Research Article

The Potential Role of Moringa Leaf Extract as Bio-Stimulant to Improve some Quality Parameters of Different Lettuce (*Lactuca sativa* L.) Genotypes

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Abstract | Improving plant quality and increasing the yield, using environmentally friendly products are essential for human health and environmental protection. This research was aimed to assess the possible role of moringa (*Moringa oleifera*) leaf extract (MLE) in improving some quality parameters of lettuce (*Lactuca sativa* L.) grown under plastic tunnel conditions. In this regard, three different lettuce varieties (May King, Kobak and Great Lakes) were grown in spring 2019 and 2020. The concentration of 6% MLE was sprayed as a biostimulant every two weeks from transplanting with the amount of 25 mL plant^{-1}. The results indicated that lettuce vegetation quality (head structure, head weight and root weight) was significantly improved by the use of 6% MLE, where their combination of MLE and plant variety has also improved some of the vegetation quality parameters. However, significantly the morphological quality parameters were least influenced by the plant variety alone. On the other hand, MLE did not significantly impact the bioactive compounds as total polyphenols and vitamin C. However, the treatment of 6% MLE significantly reduced nitrate content in the plant leaves of both experiments by 4% in 2019 and 20% in 2020. In both experiments, the significantly influenced factors on the bioactive compounds (total polyphenols and vitamin C) were mainly due to the plant varieties and the interaction of 6% MLE and plant varieties. In our experiments, physical parameters like relative chlorophyll content, normalized difference vegetation index (NDVI) were also evaluated. The results indicate that 6% MLE can have a potential role in improving vegetative quality parameters and reducing nitrate content in lettuce leaves, while bioactive compounds were most influenced by plant variety and the interaction of plant variety and foliar application with MLE.

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Introduction

Lettuce (*Lactuca sativa* L.) is the most popular cool-season grown fresh-cut vegetables in the world because it is now the main ingredient of fast food, especially dressing up hamburgers (Chiesa *et al*., 2004). Furthermore, there is an increase demand for fresh cut lettuce leaves in the salad bars and supermarkets. Lettuce is rich in vitamins (A, B, C and E), fibre, minerals as iron (Fe), calcium (Ca) and potassium (K) and very low calories (54-67 kJ), high amount of water above 95% (Kim *et al*., 2016). It plays an important role in the human diet because it contains very low fat, sodium (Na) and a valuable source of many bioactive compounds (phenol and flavonoid). Lettuce varieties are different for their nutrient content for example, romaine lettuce contains higher amount of iron, calcium, β-carotene than Crisphead lettuce (Mou and Ryder, 2002). Mattila and Hellström (2007) have confirmed higher soluble phenolic acids content in Pot-grown lettuce Lollo Rosso (*Lactuca sativa* var. *crispa*) 52 mg 100 g^{-1} than Iceberg (*Lactuca sativa* var. *capitata*) 5.1 mg 100 g^{-1}. Also, in red loose-leaf lettuce 'Red sails' variety (0.18–0.4 mg g^{-1} FW or 3.5–7.9 mg g^{-1} DW) and in green romaine 'Kalura' (0.03–0.1 mg g^{-1} FW or 0.6–2.0 mg g^{-1} DW), this is higher in cultivation in the open field than in high tunnel system due to high radiation and light intensity (Tsormpatsidis *et al*., 2008; Zhao *et al*., 2007). Flavonoid in lettuce is also varied depending on the variety and pigment content in the leaves which is mostly ranged from 2.8 mg g^{-1} FW or 56 mg g^{-1} DW in Lollo Rosso variety to 0.01 mg g^{-1} FW or 0.2 mg g^{-1} DW in green iceberg variety (Kim *et al*., 2016).

Many researchers have shown the importance of moringa leaf extract (MLE) as a plant hormone or growth promoter in improving plant growth and yield (Kim *et al*., 2016; Merwad, 2018; Phiri, 2010). This is because MLE contains many phytohormones like zeatin, indoleacetic acid (IAA), gibberellins and indole butyric acid (IBA) (Elzaawely *et al*., 2017; Rady and Mohamed, 2015), amino acid, ascorbate, minerals and beneficial compounds as plant growth promoters (Foild *et al*., 2001; Fuglie, 1999). Foliar spray with MLE can also improve quality and reduce nitrate accumulation in plant leaves. Based on the result by Elbagory (2018), nitrate accumulation (NO_3^-) in the lettuce leaves, grown in soilless culture reduced from 497.14 and 476.8 mg $plant^{-1}$ to 463.46 and 450.16 mg $plant^{-1}$ through the foliar spray of MLE and improved vegetative growth, chemical content and yield of the plant. A two-year experiment showed the highest yield from the combination of MLE with 100 kg ha^{-1} of NPK in *Solanium menlongena* at 9.5 t ha^{-1} in 2013 and 10.5 t ha^{-1} in 2013 (Ozobia, 2014). Culver *et al*. (2013) has demonstrated that the best result for growth and yield of beans planted in greenhouse and maize in open field was from spraying with MLE every two weeks from emergence until harvest. Plant height, leaf number, root and shoot dry matter, chlorophyll content, fresh pod yield and yield components increased in snap bean plants (*Phaseolus vulgaris* L.) treated with MLE at the concentration of 11, 20, 33 and 50% (Emongor, 2015). Moringa leaf extract improved the yield and growth of tomatoes grown in open field and greenhouse production (Hacişevki, 2009).

Lettuce is known for high ability to accumulate nitrate in the leaves which is accounted as a negative quality parameter (Santamaria, 2006). Having a high daily intake of nitrate more than the acceptable range of 0-3.7 mg kg^{-1} body weight (Santamaria, 2006) may lead to severe pathologies in humans (McKinney *et al*., 1999). So that, increasing the yield and reducing nitrate content using natural plant growth enhancer or growing low nitrate content varieties can be the solution for the human health to reduce the risk of some chronic diseases, which may come from the excessive daily vegetable consumption. Therefore, this study aimed to evaluate the use of moringa leaf extract (6%MLE) in improving the growth, yield and quality of different lettuce genotypes growing under plastic tunnel.

Materials and Methods

Experiment set up and design

This study was carried out under plastic tunnel in spring 2019 and 2020 at the University of Debrecen, Farm and Regional Research Institute in its Botanical and Exhibition Garden, Hungary. Seeds of three different lettuce varieties (*May King, Kobak and Great Lakes*) were sown in 200-cell trays. 35 days after sowing, the seedlings were transplanted to direct soil with the distance of 30 cm between two plants. The experiment was designed as randomized complete block (RCBD) with 3 replications (total of 20 plants per variety). Some important chemical and physical properties of soil was measured (Table 1).

Fertilizer application

Lettuce is known as a sensitive vegetable for the nitrogen fertilizer deficiency. Lack of nitrogen causes decrease in the growth, induces abnormality of the head and yellowing of the older leaves (Garcia *et al*., 1982). The plant requires a moderate amount of N fertilizer from transplanting to harvest. Thus, equal amount of N fertilizer (FERTICARE 24-8-16+microelements) was applied with the irrigation water to the plants 3 times after transplanting every 20 days interval with the amount of 225 kg ha^{-1} which is recommended by Hoque *et al*. (2010).

Preparation of moringa leaf extract (MLE)

The extract was prepared based on the method by

Makkar and Becker (1996) and developed by Culver *et al.* (2012), where fresh leaves (less than 40 days old) of moringa were collected from the young tree grown at the University of Debrecen, Farm and Regional Research Institute in its Botanical and Exhibition Garden, Hungary. After drying in a shaded area at room temperature (25-27 °C) for about 72 hours then it was grounded to powder form using a grinder. Afterwards, 20 g of the leaf powder was mixed with 225 mL of 80% ethanol and set aside for three hours then added another 225 mL of 80% ethanol continuously until we achieved 625 mL of the extract. After stirring the extract using homogenizer it was placed in 10 000 rpm ultra-turrax for 10 minutes then filtered using No. 2 Whatman filter paper. The extract was diluted with distilled water to the recommended concentration of 6% MLE by Thanaa *et al.* (2017) to spray directly onto the plant leaves using hand sprayer at the second week from transplanting with the amount of 250 mL m^{-2} or 25 mL $plant^{-1}$. Important chemical ingredients of the extract were determined as it is shown in (Table 2).

Table 1: *Some chemical and physical properties of soil.*

Soil sample	Value
pH value	7.18
Gold bound number	38
Total water-soluble salts (m/m) %	0.04
Carbonate Calcium $CaCO_3$ (m/m) %	1.62
Organic carbon (humus content) (%)	3.04
Phosphorus pentoxide P_4O_{10} (mg/kg)	314
Potassium oxide K_2O (mg/kg)	381
Nitrate NO_3^- (mg/kg) (KCL extract)	89.9
Sodium Na (mg/kg) (Al extract)	155
Magnesium Mg (mg/kg)	192
Sulphur S (mg/kg)	13.7
Manganese Mn (mg/kg) (EDTA extract)	31.4
Zinc Zn (mg/kg) (EDTA extract)	3.27
Copper Cu (mg/kg) (EDTA extract)	2.57

Source: *Agricultural Laboratory Centre, University of Debrecen.*

Morphological parameters

Parameters like head structure was measured based on the scale from 1 to10 where 1 is for loose and 10 is for hard head structure. Also, head weight and root weight (g $head^{-1}$) were measured by a digital scale, whereas head diameter and internal stem size was determined by the use of ruler (cm).

Table 2: *Chemical composition of Moringa oleifera leaf extract (MLE) per fresh weight (FW).*

MLE	**Value** (mg L^{-1})
Boron (B)	0,581
Phosphorus (P)	100
Iron (Fe)	2,24
Potassium (K)	350
Magnesium (Mg)	86,8
Sulphur (S)	267
Calcium (Ca)	326
Sodium (Na)	11,4
Cupper (Cu)	0,319
Zinc (Zn)	0,477

Source: *Agricultural Laboratory Centre, University of Debrecen. MLE: Moringa Leaf Extract.*

Total polyphenols, vitamin C and nitrate content

Total polyphenols in mg GAE 100 g^{-1} fresh product were determined by Folin Ciocalteu colorimetric method, where the results were given in *gallic acid* equivalent value (Singleton, *et al.*, 1999). Whereas, Vitamin C content was determined by redox titration using iodine solution following the method by Ciancaglini *et al.* (2001). 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 ($NO_3^- + 2e^-$ ® NO_2^-) to form diazo compound.

Correlation measurements and data analysis

To find the correlation between the chlorophyll content, plant vegetation growth and bioactive contents, relative chlorophyll content and normalized difference vegetation was determined the day before harvesting using SPAD-502 (Spectrum Technologies) and Greenseeker (handled crop sensor). These devices are used as non-destructive reading methods which are relying on the light intensity based on the used wavelengths approximately 650 nm: red LED for SPAD-502 and optical transmittance sensor (Optical Density Difference at two wavelengths: 650 nm and 940 nm) for the NDVI device. SPAD-502 is recommended as an accurate device for the non-destructive methods of measuring relative chlorophyll content (León *et al.*, 2007; Netto *et al.*, 2005), whereas they have reviewed the valuable performance of Greenseeker related to the nitrogen status and biomass of cereal crops (Bijay-Singh and Ali, 2020).

Table 3: *Effect of foliar application of MLE and plant varieties on vegetative parameters of lettuce during the spring seasons of 2019 and 2020.*

Varieties	Treat-ments	Vegetative parameters											
		Head structure (1….10)		Head weight (g)		Head diameter (cm)		Number of leaves		Size of internal stem (cm)		Root weight (g)	
		2019	2020	2019	**2020**	2019	2020	**2019**	2020	2019	2020	2019	**2020**
Interaction between MLE foliar application and plant variety													
May King	Control	5.83^{a}	6.33^{c}	293.70^{b}	219.33^{c}	24.00^{a}	28.66^{b}	33.00^{ab}	26.33^{d}	2.63^{ab}	2.66^{ab}	13.43^{bc}	12.03^{c}
	MLE	8.5^{a}	9.66^{a}	295.90^{b}	391.66^{a}	25.00^{a}	34.00^{a}	37.00^{a}	43.33^{b}	2.83^{ab}	2.33^{ab}	16.60^{a}	22.56^{a}
Kobak	Control	5.26^{a}	8.26^{ab}	296.10^{b}	242.00^{c}	25.33^{a}	29.66^{ab}	29.66^{b}	40.33^{b}	2.83^{ab}	4.00^{a}	12.63^{c}	16.33^{bc}
	MLE	8.36^{a}	9.66^{a}	296.26^{b}	307.03^{b}	25.00^{a}	27.33^{b}	31.33^{b}	50.33^{a}	3.26^{a}	3.66^{a}	14.26^{b}	19.63^{ab}
Great Lakes	Control	6.26^{a}	7.66^{bc}	295.90^{b}	307.03^{b}	29.00^{a}	27.66^{b}	19.66^{c}	21.00^{d}	2.56^{b}	1.36^{b}	13.50^{bc}	12.76^{c}
	MLE	8.86^{a}	8.66^{ab}	319.9^{3}a	330.43^{b}	25.33^{a}	27.33^{b}	19.33^{c}	35.33^{c}	2.53^{b}	4.33^{a}	14.20^{b}	19.63^{ab}
Foliar applications of MLE													
In the mean of varieties	Control	5.78	7.42	303.24	263.92	26.11	28.66	27.44	29.22	2.83	2.67	13.18	13.71
	MLE	8.57*	9.33*	296.02	335.24*	25.11	29.55	29.22	43.00*	2.87	3.44	15.02*	20.61*
Plant variety													
May King	In the mean of treat-ments	7.16^{a}	8.00^{a}	294.8^{b}	305.50^{a}	24.50^{a}	31.33^{a}	35.00^{a}	34.83^{b}	2.73^{b}	2.50^{b}	15.01^{a}	17.30^{a}
Kobak		6.81^{a}	8.10^{a}	296.1^{b}	274.51^{b}	25.16^{a}	28.50^{b}	30.50^{b}	45.33^{a}	3.28^{a}	2.85^{ab}	13.45^{b}	17.98^{a}
Great Lakes		7.56^{a}	8.96^{a}	307.9^{a}	318.73^{a}	27.16^{a}	27.50^{b}	19.50^{c}	28.16^{c}	2.55^{b}	3.83^{b}	13.85^{b}	16.20^{a}

Statistical analyses

The data were statistically analysed using IBM SPSS computer software version 25. The differences between treatment means were compared according to Tukey HSD^{a,b} Multiple Range Test at 5% level of probability. The independent sample t-test were also observed for some data comparisons in the treatments. Also, correlation and regression analysis was performed according to Weinberg and Abramowitz (2008).

Results and Discussion

Effect of foliar application of 6% MLE and plant variety on some vegetative parameters of lettuce

The data in Table 3 indicates the vegetative growth of lettuce under the influence of 6% MLE treatment and lettuce varieties in spring 2019 and 2020. In both seasons, the lettuce varieties reacted differently to the treatments. Significant differences in the interaction of plant varieties and treatments are found mostly in the spring season of 2020, where the greatest results were recorded for the varieties treated with 6% MLE compared to control.

Foliar spray with 6% MLE was the main factor influencing the vegetative quality parameters of lettuce. Except for the head diameter and size of internal stem, all the other vegetative parameters like head structure, head weight, number of leaves and root weight were significantly greater in the plants treated with 6% MLE. Similar results were stated by El-saady and Omar (2017) on lettuce, Rady and Mohamed (2015) on common beans and Culver *et al.* (2013) on tomatoes. The rapid improvement of internal stem size in lettuce is related to bolting which leads to decrease the plant quality and causes products unsaleable (Chen *et al.*, 2018).

Plant varieties, on the other hand, was the least influenced factor affecting the vegetative growth of the plant. In both seasons, the major and significant differences among the plant varieties were recorded in head weight, leaf number and size of internal stem. The average head weight varied between 219-391 g, which is similar to the results by Hoque *et al.* (2010) where they found that lettuce head weight ranged from 243-356 g grown under different fertilization and the result by Kopta *et al.* (2018) who they reported their average weight of leaf lettuce between 268 g head^{-1} and 415 g head^{-1} and 346 g head^{-1} as an average head weight by Esringu *et al.* (2015) research results. However, no significant difference was recorded for the head structure in both seasons, neither for the head diameter nor for root weight in 2020. This might be due to the temperature and light intensity

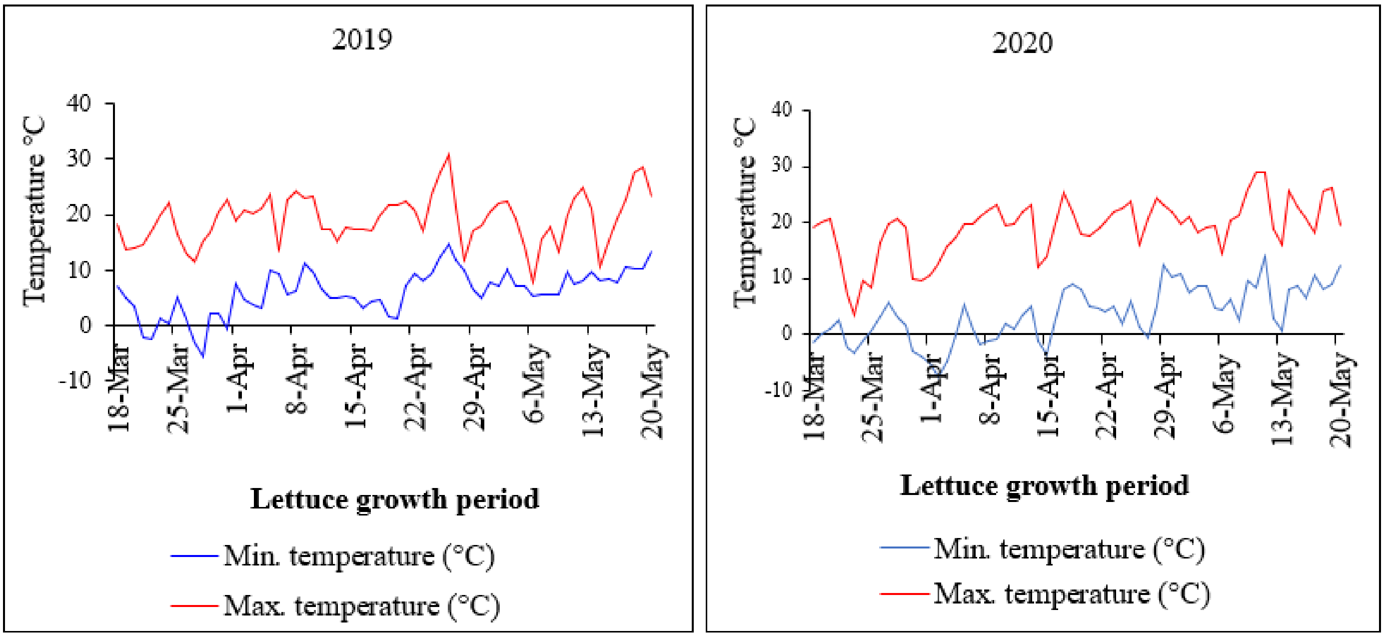


Figure 1: *Minimum and maximum temperature during the experiment period, under controlled conditions.*

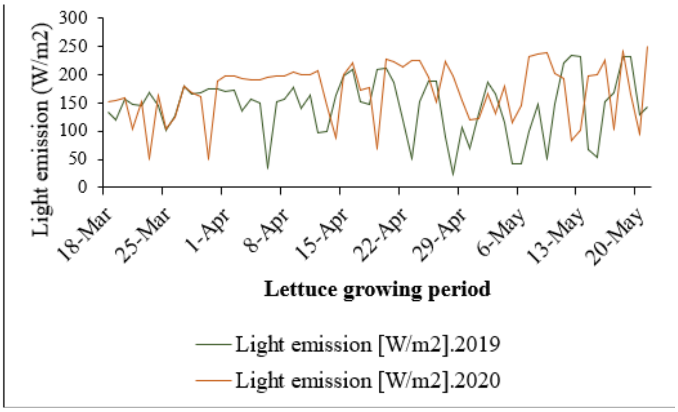


Figure 2: *Light intensity during the lettuce growing period in both 2019 and 2020.*

differences from a season to another (Figure 1 and 2). Lettuce varieties were different for their growth and physical appearance, this is mainly due to their generic factors (Rouphael *et al*., 2012), *Great Lakes* variety for example has less leaf number and darker green colour of leaves than other varieties, while *May King* and *Kobak* varieties have quite similar formations (Figure 3).

The major influence of the morphological parameters was found in the interaction of plant variety and foliar application with 6% MLE in 2020. This might be because the temperature and light emission was less fluctuated during the head formation of the plant compared to the season of 2019 (Figure 1 and 2), besides the genetic and agronomic factors are the main factors influencing the lettuce vegetation growth (Silva *et al*., 1999). No significant differences were recorded for the head structure in the experiment of Spring 2019; but greater head structure was recorded in all the varieties treated with 6% MLE compared to control. Though, all three varieties treated with 6% MLE had significantly higher head structure in the experiment of 2020 at 9.66, 9.66 and 8.66 (on the scale of 1……10), where 1 is for loose head and 10 is for hard head structure. The head weight varied between the plant genotypes, in 2019 the marketable head weight was recorded in the interaction of *Great Lakes* variety with 6% MLE at 319.93 g head $^{-1}$, while in 2020 the significantly greater head weight was in the interaction between *May King* and 6%MLE at 391.66 g head $^{-1}$. The only significant difference in the head diameter was in the interaction of *May King* variety sprayed with 6% MLE in 2020 at 34.00 cm. Similar results were recorded for both experiments of 2019 and 2020 on the leaf number, internal stem size cm and root weight g plant^{-1}. However, amongst the varieties the lowest internal stem size was measured in the *Great Lakes* variety of control treatment in 2020 at 1.36 cm followed by 2.53 cm in the interaction of *Great Lakes* variety with 6% MLE. This might be because *Great Lakes* variety is a day-neutral variety for the bolting or flowering requirements (Ryder, 1979).

The effect of foliar application (MLE) and lettuce varieties on the total polyphenol, vitamin C and nitrate content

Lettuce is considered as the most important green vegetable in the modern human healthy diet program. The plant is rich in phytochemical compounds as polyphenols, flavonoids and vitamin C which is playing a vital role in the human health because it is

Table 4: *Effect of foliar application of MLE and plant varieties on total polyphenol, vitamin C and nitrate content of lettuce during the spring seasons of 2019 and 2020.*

Varieties	Treatments	Leaf contents					
		Total polyphenol (mg GAE/100g product FW)		Vitamin C (mg/100g product FW)		Nitrate (mg kg^{-1})	
		2019	2020	2019	2020	2019	2020
Interaction between MLE foliar application and plant variety							
May King	Control	33.50^{a}	30.69^{cd}	1.88^{a}	1.86^{abc}	466.00^{ab}	560.00^{c}
	MLE**	27.90^{b}	34.84^{b}	2.65^{a}	2.04^{a}	461.00^{b}	433.89^{e}
Kobak	Control	32.50^{a}	33.01^{bc}	2.41^{a}	1.72^{c}	492.00^{a}	653.69^{a}
	MLE**	25.30^{b}	30.03^{d}	1.60^{a}	1.69^{c}	457.00^{b}	586.66^{b}
Great Lakes	Control	33.60^{a}	39.36^{a}	2.63^{a}	1.96^{ab}	485.00^{ab}	481.28^{d}
	MLE**	26.40^{b}	35.30^{b}	1.73^{a}	1.81^{bc}	476.00^{ab}	337.53^{f}
Foliar applications of MLE							
In the mean of varieties	Control	31.00	32.84	2.31	1.87	481.00*	588.32*
	MLE**	28.43	34.89	1.98	1.82	463.77	452.69
Plant variety							
May King	In the mean of treatments	30.00^{a}	32.76^{b}	2.26^{a}	1.95^{a}	474.50^{ab}	496.94^{b}
Kobak		28.90^{a}	31.52^{b}	2.00^{a}	1.70^{b}	480.50^{a}	620.18^{a}
Great Lakes		30.70^{a}	37.33^{a}	2.18^{a}	1.88^{a}	463.50^{b}	399.40^{c}

*Means within the same column followed by the same letter(s) are not significantly different at the probability level of ($p \leq 0.05$) according to Tukey HSD[a,b] Multiple Range Test.
*Means within the same column followed by symbol (*) are significantly different at the probability level of ($p \leq 0.05$) according to the independent sample T-Test.
**MLE= Moringa Leaf Extract.

needed for biosynthesis of collagen hormone activities as well as having a therapeutic potential in cancer and heart disease (Dillard and Bruce German, 2000; Li and Schellhorn, 2007). Polyphenol is the compound which is largely found in the lettuce leaves in different forms as chlorogenic acid, 3-O-glucoside of kaempferol, quercetin-3-O-glucoside, di caffeoyl tartaric acid (Rouphael *et al*., 2012). The growing environment and genetic factors are the two main factors, which can significantly influence the content level of the bioactive compounds in lettuce plant (Riga *et al*., 2019; Sytar *et al*., 2018).

The results in the Table 4 show that significantly the most influenced factors affecting the total polyphenols, vitamin C and the nitrate, is the combination of the plant varieties and 6% MLE treatment, especially for the 2020 experiment. In general, except for the *May King* variety in 2020 experiment, the interaction of plant varieties and MLE has reduced the total polyphenol and vitamin C as well as nitrate content in the lettuce leaves (Table 4). Significantly the highest total polyphenols were found in the control treatment of *Great Lakes* variety in both years 2019 and 2020 at 33.60 and 39.36 mg GAE 100g^{-1} product, respectively; whereas the lowest total polyphenol was recorded in the *Kobak* variety treated with MLE at 25.30 and 30.03 mg GAE 100g^{-1} product for 2019 and 2020, respectively. This reduction might be because MLE is known as an effective bio-pesticide against aphids (Shah *et al*., 2017; Yaseen and Hájos, 2020), so that polyphenols are usually increased under stress conditions (Boo *et al*., 2011) where these plants were found much healthier in these two experiments.

Vitamin C was the least influenced parameter by the variety and treatment of 6% MLE. In the 2019 experiment, there were no significant difference for the interaction of plant varieties and MLE treatment, where the highest vitamin C was found in the *May King* variety treated with 6% MLE at 2.65 mg 100g^{-1} product and the lowest was in the *Kobak* treated with 6% MLE (1.60 mg 100g^{-1} product). This might be because of the plant genotypes, where (Mou and Ryder, 2002) demonstrated that plants with opened head structure contain higher vitamin C than closed heads, thus *May King* variety is a leafy lettuce and have much opened head structure than *Kobak* and *Great Lakes*

Figure 3: *Lettuce varieties (a. May King. B. Kobak and c. Great Lakes) of the experiment.*

varieties. Martínez-Sánchez *et al*. (2008) have shown that same species of baby leaf lettuce vegetables are different in their antioxidant compounds, vitamins and phenols. Some significant differences were recorded in the 2020 experiment. Similar to the results in 2019, the *May King* variety treated with 6% MLE recorded significantly the greatest vitamin C content of 2.04 mg $100g^{-1}$ product, where again significantly the lowest vitamin C content was in the *Kobak* variety in both treated with MLE and control at 1.72 and 1.69 mg $100g^{-1}$ product, respectively.

Lettuce is known for extreme ability to accumulate nitrate in their leaves. The interaction of genotype (G) × environment (E) is crucial to be clarified while evaluating nitrate accumulation in the lettuce plant because it is a complex trait (Burns *et al*., 2011). The accumulation of nitrate is typically due to the low light intensity, plant genotypes and in response to N supply (Burns *et al*., 2011; Escobar-Gutierrez *et al*., 2016). The reduction of nitrate level through plant genotype cultivation has long being practiced especially in the countries with low light intensity as in the UK. However, in the very early days plant biostimulants are recognized an efficient tool to enhance yield, increase nutrient uptake and improve quality by reducing unwanted substance content in plant leaves as nitrate and heavy metals (Colla and Rouphael, 2015; Rouphael and Colla, 2018). It is important to reduce the nitrate level through agronomic practices using plant bio stimulants since this will take less time, results faster and can be practiced everywhere. The results in Table 4 indicates the great performance of the interaction of lettuce varieties and 6% MLE treatment on the nitrate reduction in the plant leaves. In both experiments (2019 and 2020), the lowest nitrate content was found in the varieties treated with 6% MLE and the highest level was in the untreated plants in all three varieties. *Kobak* variety, for example, recorded significantly lower nitrate in the plants treated with 6% MLE in 2019 at 457.00 mg kg^{-1}, whereas in 2020 *Great Lakes* variety treated with 6% MLE recorded significantly lowest nitrate level in the leaves at 337.53 mg kg^{1}.

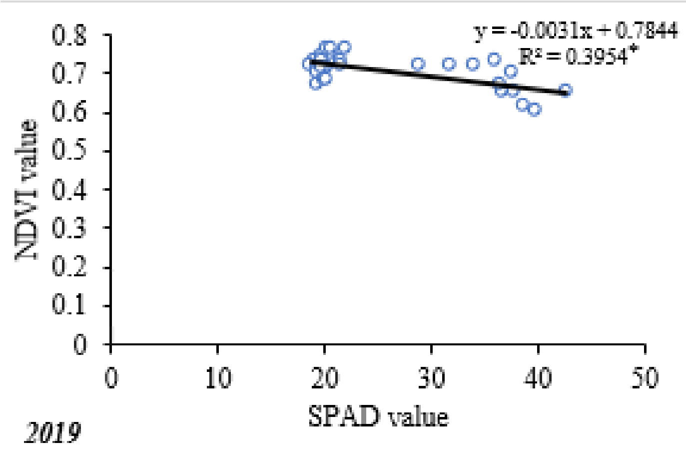
Total polyphenols and vitamin C were mostly influenced by plant varieties and environmental factors rather than the foliar application with MLE (Table 4). In 2020, *Great Lakes* variety recorded significantly higher total polyphenol content compared to the other two varieties at 37.33 mg GAE $100g^{-1}$ product, whereas no significant difference was measured among them for 2019, again the highest polyphenol was found in the same variety at30.70 mg GAE $100g^{-1}$ product. Genetic factor is important to determine the polyphenol and flavonoid content in the leaves (Mou, 2005; Pernice *et al*., 2007; Yang *et al*., 2018).

The only significant difference for the vitamin C (ascorbic acid) content was in 2020 where the lowest amount was found in the *Kobak* variety at 1.70 mg $100g^{-1}$ product, conversely significantly higher vitamin C was detected in the *May King* variety at 2.26 and 1.95 mg $100g^{-1}$ product for the 2019 and 2020, respectively (Table 4).

Table 4 shows the genotypes differ in their accumulation of nitrate in their leaves. The lowest nitrate content was found in the *Great Lakes* variety at 463.50 and 399.40 mg kg^{-1} in 2019 and 2020 experiments, respectively; while the highest content was found in the *Kobak* variety at 480.50 and 620.18 mg kg^{-1} for 2019 and 2020, respectively. These studies were carried out at the same time of the year (from 18 March to 20 May), so that this proves that the combination of plant genotype with the plant bio stimulant was amongst the strongest factor affecting the nitrate content in the plant leaves. The major reduction of nitrate content was found in the experiment of 2020 in the combination of foliar application of 6% MLE and *Great Lakes* variety at 43% (avg.337.53 mg kg^{-1}) lower, as compared to control (avg. 481. 28 mg kg^{-1}). In the mean of varieties, a significant reduction of nitrate level was recorded in both years by 4% in 2019 and 20% in 2020. Similar results were found by Cozzolino *et al*. (2020) where they demonstrate that lettuce foliar sprayed with biostimulants of a tropical plant extract (PE) could reduce the nitrate content in the leaves by 23%. This finding might be related to high level of the amino acid content in the plant extract which may inhibit the root system to uptake the nitric ion in the soil while absorbing by the plant leaves. Many studies confirm our results such as on iceberg lettuce by (Bulgari *et al*., 2015), spinach by (Kunicki *et al*., 2010), greenhouse tomato quality evaluation by (Colla *et al*., 2017).

Correlation of relative chlorophyll content (SPAD) and normalized vegetation index (NDVI) to vegetation growth of lettuce

Significant positive correlation ($p \leq 0.05$) was observed in the experiment of 2019 and 2020 for the SPAD



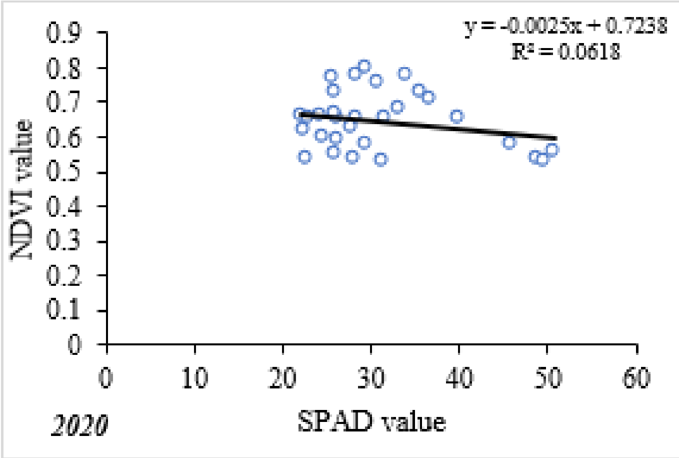


Figure 4: *Regression equation results of the studied data for SPAD vs NDVI value for 2019 and 2020.*

Table 5: *Correlation between vegetative parameters and SPAD, NDVI measurements.*

	Years	Head structure	Head weight	Head diameter	No. of leaves	Internal stem size	Root weight	SPAD	NDVI
Head structure	2019	1.000							
	2020	1.000							
Head weight	2019	0.087	1.000						
	2020	0.681**	1.000						
Head diameter	2019	0.126	0.636***	1.000					
	2020	0.147	0.363	1.000					
No. of leaves	2019	-0.030	-0.464	-0.342	1.000				
	2020	0.693**	0.231	0.249	1.000				
Internal stem size	2019	0.072	-0.057	0.165	0.360	1.000			
	2020	0.189	-0.307	-.251	0.532*	1.000			
Root weight	2019	0.506*	-0.050	-0.135	0.417	-0.044	1.000		
	2020	0.777**	0.598**	0.322	0.749**	0.207	1.000		
SPAD	2019	0.268	0.053	0.496*	-0.788**	-0.635**	-.004	1.000	
	2020	0.370	0.118	0.517*	-0.824**	-0.496*	-.0433	1.000	
NDVI	2019	0.052	0.274	0.483*	0.560*	0.649**	0.461	-0.630**	1.000
	2020	0.573*	0.685**	0.148	0.253	0.057	0.686 **	-0.069	1.000

***Correlation is significant at the 0.01 level (2-tailed); *Correlation is significant at the 0.05 level (2-tailed).*

value and head diameter at 0.496 and 0.517, whereas negative was recorded for the leaf number and internal stem size at (-)0.788, (-)0824 at $p \leq 0.01$ and internal stem size of (-)0.635 at $p \leq 0.01$ and (-)0.496 at $p \leq 0.05$ for 2019 and 2020, respectively. This result demonstrates that plants with higher relative chlorophyll content can have a better physical parameter regarding to the growth performance and delaying flowering or bolting. This might be due to the activation of the Gibberellins (GAs) hormones especially GA2-oxidase gene in the plant, because this leads to the plants inhibits elongation of hypocotyl and stem, improving chlorophyll content and delaying flowering (Yan *et al*., 2017). However, the negative correlation between the relative chlorophyll and leaf number might be due to the genetic variation of the lettuce varieties, for example *Great Lakes* variety is greener and naturally produces lower leaf number in comparing to *May King* and *Kobak* varieties (Figure 3).

However, the significant positive and negative correlation of NDVI was varied based on the parameters and the growing seasons. Significantly a positive correlation in the season of 2020 at $p \leq 0.05$ was recorded for the NDVI and head structure at 0.573, ($p \leq 0.05$), head weight 0.685 at ($p \leq 0.05$) and root weight 0.686 at ($p \leq 0.01$), this might be because plants with greater head structure have more surface to penetrate the sunlight for the photosynthesis metabolism (Kopta *et al*., 2018) and produce more nutritional value to improve other vegetative parts (Mou and Ryder, 2002). Whereas in the season of 2019, significantly positive correlation was recorded for the head diameter, number of leaves and internal stem size 0.483 at ($p \leq 0.01$), 0.560 at ($p \leq 0.05$) and 0.649 at ($p \leq 0.01$), respectively (Table 5). This shows that in some vegetative parameters there is a negative correlation between SPAD and NDVI as it is shown in Table 5.

A stronger negative regression equation was found between the SPAD and NDVI in the experiment of 2019 at R^2 equal to 0.395, also similar results was recorded in the experiment of 2020 at R^2 equal to 0.06 (Figure 4). Therefore, both measurements have to be considered while measuring physical parameters in the lettuce plant.

Conclusions and Recommendations

Based on these results, plant biostimulant (MLE) could improve vegetative parameters and quality by reducing the nitrate level of different lettuce varieties. However, this did not significantly influence the bioactive compounds as total polyphenols and vitamin C. Also, the interaction of plant variety and 6% MLE could significantly improve the morphological parameters rather than bioactive compounds and reduced 16% of nitrate level in the experiment of 2020. Concerning the environmental factors as light intensity and temperature fluctuation during the growing season of the plants, use of moringa leaf extract as a natural plant biostimulant can play an important role in improving quality parameters in leafy vegetables like lettuce.

Novelty Statement

The novelty of our research is to assess the influence of moringa leaf extract on the leafy vegetable (lettuce) to find out the alternative material for the chemical resources in organic vegetable production and quality improvement.

Author's Contribution

This research is part of the Ph. D. study of Arshad Abdulkhalq Yaseen. The technical support is by Agrar Campus at the University of Debrecen under the supervision of Mária Takácsné Hájos, CSc.

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