Journal of King Saud University – Science 34 (2022) 101916

Contents lists available at ScienceDirect

Journal of King Saud University – Science

journal homepage: www.sciencedirect.com

Original article

# Evaluation of moringa (*Moringa oleifera* Lam.) leaf extract on bioactive compounds of lettuce (*Lactuca sativa* L.) grown under glasshouse environment

Arshad Abdulkhalq Yaseen [a,b,*], Maria Takacs-Hajos [a]

[a] University of Debrecen, Faculty of the Agricultural and Food Sciences and Environmental Management, Horticulture Department, H-4032 Debrecen, Böszörményi Street 138, Hungary
[b] Salahaddin University-Erbil, College of Agricultural Engineering Sciences, Department of Horticulture, Kurdistan Region, Iraq

ARTICLE INFO

*Article history:*
Received 21 September 2021
Revised 8 February 2022
Accepted 15 February 2022
Available online 22 February 2022

*Keywords:*
Lettuce quality
Bioactive compounds
Lettuce cultivars
Moringa leaf extract
Nitrate accumulation

ABSTRACT

This study aimed to assess the novelty of moringa (*Moringa oleifera* Lam.) leaf extract (MLE) as a natural biostimulant on bioactive compounds in lettuce. In autumn season of 2019 and 2020, MLE (6%) was foliar sprayed onto lettuce grown under glasshouse conditions. Vitamin C, total polyphenols, total chlorophyll content and nitrate accumulation in three lettuce cultivars (*May King, Kobak, and Great Lakes*) and some correlations among the bioactive compounds were assessed. The results in 2019 show that there was no significant differences in the total polyphenols (mg GAE 100 $g^{-1}$ FW) and vitamin C (mg 100 $g^{-1}$ FW) between treated and untreated plants, while in 2020 significantly higher vitamin C (mg 100 $g^{-1}$ FW) and total polyphenols (mg GAE 100 $g^{-1}$ FW) were recorded in the plants treated with 6% MLE. In contrast, in 2019 application of 6% MLE lowered nitrate content (mg $kg^{-1}$) by 13% in *May King*, 23% in *Kobak*, and 44% in *Great Lakes*. Similarly, in the season of 2020 foliar application of 6% MLE reduced the leaf nitrate content by 16% in *May King*, 19% in *Kobak*, and 44% in *Great Lakes*. In the mean of varieties, the only significant difference in total polyphenol and vitamin C was in the *Great Lakes* variety grown in 2020 whereas no differences were found for the experiment of 2019. In the mean of years, the only statistical difference was recorded for the nitrate content in control of the *Great Lakes* variety by 44% lower nitrate in the treated plants with 6%MLE. Positive correlation was found between vitamin C and total polyphenol content, whereas nitrate had a negative regression slop to vitamin C and total polyphenol content in both experiments. The major total chlorophyll differences were found in the experiment of 2019, although no significant differences were found for the SPAD value of the experiment of 2020. In our 2 years study, there was a positive correlation between vitamin C and total polyphenol while negative correlation was found between vitamin C and total polyphenol to nitrate content in all the lettuce cultivars.



## 1. Introduction

Lettuce is the worlds' most popular consumed fresh leafy vegetable. It plays a critical role in the human diet and benefits for weight losses due to its low calory and high dietary fiber content (10 kcal [60 kJ]/100 g, FW) (Mampholo et al., 2016), appreciable useful compounds like phenols, vitamin A, C and E, carotenoids, iron and calcium (Romani et al., 2002; Szeto et al., 2002). Polyphenols in lettuce leaves contain many antioxidant properties which provide better human health (Lima et al., 2014; Wang et al., 1998). Many studies have described the health benefits of lettuce consumption to humans and rats in the risk reduction of cardiovascular diseases (Nicolle et al., 2004; Serafini et al., 2002).

However, leafy vegetables are considered to accumulate higher nitrate than other vegetables, e.g. bulbs, tubers, and roots (Maynard et al., 1976; Santamaria, 2006), which may threaten human health while converting to nitrite and nitric oxide by salivary enzymes and oral bacteria (Alexander et al., 2008; Khan et al., 2015). So that, the Food and Agriculture Organization (FAO) and the European Commission (EC) have provided the daily

* Corresponding author at: University of Debrecen, Faculty of the Agricultural and Food Sciences and Environmental Management, Horticulture Department, H-4032 Debrecen, room 69, Böszörményi Street 138, Hungary.

*E-mail address:* arshad.yaseen@agr.unideb.hu (A.A. Yaseen).

Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

https://doi.org/10.1016/j.jksus.2022.101916
1018-3647/

acceptance of nitrate intake of 0–3.7 mg $kg^{-1}$ body weight (Santamaria, 2006). Similarly, the U.S. Environmental Protection Agency (EPA) has set up an acceptable daily consumption of 7.0 mg $kg^{-1}$ body weight (Mensinga et al., 2003).

Vegetables have a short lifecycle and need a higher amount of fertilizer during the growing season in comparison to cereals and fruit trees, thus they need more fertilizer and intensive care till they reach the harvest. *Lactuca sativa* is a soil nutrient-depleted vegetable since it grows fast, produces a very shallow root system. So that, it requires adequate levels of fertilizer during its grown season (Thorup-Kristensen, 2001). Although, excessive fertilization of the lettuce field may cause many issues to the environment as water quality throughout leaching and runoff, eutrophication, greenhouse effect, and acid rain (Gastal and Lemaire, 2002; Heckman, 2007; Heckman et al., 2003; Wang et al., 2002) and harmful effects on human health (Ikemoto et al., 2002; Liu et al., 2014).

Fertilizers and pesticides cannot play the entire role in this concomitant challenge that the agriculture sector is facing in the future of the fast global population and environmental impacts on the ecosystem. In this sense, biostimulants are the main focussed product by scientists as an appropriate alternative for the vegetable growers besides the fertilizer and pesticides to extra care of their plants. Biostimulants are mostly made from plant extracts which are used for multipurpose reasons in crop production to alleviate plant abiotic stress (Colla and Rouphael, 2015), nutrient use efficiency, and growth improvement (Rouphael and Colla, 2020).

The reduction of nitrate accumulation in our daily food is extremely important because the nutritional product quality is accounted as the global food security where it is strategically important for the governments all around the world (Bian et al., 2020). High nitrate content or nitrate accumulation in leafy vegetables especially lettuce is a major issue facing quality assistance of the vegetable producers. Thus, plant biostimulants are important not only as a safe material to the environment and human health, but reducing fertilizer inputs to the crops with the same or even better yield and quality (Bulgari et al., 2015).

Moringa leaf extract (MLE) is a recently concerned plant extract that is applied to the plants as an environmentally friendly and safe biostimulant since it contains many beneficial bioactive compounds, macro and micronutrients, minerals, plant hormones vital amino acids, and vitamins (Rady et al., 2013; Sohaimy et al., 2015; Yaseen and Takácsné-Hájos, 2020). The benefits of MLE as a growth enhancer, improve yield and quality has been found on many different crops, green bean (Elzaawely et al., 2017), wheat (Ju et al., 2019), organic fennel (Abdel-Rahman and Abdel-Kader, 2020; El-Serafy and El-Sheshtawy, 2020), freesia plant (Ahmad et al., 2019), mandarin (Nasir et al., 2016), pepper (Aluko, 2016). MLE is also considered as an alternative to inorganic fertilizer (Saini et al., 2016), and effective biostimulant to reduce plant stress (drought and salinity) of many crops (Lucini et al., 2015; Zulfiqar et al., 2020).

However, very little research is available on the influence of moringa leaf extract in improving the quality and nitrate reduction of different lettuce cultivars. Thus, these researches aimed to evaluate the role of moringa leaf extract (MLE) in improving some bioactive components in three lettuce verities grown under glasshouse conditions.

## 2. Materials and methods

### 2.1. Growth conditions and plant materials

In September 2019 and 2020, seeds of *May King*, *Kobak*, and *Great Lakes* lettuce varieties were sown under the optimal weather condition with 70 ± 5 % relative humidity, 25/15 ± 2 ℃ day/ night temperature under glasshouse of research field at the University of Debrecen, Hungary. After about 35 days of the seed sowing, the seedlings were transplanted to the controlled environment under glasshouse weather conditions with 10/14-h day/night photoperiod, 50–75% RH, 20/13 day/night temperature (Figs. 1 and 2). The seedlings were transplanted in the prepared glasshouse soil which the soil composition is shown in Table 1. In our experiment, 40 seedlings of the same development were transplanted per variety. They were divided into two groups (treated and untreated groups). Two weeks after transplanting, the seedlings were foliar sprayed with 6% MLE every two weeks intervals, where the control plants sprayed with distilled water only. Harvesting began 45 days after transplanting. The whole heads of the lettuce plants were carefully taken out for the sample assessment. The outer, middle and inner leaves of each plant were mixed and directly transferred to the lab for total polyphenol (mg GAE 100 $g^{-1}$ FW), vitamin C (mg 100 $g^{-1}$ product), and nitrate content (mg $kg^{-1}$) measurements.

### 2.2. Moringa leaf extract (MLE) preparation

In August 2019 and 2020, young leaves were harvested from the moringa trees grown under a glasshouse at the agrar campus, University of Debrecen, Hungary. Moringa leaf extract was prepared from the fresh young leaves (about 40 days old). First, the leaves were washed and placed in a refrigerator at 4 °C for 24 h as described by Yaseen and Hájos (2020). Then the stored leaves were grinded with the amount of (1 L water/10 kg fresh material) using a kitchen blender then the mixture was squeezed and passed through muslin cloth based on the method by Foidl et al., (2001). Then the extract was placed in 8, 000 *X g* centrifuge for 15 min and the needed concentration of 6% MLE was prepared for the foliar spray treatment. Some mineral elements were measured (Table 2). The extract was then kept in the refrigerator at 4 ℃ till the plants ready to be foliar sprayed (Fig. 3).

### 2.3. Determination of total polyphenol content

The edible lettuce leaves of the inner, middle, and outer leaves of the plant samples were mixed and taken to the lab immediately after harvesting to measure the bioactive compound assessments. Total polyphenols in mg GAE 100 $g^{-1}$ fresh product were performed from the sample solutions using Folin–Ciocalteu reagent. Samples of the solution (5.0 mL) were diluted in distilled water. Only 2.0 mL of each diluted sample was mixed with 10 mL of distilled water and 1.0 mL of prepared Folin–Ciocalteu reagent and placed in 250 mL flask. The volumetric flask was filled with sodium carbonate solution $Na_2CO_3$ (Sigma-Aldrich Kft., Budapest, Hungary)

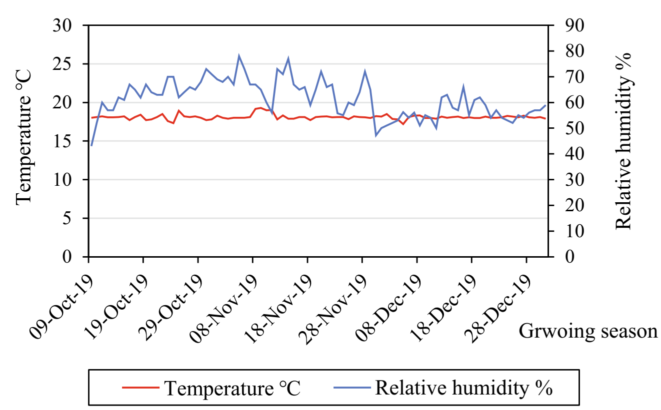


**Fig. 1.** The average temperature and humidity during the lettuce growing season of 2019.

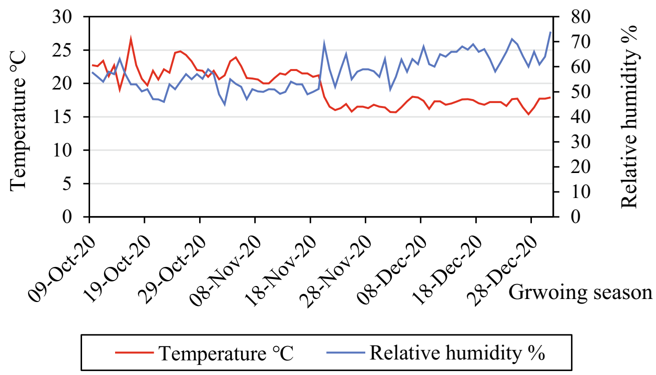


**Fig. 2.** The average temperature and humidity during the lettuce growing season of 2020.

**Table 1**
Some important parameters of the experimental soil.

| Parameters | Value |
|---|---|
| pH value (KCl) | 7.4 |
| EC (mS $cm^{-1}$) | 0.11 |
| Carbonated lime g $kg^{-1}$ | 30.3 |
| Organic carbon – humus content (%) | 1.7 |
| Phosphorus pentoxide (mg $kg^{-1}$) (AL) | 236.0 |
| Potassium oxide (mg $kg^{-1}$) | 177.0 |
| Nitrate and nitrite -N (mg $kg^{-1}$) (KCl) | 70.6 |
| Sodium (mg $kg^{-1}$) (AL) | 42.0 |
| Magnesium (mg $kg^{-1}$) (AL) | 239 |
| Sulphur (mg $kg^{-1}$) (KCl) | 50.4 |
| Manganese (mg $kg^{-1}$) (EDTA) | 22.0 |
| Zinc (mg $kg^{-1}$) (EDTA) | 1.9 |
| Copper (mg $kg^{-1}$) (EDTA) | 1.4 |

**Table 2**
Mineral element content in 100 g moringa leaf extract (MLE).

| MLE | Values (mg) |
|---|---|
| Phosphorus (P) | 100 |
| Potassium (K) | 350 |
| Sulphur (S) | 267 |
| Calcium (Ca) | 326 |
| Magnesium (Mg) | 86.8 |
| Sodium (Na) | 11.4 |
| Iron (Fe) | 2.24 |
| Cupper (Cu) | 0.319 |
| Zinc (Zn) | 0.477 |
| Boron (B) | 0.581 |

at 29% (w/v). The absorbance was measured using a spectrophotometer at 720 nm. The results were given in gallic acid equivalent (GAE) of 50 mM–2.5 mM gallic acid [in 95% (vol/vol) methanol] based on the method by Ainsworth and Gillespie (2007).

### 2.4. Ascorbic acid (Vitamin C) measurement

Vitamin C content was determined by redox titration using iodine solution following the method described by Ciancaglini et al. (2001).

$$\text{ascorbic acid} + I_2 \rightarrow 2\,I^- + \text{dehydroascorbic acid}$$

### 2.5. Nitrate determination

The nitrate content in lettuce leaves was measured based on the method by Kmecl et al. (2007) using Segmented Flow Analyzer (AA II, Bran + Luebbe) at the wavelength of 540 nm after the reduction in copper coated cadmium column (NO3– + 2e– ® NO2–) to form a diazo compound.

### 2.6. Statistical analysis

Data were subjected to one-way analysis of variance (ANOVA) at the confidence level $P \leq 0.05$ using SPSS version 25 (SPSS Inc., Chicago, IL, USA). The means of 12 samples/replication were subjected to the independent sample *t*-test to determine significant differences between the two groups.

SPAD-502 (Spectrum Technologies) device was used to perform the correlation between chlorophyll content, nitrate and bioactive compounds in lettuce leaves based on the method by Madeira et al. (2003). The data were statistically analysed using pearson correlation method by Weinberg and Abramowitz (2008) at $p \leq 0.05$ and $p \leq 0.01$.

### 2.7. Fertilizer application

Unlike other vegetables, lettuce growth, yield and some chemical contents as nitrate content are most affected by nitrogen fertilizer. Based on the recommended nitrogen fertilizer for lettuce as discussed by Liu et al., (2014), the inorganic fertilizer FERTICARE 24–8-16 (NPK) was applied with the amount of 200 kg N $ha^{-1}$ to our plants.

## 3. Results and discussions

### 3.1. Total polyphenol content (mg GAE 100 $g^{-1}$ FW)

The influence of the application of moringa leaf extract varied considering the environmental factors and plant genotypes. In the autumn season of 2019, statistically higher total polyphenol was measured in untreated *Great Lakes* variety, while non of the other varieties (*May King* and *Kobak*) were significantly different. However, in the season of 2020, foliar spray with 6% MLE could significantly enhance the total polyphenol content in both *May King* and *Great Lakes*, whereas no significant difference was observed for the *Kobak* variety (Table 3). Except for genetic factors many other factors can change the polyphenol quantity in the plant leaves as leaf position (inner or outer leaves), agronomical factors as well as plant tissue type (green, yellow or white) (Bilyk and Sapers, 1985; Romani et al., 2002).

In general, plants in 2020 had more temperature fluctuation stress than in 2019, for example, a dramatic drop in temperature of roughly 5 °C occurred towards the end of November 2020 and continued until the end of the season (Figs. 1 and 2). Thus, this might be the reason for having higher total polyphenol in the experiment of 2020. Many research studies have been provided on the abiotic stress factor affecting total polyphenol in plants (Abdallah et al., 2013; Helyes et al., 2015). The temperature in 2020 was much fluctuated and decreased in the last two growing months (November and December) than in 2019, thus the polyphenol was also greater in 2020. This result agrees with the result by Boo et al., (2011) where they found higher total polyphenol and anthocyanin contents in lettuce grown under day/night temperature of 13/10 °C and 20/13 °C, followed by 25/20 °C and 30/25 °C.

Nasir et al., (2020) have detected the improvement of polyphenol in mandarin fruit by 1.4-fold in the trees treated with 3% MLE in comparison to untreated trees. This might be owing to the zeatin content of the moringa leaves (Basra et al., 2011) because this enhances the antioxidant content of the plants through the enzymes in the plant cells (Zhang and Ervin, 2004). Yasmeen et al., (2013) have also found a maximum value of total phenolic

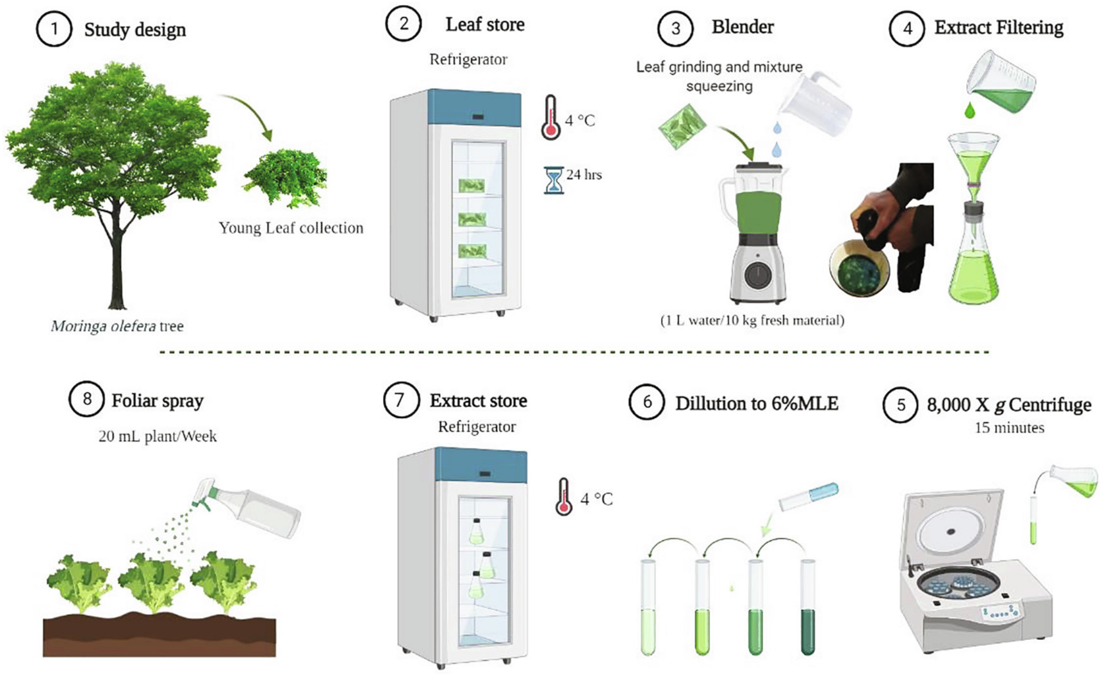


**Fig. 3.** Moringa leaf extract (MLE) preparation from fresh leaves Created with BioRender.com.

**Table 3**
Effect of lettuce varieties, foliar treatments and growth season on total polyphenol content (mg GAE 100 $g^{-1}$ FW) in lettuce leaves for the season of 2019 and 2020.

| Lettuce cultivars | *Total polyphenol content (mg GAE 100 $g^{-1}$ FW)* | | | | | |
|---|---|---|---|---|---|---|
| | Growing seasons | | | | In the mean of years Mean ± Std. Deviation | |
| | 2019 Mean ± Std. Deviation | | 2020 Mean ± Std. Deviation | | | |
| | Control | 6% MLE | Control | 6% MLE | Control | MLE |
| *May King* | 37.65 ± 2.04 | 35.59 ± 1.53 | 51.63 ± 2.00 | 65.83 ± 10.00 | 44.64± 7.86 | 50.71± 17.75 |
| *Kobak* | 37.95 ± 1.00* | 35.61 ± 1.00 | 65.40 ± 4.07 | 70.88 ± 2.00 | 51.67± 15.26 | 53.24± 19.36 |
| *Great Lakes* | 41.73 ± 1.01* | 37.26 ± 0.97 | 55.69 ± 3.00 | 73.03 ± 5.00* | 48.71± 7.90 | 55.14± 19.85 |
| In the mean of varieties | 39.11 ± 2.33 | 36.15 ± 1.33 | 58.93 ± 9.24 | 69.91*± 6.5 | – | |

Means in the same column followed by the same symbol (*) are not significantly different at probability level ($p \leq 0.05$) according to the independent sample *t*-test

content in the wheat grown under moderate saline growth conditions.

Elzaawely et al. (2017) further mentions that the quality enhancement in plants treated with MLE is related to the hormon concentration in moringa leaves, particularly gibberellins ($GA_7$).

In the mean of years, the total polyphenol content in lettuce leaves was significantly improved in the foliar sprayed plants by 8% compared to the control plants. Where in the mean of plant varieties, there were no significant differences in the season of 2019 among the varieties, while in 2020 plants foliar sprayed with 6% MLE were significantly affected by the biostimulants in comparison to non-treated plants (Table 3).

### *3.2. Vitamin C (mg 100 $g^{-1}$ fresh weight)*

The ascorbic acid content of the lettuce cultivars ranged from 4.81 to 10.3 mg100 $g^{-1}$ FW in both seasons 2019 and 2020. The highest amount of the vitamin C (10.3 mg100 $g^{-1}$) was found in the *Great Lakes* variety in 2020 in the plants foliar sprayed with 6% MLE, while the lowest was in the *Kobak* and *May King* treated with 6% MLE in 2019 (4.81 and 4.91 mg100 $g^{-1}$) fresh product, respectively (Table 4). The experiment results indicate the variation of the vitamin C content under the influence of the interaction of plant variety and the treatments from seasons 2019 and 2020. Lower vitamin C was found in the treated plants with 6% MLE for the season of 2019, however statistically no significant difference was found between treated and untreated cultivars. This shows that the used plant biostimulant (6%MLE) could perfectly work on the plants in 2020 where the treated plants could improve better under less abiotic stress by producing higher vitamin C than in 2019. Plant biostimulants can improve growth and reduce abiotic stress in plants (du Jardin, 2015; Rouphael and Colla, 2018). Whereas, except for the *Kobak* cultivar there was a significantly higher vitamin C in *May King* and *Great Lakes* cultivars treated with 6% MLE in the grown season of 2020. Nasir et al., (2020) demonstrated the improvement of vitamin C by 2.3-fold in mandarin fruit treated with 3% MLE comparing to the control. Moringa leaf extract (MLE; 30 times diluted) resulted in the maximum ascorbic acid content in the wheat grown under highly saline (12 dS $m^{-1}$) growth conditions (Yasmeen et al., 2013). In the mean of years, plants were not affected by 6% MLE for the vitamin C content. Similarly, in 2019 no significant results appear between treated and non-treated plants among the varieties. In contrast, in the mean of varieties in 2020 plants treated with 6% MLE recorded 26% greater vitamin C content than non-treated plants (Table 4).

**Table 4**
Effect of lettuce varieties, foliar treatments and growth season on Vitamin C (mg 100 g$^{-1}$ fresh weight) content in lettuce leaves for the season of 2019 and 2020.

| Lettuce cultivars | *Vitamin C (mg 100 g$^{-1}$ fresh wieght)* | | | | | |
|---|---|---|---|---|---|---|
| | Growing seasons | | | | In the mean of years Mean ± Std. Deviation | |
| | 2019 Mean ± Std. Deviation | | 2020 Mean ± Std. Deviation | | | |
| | Control | 6% MLE | Control | 6% MLE | Control | MLE |
| *May King* | 5.85 ± 0.90 | 4.81 ± 0.96 | 6.03 ± 0.76 | 9.21 ± 0.09* | 5.94 ± 0.75 | 7.01 ± 2.48 |
| *Kobak* | 5.83 ± 0.17 | 4.91 ± 0.94 | 8.87 ± 0.75 | 9.92 ± 1.01 | 7.35 ± 1.73 | 7.41 ± 2.87 |
| *Great Lakes* | 8.61 ± 1.17 | 8.30 ± 0.69 | 6.95 ± 0.15 | 10.30 ± 0.30* | 7.78 ± 1.17 | 9.30 ± 1.19 |
| In the mean of varieties | 6.76 ± 1.57 | 6.01 ± 1.88 | 7.28 ± 1.36 | 9.81 ± 0.71* | – | |

Means in the same column followed by the same symbol (*) are not significantly different at probability level ($p \leq 0.05$) according to the independent sample t-test

### 3.3. Nitrate content in lettuce leaves (mg kg$^{-1}$)

Nitrate content in lettuce leaves was varied from year to year and among the varieties. Many factors can influence these differences as genetic, environmental impacts as light, temperature, and $CO_2$ differences between the seasons (Bian et al., 2020; Maynard et al., 1976; Prugar et al., 1991), as well as plant age – e.g. younger plants, contain higher nitrate content than older ones (Geiger et al., 1998) as well as agronomical factors (Al-Gburi, 2021). Our result indicates that factors (genetic and biostimulants) could mitigate the nitrate concentration in lettuce fresh mass. Nitrate accumulation was varied among different lettuce genotypes, lower in *May King* followed by *Kobak* and *Great Lakes* variety. This is similar to the result by Nádasy and Nádasy (2006) where they found that *Great Lakes* was more accumulating nitrate than *Kobak* and *May King* varieties in their pot experiment of adding 100 mg N-fertilizer per kg of soil.

In recent years, many researchers have reported the positive influence of plant biostimulants, organic extracts, amino acids, and vitamins on nitrate reduction in plants. Examples are given on reduction of nitrate in lettuce, swiss chard, spinach, and radish using protein hydrolysates as biostimulants (Colla and Rouphael, 2015). Also, Amanda et al., (2009) have shown the positive influence of plant spraying with the biostimulant of Actiwave®, Valagro SpA at a concentration of 6 mL m$^{-2}$ on nitrate reduction of lettuce grown under controlled environment. Table 5 shows that in the season of 2019, foliar application of 6% MLE lowered the leaf nitrate content by 13% in *May King*, 23% in *Kobak*, and 44% in *Great Lakes*. Similarly, in the season of 2020 foliar application of 6% MLE reduced the leaf nitrate content by 16% in *May King*, 24% in *Kobak*, and 44% in *Great Lakes*. Other researchers have shown that biostimulants as double irrigation with 1.0% Bio-algeen S-90 (200 mL per plant) extracted from the brown seaweed and 0.3% Megagreen (100 mL m$^{-2}$) reduced nitrate content in lettuce leaves (Dudaš et al., 2016). Our results agree with the result by Fu et al., (2017) where the increase of vitamin C content in lettuce leaves the nitrate level decreases. Based on our previous results of the experiment of (Yaseen and Takácsné-Hájos, 2021), the high amount of amino acid content in the MLE could also inhibit the nitric ion in the soil while being absorbed by the plant leaves.

In the mean of varieties, the application of 6% MLE could significantly reduce nitrate content by 27.5% in 2019 and 27.8 % in 2020. Whereas in the mean of years, the only significant difference was in *Great Lakes* variety with the highest reduction (44%) which was followed by *Kobak* and May King with 24 and 14 % (Table 5).

### 3.4. Chlorophyll content in related to MLE treatment

Many studies have shown that moringa leaf extract (MLE) can improve photosynthetic pigments under normal and stress environmental conditions. Based on our two-year research, 6% MLE colud considerably enhance total chlorophyll content in several lettuce cultivars. In the season of 2019, all the cultivars produced significantly higher chlorophyll content in the treated plants compared to untreated plants, whereas in the 2020 experiment, the treated lettuce cultivars produced higher chlorophyll pigment content, but there was no significant difference between control and treated plants with 6% MLE (Table 6.). The pigment concentration was varied among the lettuce varieties. For example, the Great Lakes variety had the greatest pigment concentration among the lettuce cultivars, which is owing to the fact that this variety is inherently greener in color, whilst others are lighter green to yellow. Similar findings were made by Ahmad et al. (2019), whom discovered a substantial increase in total chlorophyll in freesia (bulbous cut flower) plants. Other researchers show that foliar application of MLE dramatically increase phenolic antioxidants, other biochemicals, and total chlorophyll content in spinach (*Spinacia oleracea* L.) leaves (Aslam et al., 2016). Also, Elzaawely

**Table 5**
Effect of the lettuce varieties, foliar treatments and growth season on nitrate in lettuce leaves (mg kg$^{-1}$) content for the season of 2019 and 2020.

| Lettuce cultivars | *Nitrate accumulation in lettuce leaves (mg kg$^{-1}$)* | | | | | |
|---|---|---|---|---|---|---|
| | Growing seasons | | | | In the mean of years Mean ± Std. Deviation | |
| | 2019 Mean ± Std. Deviation | | 2020 Mean ± Std. Deviation | | | |
| | Control | 6% MLE | Control | 6% MLE | Control | MLE |
| *May King* | 784.00 ± 22.00* | 692.00 ± 16.00 | 439.00 ± 3.00* | 379.00 ± 15.00 | 611.50± 189.48 | 535.50± 171.99 |
| *Kobak* | 950.00 ± 42.00* | 772.00 ± 20.00 | 642.00 ± 3.00* | 517.00 ± 8.00 | 796.00± 170.78 | 644.50± 140.33 |
| *Great Lakes* | 1180.66 ± 13.50* | 820.00 ± 22.00 | 702.00 ± 8.00* | 489.00 ± 4.00 | 941.33± 262.36* | 654.50± 181.84 |
| In the mean of varieties | 971.55± 174.27* | 761.33 ± 58.48 | 594.33 ± 119.44* | 461.66 ± 63.77 | – | |

Means in the same column followed by the same symbol (*) are not significantly different at probability level ($p \leq 0.05$) according to the independent sample *t*-test

**Table 6**
Chlorophyll content in three lettuce cultivars in related to plant biostimulants in the experiments of autumn 2019 and 2020.

| Treatments | Cultivars | Total chlorophyll (SPAD value) | | |
|---|---|---|---|---|
| | | Growing Seasons | | Mean of Years |
| | | 2019 | 2020 | |
| untreated | May King | 13.16 | 19.43 | 16.30 |
| | Kobak | 16.23 | 20.30 | 18.26 |
| | Great Lakes | 32.76 | 36.06 | 34.41 |
| treated | May King | 17.56* | 23.33 | 20.45* |
| | Kobak | 23.90* | 20.56 | 22.23* |
| | Great Lakes | 40.03* | 38.10 | 39.06* |

**Table 7**
Correlation between bioactive compounds and SPAD, NDVI measurements.

| | Years | Total polyphenol | Vitamin C | Nitrate | SPAD |
|---|---|---|---|---|---|
| Total polyphenol | 2019 | 1.000 | | | |
| | 2020 | 1.000 | | | |
| Vitamin C | 2019 | 0.469* | 1.000 | | |
| | 2020 | 0.757** | 1.000 | | |
| Nitrate | 2019 | 0.562* | 0.488* | 1.000 | |
| | 2020 | −0.082 | −0.172 | 1.000 | |
| SPAD | 2019 | 0.279 | 0.708** | 0.170 | 1.000 |
| | 2020 | 0.096 | 0.143 | 0.309 | 1.000 |

**Correlation is significant at the 0.01 level (1-tailed).
*Correlation is significant at the 0.05 level (1-tailed).

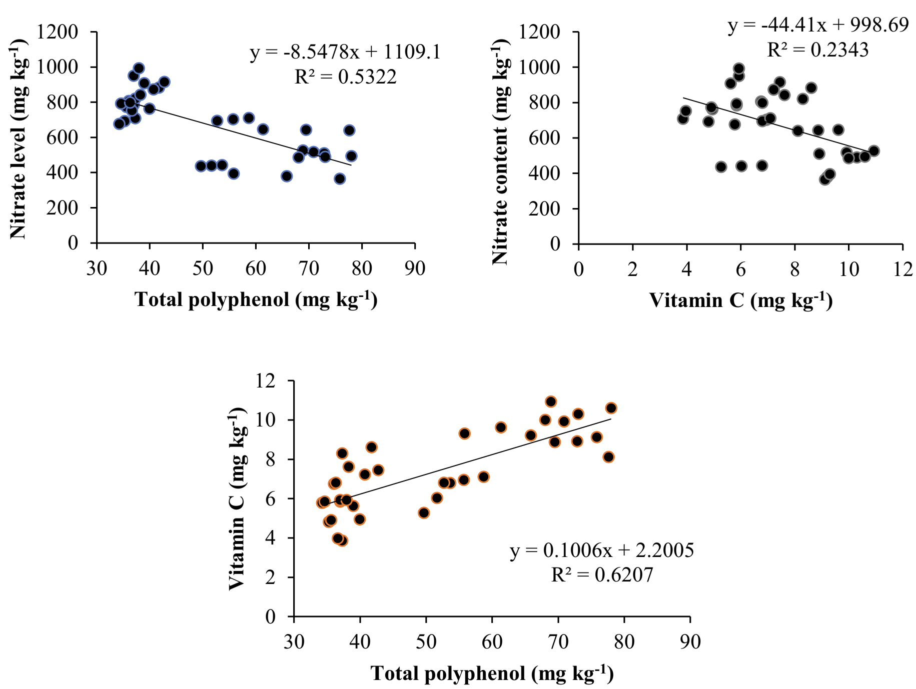


**Fig. 4.** Regression equation results of the studied data for the mean of bioactive compound value of 2019 and 2020.

et al. (2017) discovered a substantial increase in chlrophyll a, b, and carotinoides in snap beans treated with 1:20 and 1:30 foliar sprays of MLE across two growth seasons. Similarly, our findings show that plants treated with 6% MLE had considerably greater chlorophyll pigmentation than untreated plants over the course of years.

### 3.5. Correlation & regression analysis results

Our findings reveal that the primary bioactive compounds in the 2019 trial showed a positive correlation with SPAD values. Total polyphenol, for example, showed a strong positive connection with vitamin C and Nitrate, but no relationships with SPAD value (chlorophyll content) were identified. Vitamin C, on the other hand, exhibited a positive association with nitrate content and SPAD value of 0.757** at $p \leq 0.05$ for 2019 and 2020. Some of the experiment's 2020 results were entirely different from those in 2019. For example, there was no connection between vitamin C, nitrate, and SPAD (Table 7). In the experiment of 2019, vitamin C had a positive correlation with nitrate content at $p \leq 0.01$, whereas in the season of 2020 there was no or negative correlation between vitamin C and nitrate content at (-) 0.172. The strongest correlation among bioactive chemicals was discovered during the growth season of 2019, but minimal correlation was observed during the growing season of 2020, this might be due to meteorological variations, particularly temperature and light.

In the mean of years, total polyphenol and vitamin C had a negative correlation regression with the nitrate content in lettuce cultivars at $R^2$ = 0.532 and $R^2$ = 0.234 respectively. Nonetheless a positive correlation regression equation between vitamin C and total polyphenol was found for the mean of the experiment of 2019 and 2020 at $R^2$ = 0.6207 (Fig. 4).

## 4. Conclusion

In the consideration of climatic and genetic variables, Moringa (*Moringa oleifera* Lam.) leaf extract (MLE) can be a reliable source of safe and natural biostimulants to improve certain bioactive components and reduce nitrate in the edible part of lettuce. Our experiment gives a safe and simple method for farmers to obtain the extract, in which they may grow the tree in their field and make the extract for their plants. The use of plant biostimulants (6% MLE) decreased nitrate accumulation in the lettuce cultivars grown in autumn 2019 and 2020. By reducing nitrate concentration in plant leaves, the leaf extract can play a significant role in lowering human health risks as nitrite intake via vegetable consumption. Also, Whereas vitamin C and total polyphenols showed very little improvement. This extract is critical for reducing nitrate without losing other quality factors such as bioactive components (vitamin C and polyphenols).

## 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.

## References


Abdallah, S.B., Rabhi, M., Harbaoui, F., Zar-kalai, F., Lachâal, M., Karray-Bouraoui, N., 2013. Distribution of phenolic compounds and antioxidant activity between young and old leaves of Carthamus tinctorius L. and their induction by salt stress. Acta Physiol. Plant. 35 (4), 1161–1169. https://doi.org/10.1007/s11738-012-1155-z.

Abdel-Rahman, S.S.A., Abdel-Kader, A.A.S., 2020. Response of Fennel (Foeniculum vulgare, Mill) plants to foliar application of moringa leaf extract and benzyladenine (BA). South African J. Bot. 129, 113–122. https://doi.org/10.1016/j.sajb.2019.01.037.

Ahmad, I., Tanveer, M.U., Liaqat, M., Dole, J.M., 2019. Comparison of corm soaks with preharvest foliar application of moringa leaf extract for improving growth and yield of cut Freesia hybrida. Sci. Hortic. (Amsterdam) 254, 21–25. https://doi.org/10.1016/j.scienta.2019.04.074.

Ainsworth, E.A., Gillespie, K.M., 2007. Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin-Ciocalteu reagent. Nat. Protoc. 2 (4), 875–877. https://doi.org/10.1038/nprot.2007.102.

Al-Gburi, B.K.H., 2021. Effect of different control applications on Cuscuta campestris, and biochemical content of eggplant. J. Saudi Soc. Agric. Sci. 20 (4), 209–216. https://doi.org/10.1016/j.jssas.2021.01.007.

Alexander, J., Benford, D., Cockburn, A., Cravedi, J.P., Dogliotti, E., Domenico, A.D., Fernández-Cruz, M.L., Fink-Gremmels, J., Fürst, P., Galli, C., Grandjean, P., 2008. Nitrate in vegetables - Scientific Opinion of the Panel on Contaminants in the Food chain. EFSA J. 689, 1–79. https://doi.org/10.2903/j.efsa.2008.689.

Aluko, M., 2016. Moringa leaf extract on the growth and yield of pepper (Capsicum annuum L.). ARPN J. Agric. Biol. Sci. 11, 107–109.

Amanda, A., Ferrante, A., Valagussa, M., Piaggesi, A., 2009. Effect of biostimulants on quality of baby leaf lettuce grown under plastic tunnel. Acta Hortic. 807, 407–412. https://doi.org/10.17660/ActaHortic.2009.807.58.

Aslam, M., Sultana, B., Anwar, F., Munir, H., 2016. Foliar spray of selected plant growth regulators affected the biochemical and antioxidant attributes of spinach in a field experiment. Turkish J. Agric. For. 40, 136–145. https://doi.org/10.3906/tar-1412-56.

Basra, S.M.A., Iftikhar, M.N., Afzal, I., 2011. Potential of moringa (Moringa oleifera) leaf extract as priming agent for hybrid maize seeds. Int. J. Agric. Biol. 13, 1006–1010.

Bian, Z., Wang, Y.u., Zhang, X., Li, T., Grundy, S., Yang, Q., Cheng, R., 2020. A Review of Environment Effects on Nitrate Accumulation in Leafy Vegetables Grown in Controlled Environments. Foods 9 (6), 732. https://doi.org/10.3390/foods9060732.

Bilyk, A., Sapers, G.M., 1985. Distribution of Quercetin and Kaempferol in Lettuce, Kale, Chive, Garlic Chive, Leek, Horseradish, Red Radish, and Red Cabbage Tissues. J. Agric. Food Chem. 33 (2), 226–228. https://doi.org/10.1021/jf00062a017.

Boo, H.-O., Heo, B.-G., Gorinstein, S., Chon, S.-U., 2011. Plant Science Positive effects of temperature and growth conditions on enzymatic and antioxidant status in lettuce plants. Plant Sci. 181 (4), 479–484. https://doi.org/10.1016/j.plantsci.2011.07.013.

Bulgari, R., Cocetta, G., Trivellini, A., Vernieri, P., Ferrante, A., 2015. Biostimulants and crop responses: A review. Biol. Agric. Hortic. 31 (1), 1–17. https://doi.org/10.1080/01448765.2014.964649.

Ciancaglini, P., Santos, H.L., Daghastanli, K.R.P., Thedei, G., 2001. Using a classical method of vitamin C quantification as a tool for discussion of its role in the body. Biochem. Mol. Biol. Educ. 29, 110–114. https://doi.org/10.1016/S1470-8175(01)00039-X.

Colla, G., Rouphael, Y., 2015. Biostimulants in horticulture. Sci. Hortic. (Amsterdam) 196, 1–134. https://doi.org/10.1016/j.scienta.2015.10.044.

du Jardin, P., 2015. Plant biostimulants: Definition, concept, main categories and regulation. Sci. Hortic. 196, 3–14.

Dudaš, S., Šola, I., Sladonja, B., Erhatić, R., Ban, D., Poljuha, D., 2016. The effect of biostimulant and fertilizer on "Low Input" lettuce production. Acta Bot. Croat. 75, 253–259. https://doi.org/10.1515/botcro-2016-0023.

El-Serafy, R.S., El-Sheshtawy, A.A., 2020. Effect of nitrogen fixing bacteria and moringa leaf extract on fruit yield, estragole content and total phenols of organic fennel. Sci. Hortic. 265, 109209. https://doi.org/10.1016/j.scienta.2020.109209.

Elzaawely, A.A., Ahmed, M.E., Maswada, H.F., Xuan, T.D., 2017. Enhancing growth, yield, biochemical, and hormonal contents of snap bean (Phaseolus vulgaris L.) sprayed with moringa leaf extract. Arch. Agron. Soil Sci. 63 (5), 687–699. https://doi.org/10.1080/03650340.2016.1234042.

Foidl, N., Makkar, H.P.S., Becker, K., Foild, N., Km, S., 2001. the Potential of Moringa Oleifera for Agricultural and Industrial Uses. What Dev. potential Moringa Prod., 1–20

Fu, Y., Li, H.Y., Yu, J., Liu, H., Cao, Z.Y., Manukovsky, N.S., Liu, H., 2017. Interaction effects of light intensity and nitrogen concentration on growth, photosynthetic characteristics and quality of lettuce (Lactuca sativa L. Var. youmaicai). Sci. Hortic. (Amsterdam) 214, 51–57. https://doi.org/10.1016/j.scienta.2016.11.020.

Gastal, F., Lemaire, G., 2002. N uptake and distribution in crops: An agronomical and ecophysiological perspective. J. Exp. Bot. 53, 789–799. https://doi.org/10.1093/jexbot/53.370.789.

Geiger, M., Walch-Liu, P., Engels, C., Harnecker, J., Schulze, E.-D., Ludewig, F., Sonnewald, U., Scheible, W.-R., Stitt, M., 1998. Enhanced carbon dioxide leads to a modified diurnal rhythm of nitrate reductase activity in older plants, and a large stimulation of nitrate reductase activity and higher levels of amino acids in young tobacco plants. Plant, Cell Environ. 21 (3), 253–268. https://doi.org/10.1046/j.1365-3040.1998.00277.x.

Heckman, J.R., 2007. Sweet corn nutrient uptake and removal. Horttechnology 17, 82–86. https://doi.org/10.21273/horttech.17.1.82.

Heckman, J.R., Sims, J.T., Beegle, D.B., Coale, F.J., Herbert, S.J., Bruulsema, T.W., Bamka, W.J., 2003. Nutrient removal by corn grain harvest. Agron. J. 95 (3), 587–591. https://doi.org/10.2134/agronj2003.5870.

Helyes, L., Nagy, Z.S., Daood, H., Pék, Z., Lugasi, A., 2015. The simultaneous effect of heat stress and water supply on total polyphenol content of eggplant. Appl. Ecol. Environ. Res. 13, 583–595. https://doi.org/10.15666/aeer/1302_583595.

Ikemoto, Y., Teraguchi, M., Kobayashi, Y., 2002. Plasma levels of nitrate in congenital heart disease: Comparison with healthy children. Pediatr. Cardiol. 23 (2), 132–136. https://doi.org/10.1007/s00246-001-0036-9.

Ju, A., Baek, S.-K., Kim, S., Song, K.B., 2019. Development of an antioxidative packaging film based on khorasan wheat starch containing moringa leaf extract. Food Sci. Biotechnol. 28 (4), 1057–1063. https://doi.org/10.1007/s10068-018-00546-9.

Khan, S., Karmokar, A., Howells, L., Thomas, A., Bayliss, R., Gescher, A., Brown, K., 2015. Nitrate and nitrite in the diet: how to assess their benefit and risk for human health. Mol. Nutr. Food Res. 59, 106–128. https://doi.org/10.1002/mnfr.201400286.This.

Kmecl, V., Knap, T., Žnidarčič, D., 2007. Evaluation of the nitrate and nitrite content of vegetables commonly grown in Slovenia. Ital. J, Agron, p. 12.

Lima, G.P.P., Vianello, F., Corrêa, C.R., da Silva Campos, R.A., Borguini, M.G., 2014. Polyphenols in Fruits and Vegetables and Its Effect on Human Health. Food Nutr. Sci. 5, 1065–1082. https://doi.org/10.4236/fns.2014.511117.

Liu, C.W., Sung, Y., Chen, B.C., Lai, H.Y., 2014. Effects of nitrogen fertilizers on the growth and nitrate content of lettuce (Lactuca sativa L.). Int. J. Environ. Res. Public Health 11, 4427–4440. https://doi.org/10.3390/ijerph110404427.

Lucini, L., Rouphael, Y., Cardarelli, M., Canaguier, R., Kumar, P., Colla, G., 2015. The effect of a plant-derived biostimulant on metabolic profiling and crop performance of lettuce grown under saline conditions. Sci. Hortic. (Amsterdam) 182, 124–133. https://doi.org/10.1016/j.scienta.2014.11.022.

Madeira, A.C., Ferreira, A., de Varennes, A., Vieira, M.I., 2003. SPAD meter versus tristimulus colorimeter to estimate chlorophyll content and leaf color in sweet pepper. Commun. Soil Sci. Plant Anal. 34 (17-18), 2461–2470. https://doi.org/10.1081/CSS-120024779.

Mampholo, B.M., Maboko, M.M., Soundy, P., Sivakumar, D., 2016. Phytochemicals and Overall Quality of Leafy Lettuce (Lactuca sativa L.) Varieties Grown in Closed Hydroponic System. J. Food Qual. 39 (6), 805–815. https://doi.org/10.1111/jfq.12234.

Maynard, D.N., Barker, A.V., Minotti, P.L., Peck, N.H., 1976. Nitrate Accumulation in Vegetables. Adv. Agron. 28, 71–118.

Mensinga, T.T., Speijers, G.J.A., Meulenbelt, J., 2003. Health implications of exposure to environmental nitrogenous compounds. Toxicol. Rev. 22 (1), 41–51. https://doi.org/10.2165/00139709-200322010-00005.

Nádasy, E., Nádasy, M., 2006. Some harmful or useful environmental effect of nitrogen fertilisers. Cereal Res. Commun. 34, 49–52.

Nasir, M., Khan, A.S., Basra, S.M.A., Malik, A.U., 2020. Improvement in growth, productivity and quality of 'Kinnow' mandarin fruit after exogenous application of Moringa olifera leaf extract. South African J. Bot. 129, 263–271. https://doi.org/10.1016/j.sajb.2019.07.042.

Nasir, M., Khan, A.S., Basra, S.M.A., Malik, A.U., 2016. Foliar application of moringa leaf extract, potassium and zinc influence yield and fruit quality of 'Kinnow' mandarin. Sci. Hortic. (Amsterdam) 210, 227–235. https://doi.org/10.1016/j.scienta.2016.07.032.

Nicolle, C., Cardinault, N., Gueux, E., Jaffrelo, L., Rock, E., Mazur, A., Amouroux, P., Rémésy, C., 2004. Health effect of vegetable-based diet: Lettuce consumption improves cholesterol metabolism and antioxidant status in the rat. Clin. Nutr. 23 (4), 605–614. https://doi.org/10.1016/j.clnu.2003.10.009.

Prugar, J., Vanek, V., Sokolov, O.A., Semenov, V.M., 1991. Nitrates in plants. Nitrogen Cycles in the Present Agriculture. EdBielek, P., Kundeyarov, VN, Prir. Bratislava, Czecho-Slovakia 127–167.

Rady, M.M., Varma, C.B., Howladar, S.M., 2013. Common bean (Phaseolus vulgaris L.) seedlings overcome NaCl stress as a result of presoaking in Moringa oleifera leaf extract. Sci. Hortic. (Amsterdam) 162, 63–70. https://doi.org/10.1016/j.scienta.2013.07.046.

Romani, A., Pinelli, P., Galardi, C., Sani, G., Cimato, A., Heimler, D., 2002. Polyphenols in greenhouse and open-air-grown lettuce. Food Chem. 79 (3), 337–342. https://doi.org/10.1016/S0308-8146(02)00170-X.

Rouphael, Y., Colla, G., 2020. Biostimulants in Agriculture. Front. Plant Sci. 11, 40. https://doi.org/10.3389/fpls.2020.00040.

Rouphael, Y., Colla, G., 2018. Synergistic biostimulatory action: Designing the next generation of plant biostimulants for sustainable agriculture. Front. Plant Sci. 9, 1655. https://doi.org/10.3389/fpls.2018.01655.

Saini, R.K., Sivanesan, I., Keum, Y.S., 2016. Phytochemicals of Moringa oleifera: a review of their nutritional, therapeutic and industrial significance. 3. Biotech 6, 1–14. https://doi.org/10.1007/s13205-016-0526-3.

Santamaria, P., 2006. Nitrate in vegetables: Toxicity, content, intake and EC regulation. J. Sci. Food Agric. 86 (1), 10–17. https://doi.org/10.1002/jsfa.2351.

Serafini, M., Bugianesi, R., Salucci, M., Azzini, E., Raguzzini, A., Maiani, G., 2002. Effect of acute ingestion of fresh and stored lettuce (Lactuca sativa) on plasma total antioxidant capacity and antioxidant levels in human subjects. Br. J. Nutr. 88 (6), 615–623.

Sohaimy, S.A.E., Hamad, G.M., Mohamed, S.E., Amar, M.H., Al-hindi, R.R., 2015. Biochemical and functional properties of Moringa oleifera leaves and their potential as a functional food. Glob. Adv. Res. J. Agric. Sci. 4, 188–199.

Szeto, Y.T., Tomlinson, B., Benzie, I.F.F., 2002. Total antioxidant and ascorbic acid content of fresh fruits and vegetables: implications for dietary planning and food preservation. Br. J. Nutr. 87 (1), 55–59.

Thorup-Kristensen, K., 2001. Root growth and soil nitrogen depletion by onion, lettuce, early cabbage and carrot. Acta Hortic. 563, 201–206. https://doi.org/10.17660/ActaHortic.2001.563.25.

Wang, H.K., Xia, Y., Yang, Z.Y., Natschke, S.L., Lee, K.H., 1998. Recent advances in the discovery and development of flavonoids and their analogues as antitumor and anti-HIV agents. Adv. Exp. Med. Biol. 439, 191–225.

Wang, Z., Zong, Z., Li, S., Chen, B., 2002. [Nitrate accumulation in vegetables and its residual in vegetable fields]. Huan jing ke xue= Huanjing kexue 23, 79–83.

Weinberg, S.L., Abramowitz, S.K., 2008. Statistics using SPSS: An integrative approach. Cambridge University Press, Cambridge, UK.

Yaseen, A., Takácsné-Hájos, M., 2020. Study on moringa tree (Moringa oleifera lam.) leaf extract in organic vegetable production: A review. Res. Crop. 21, 402–414. https://doi.org/10.31830/2348-7542.2020.067.

Yaseen, A.A., Takácsné-Hájos, M., 2021. The Potential Role of Moringa Leaf Extract as Bio-Stimulant to Improve some Quality Parameters of Different Lettuce (Lactuca sativa L.) Genotypes. Sarhad J. Agric. 37, 1107–1119. https://doi.org/10.17582/journal.sja/2021/37.4.1107.1119.

Yasmeen, A., Basra, S.M.A., Farooq, M., Rehman, H.u., Hussain, N., Athar, H.u.R., 2013. Exogenous application of moringa leaf extract modulates the antioxidant enzyme system to improve wheat performance under saline conditions. Plant Growth Regul. 69 (3), 225–233. https://doi.org/10.1007/s10725-012-9764-5.

Zhang, X., Ervin, E.H., 2004. Cytokinin-containing seaweed and humic acid extracts associated with creeping bentgrass leaf cytokinins and drought resistance. Crop Sci. 44 (5), 1737–1745. https://doi.org/10.2135/cropsci2004.1737.

Zulfiqar, F., Casadesús, A., Brockman, H., Munné-Bosch, S., 2020. An overview of plant-based natural biostimulants for sustainable horticulture with a particular focus on moringa leaf extracts. Plant Sci. 295, 110194. https://doi.org/10.1016/j.plantsci.2019.110194.