected by nitrogen fertilizer at 100%with foliar application of Mo.

3. Quality parameters of spinach plants:

A. Nitrate, nitrite and nitrate reductase enzyme activity:

- The highest values of nitrate and nitrite accumulation were associated with the plants treated with N-fertilizer at the rate of 150% RD, while such effect has been recorded the lowest level of nitrate reductase activity.
- The highest values of nitrate and nitrite in both seasons were recorded for the untreated plants, while the lowest values were connected with the plants treated with Fe+Mo. Furthermore, the highest N.R activity was also, influenced by the application of Fe+Mo by foliar way. While, the lowest level of N.R enzyme activity was realized for the untreated plants.
- A positive effect was happened on the mean values of all the aform mentioned traits due to using the combination between the studied parameters. In this respect, the highest values of NO_3-N and NO_2-N, were obtained for the treatment of ammonium nitrate 150% alone, while

the highest values of N.R.A was recorded for the untreated plants in the presence of (Fe+Mo) in both seasons. On the contrary of this trend the lowest values of nitrate and nitrite ppm were recorded for the untreated plants + (Fe+Mo), which realized the highest rate of nitrate reductase activity.

B. Soluble, insoluble and total oxalate mg $100g^{-1}$ FW:

- There were significant increases in soluble and total oxalate mg $100g^{-1}$ F.W recorded with ammonium nitrate at 100% of recommended dose, while the highest values of insoluble oxalate mg $100g^{-1}$ F.W was recorded of 50% of nitrogen fertilizer in both seasons.
- The foliar application of Fe+Mo on spinach plants significantly decreased the average values of insoluble oxalate mg $100g^{-1}$, and increased soluble and total oxalate over the control.
- The highest values of soluble and total oxalate mg $100g^{-1}$ were recorded for the plants treated with ammonium nitrate at 100% and foliar application of Fe+Mo. Moreover, the mean value of insoluble oxalate mg $100g^{-1}$ significantly obtained from using 125% ammonium nitrate in the absence of Fe or Mo.

C. Total phenol, VC mg $100g^{-1}$ and total soluble solids (TSS %):

- a higher amount of VC mg $100g^{-1}$ FW and TSS% of spinach plant were accumulated due to an addition of ammonium nitrate at the rate of 100% to soil more than those obtained from the plants treated with other levels of nitrogen fertilization, while total phenol significantly decreased as the level of N-fertilizer was increased and recorded the highest level of such

traits in the plants treated with N level at 150% compared with the untreated plants.

- Treating spinach plant with Fe+Mo as foliar way significantly increased the average values of the previously mentioned traits than those obtained from the control treatment.
- Adding N level at 100% with foliar application of Fe+Mo significantly increased the average values of VC mg $100g^{-1}$ FW and TSS; while the highest increase in total phenol was recorded with 75% N level with Fe+Mo in the first season, but in the second season the highest value was recorded with foliar application of Fe+Mo in the absence of N-fertilization.

CONCLUSION:

Under the same conditions of this experiment it can be concluded that, applying N fertilization in doses higher than the recommended one (100%) for spinach plant gave increases in the fresh yield and nutrients content but at the same time increased oxalate, nitrate and nitrite accumulation over the safe limit for human health. This means that, the soil supplying power for Fe and Mo was not enough to supply those elements to the studied vegetables under high doses of nitrogen.

The foliar application of Fe and Mo to the studied spinach crop decreased oxalate and nitrate accumulation under the high N fertilization rates which may be attributed to the increase in the formation and/or the activity of nitrate reductase enzymes which reduced NO_3-N to NH_4-N leading to the formation of amino acids and protein.