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Head of Doctoral School:

Professor Dr. Holb Imre

University professor, doctor of the Hungarian Academy of Sciences

Supervisor (s):

Dr. Mária Takács-Hájos

Associate professor, CSc

EFFECT OF SOME NATURAL EXTRACTS ON DIFFERENT QUALITY PARAMETERS OF LETTUCE (*Lactuca sativa* L.) VARIETIES

by:

Arshad Abdulkhalq Yaseen

doctoral candidate

Debrecen

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Written by Arshad Abdulkhalq Yaseen, certified MSc Horticulture (Crop production)

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Dissertation supervisor:
Dr. Mária Takács-Hájos, associate professor (CSc)

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Members:	Dr.............................	....	....
	Dr.............................	....	....
	Dr.............................	....	....
	Dr.............................	....	....

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1.0. INTRODUCTION

Lettuce (*Lactuca sativa* L.) is among the most common leafy vegetable in the family of (*Asteraceae*), the largest plant family with 23,000 to 30,000 spices (Bayer and Starr, 1998). Lettuce is self-fertile diploids with 2n = 2x = 18 chromosomes. It is a cool-season vegetable which is cultivated in all continents, particularly in temperate and subtropical regions. Lettuce is widely grown for commercial purposes all over the world, despite it is produced in home gardens (Rubatzky and Yamaguchi, 1997), too. The annual lettuce and chicory production in 2019 was around 29.13 million metric tons, in Asia producing the most (nearly 19.6 million tons), followed by America (4.91 million tons), Europe (3.86 million tons), Africa (537.3 thousand tons), and Australia (173.6 thousand tons) (FAOstat, 2021).

Spain (961.94 thousand tones), Turkey (520.0 thousand tones), Italy (487.02 thousand tones), Germany (219.03 thousand tones), and France (214.49 thousand tones) led the European Union in lettuce production in 2020, while Hungary produced just 7.80 thousand tones (Eurostat, 2021). Hungary has dropped lettuce output by 76% since 2013. The total production in 2013 was 13.69 thousand tons, but only 7.80 thousand tons of lettuce were produced in 2020.

The plant is dating back to long cultivation historical background. The signs on Egyptian tombs, according to Lindqvist (1960a), indicate that it was grown by ancient Egypt around 4500 BC. It is a leafy annual vegetable which is one of the most popular salad veggies. It grows well in temperate and tropic, and sub-tropic regions of the world. The ideal temperature for the cultivation of lettuce is 18-25°C and the night temperature is 10-15°C (Lindqvist, 1960; Ryder, 1998). The plant is the most consumed vegetable in the human diet (Kim, et al., 2016). The nutritious value of lettuce is not appreciated since the plant is rich in water (around 95%) but low in calories (Kim, et al., 2016). Lettuce is commonly consumed raw due to its high vitamin (B_9, C and E), polyphenol, flavonoids, carotenoids, and mineral content (Mou, 2009, 2012a).

The human body needs an appropriate daily intake of vitamins, micro-macro nutrients from a variety of sources, both animal and plant, on a regular basis. As a result, researchers are now interested in research studies about vitamin fortification and nutritional value in plants. Thus, numerous technological advancements have been proposed in recent decades to increase agricultural production system sustainability by drastically lowering synthetic agrochemicals such as pesticides and fertilizers.

Plant biostimulants are natural and environmentally friendly innovation that improves flowering, plant growth, fruit development, crop yield, and nutrient utilization efficiency, as well as resistance to a wide range of abiotic stresses (Rouphael and Colla, 2020).

Because lettuce is a short and cool season plant growth habit, it requires a sufficient amount of fertilizer. However, it is apparent that over fertilization caused a variety of environmental problems, such as water quality through 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 especially due to nitrate accumulation in the plant leaves (Ikemoto et al., 2002; Liu et al., 2014). As a result, in order to reduce the environmental and human health impact of chemical fertilizers, biostimulants can be used as a supplementary fertilizer to improve plant quality and reduce the amount of chemical fertilizers required in plant production while also improving the plant's ability to absorb and translocate nutrients (Pascale et al., 2017; Zodape et al., 2011). To do this, three plant extracts, moringa leaf extract (MLE), willow bark extract (W) which are home-made extracts, and a manufactured extract Bistep (B), as well as the interaction of Willow and Bistep (W+B), are administered to three lettuce cultivars, "May King," "Kobak," and "Great Lakes." Biostimulants, depending on their content and composition, can be administered to plants at low quantities to the soil or the leaves (Kunicki et al., 2010).

Moringa leaf extract (MLE) is a recently discovered plant extract that is applied to plants as an environmentally friendly and safe biostimulant due to its high concentration of useful bioactive components, macro and micronutrients, minerals, plant hormones, essential amino acids, and vitamins (Rady et al., 2013; Sohaimy et al., 2015; Yaseen and Takácsné-Hájos, 2020). Moringa leaf extract (MLE) has been used to increase yield and quality in a variety of plants, including green beans (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) and peppers (Aluko, 2016). However, there is relatively little study on the effect of moringa leaf extract (MLE) on the quality and growth of various lettuce cultivars.

Willow bark extract (W), on the other hand, was recently certified for agricultural applications by the European Union (EU) pesticide legislation, European Council

Regulation (EC) No 1107/2009, as a basic component having fungicidal property (Deniau et al., 2019; Marchand, 2016). This extract is typically made from one-year-old weeping willow branches and/or willow leaves (*Salix babylonica*) (Mutlu-Durak and Kutman, 2021). The preparation techniques are detailed in depth in the following sections. Willow bark extract (W) has been applied to treat a variety of plants, mostly those suffering from fungal infections. However, there have been few or very limited studies on the influence of Willow bark extract on nutrient absorption and quality improvement in lettuce.

Bistep is manufactured humic acid that is produced as an organic material to promote plant development, shorten vegetative duration, and aid in nutrient absorption by plants by increasing nutrient availability and improving soil structure (Cimrin and Yilmaz, 2005). So far, research on the influence of willow bark extracts on plants has mostly concentrated on root-enhancing and fungicidal qualities. As a fundamental component in plant nutrient absorption, Willow bark extract has never been proved to exhibit biostimulant activities. Furthermore, there has been minimal research on nutrient absorption and ion ratio in lettuce plants in relation to Bistep humic acid or the combination of two plant biostimulants.

The purpose of these experiments was to determine the effect of a manufactured biostimulant (Bistep) and a home-made basic substance (Willow), Moringa leaf extract (MLE), as well as the interaction of Willow + Bistep (W+B), on some quality parameters in different lettuce cultivars. We also aimed to investigate the impact of Moringa leaf extract (MLE) on the nitrate accumulation in lettuce leaves, resulted by reduce light intensity mainly in autumn season. Willow bark extract (W) is known as a biofungicide; however, its influence on physical quality parameters in lettuce has not yet been demonstrated. As a result, we wanted to test the effect on various lettuce varieties. The Bistep (B), which contains humic acid, but little information is available on how to combine it with other biostimulants such as (Willow). We wanted to demonstrate the effect of combination Bistep (B) and Willow bark extract (W) on nutrient uptake and bioactive compounds in lettuce. Finally, we try to investigate the interaction of genetic and climate factors on the evaluation of physical parameters, bioactive compounds, and mineral contents for successful lettuce production.

2.0. LITERATURE REVIEW

2.1. Origin and distribution of lettuce

Lettuce has a long historical background; the plant was domesticated from the wild spices *Lactuca serriola* L.; however, only three of the hundred spices (*L. serriola* L., *L. saligna* L., and *L. virosa* L.) may be crossed (Mou, 2011). Having wild lettuce spices around Tigris and Euphrates rivers proofs that the origin of this vegetable is West Asia-the Middle east-Iraq and Iran (Zohary, 1991). Also, according to the breeding tests and gene mapping, the genus *L. sativa* fully attaches and interfertile with wild *L. serriola*; only partially similarity with other spices like *L. saligna*; and cross sterile with *L. virosa*, this is a strong evidence to show south East Asia is the centre for the diversity of wild *L. sativa* (Zohary, 1991). On the other hand, having images on the Egyptian tombs shows the lettuce origin and farming date back to the 4500BC by the Egyptian empires where they used for either extracting oil from the seeds or feeding animals (Lindqvist, 1960).

2.2. Lettuce classification in related to the quality parameters

Lettuce quality standards is varied based on the growth nature of the plant (head or leafy) and variety. The common scientific name of lettuce is *Lactuca sativa* L. which comes from the word ‘milk forming’ *Lactuca* means ‘milk’ and *sativa* means ‘common’ this is because of the creamy substance in the lettuce stem (Weaver, 1997). The main consumed part of lettuce is the leaves. In this case, lettuce leaves representing species polymorphic characteristic, so that the marketable classification of lettuce is mainly based on the leaf formation and colour. Lettuce leaves are smooth, crumpled and savoy (Rubatzky and Yamaguchi, 1997). The colour and form of the leaves differ from one variety to another, as well as between species (Figure 1). The colour of lettuce leaves ranges from green (pale or dark) to red-purple. The leaf shape of cultivars produce head forming as in iceberg lettuce which is generally spherical, whilst those producing non-head forming as in Romaine or cos lettuce have lengthy dark green leaves. The most popular lettuce types is Romaine and iceberg, however there are other varieties which are different in texture, colour and flavour (Kim, et al., 2016). Because lettuce types have appealing forms and colours, lettuce leaves are increasingly consumed not just for their nutritional content but also to make dishes and salad plates more delicious, fresh, and colourful.

Figure 1. Types of lettuce leaves
Source: Government of western Australia (2020)

Botanically, lettuce is classified as follows: USDA (2019)

Family: *Asteraceae*

Genus: *Lactuca*

Species: *Lactuca sativa*

Marketable classification of lettuce is divided into two main groups:

1. Head forming lettuce
2. Non-head forming or leafy lettuce Ciju (2019)

Based on these two main groups, lettuce is divided into four main popular types:

- Crisp-head often referred to Iceberg
- Butterhead lettuce as Boston and Bibb lettuces
- Cos or Romaine lettuce, with elongated, crispy textured leaves

- Loose leaf or bunching, with large variation in leaf size, shape, colour, and texture as Royal Oak Leaf, and Red Deer Tongue varieties (Kiple and Ornelas, 2000)

Many kinds of lettuce are available in markets; the most popular varieties are seen in Figure 2, and they are classified into several major categories based on morphological features such as form, colour, and size (Kim, et al., 2016). Lettuce have been grouped into seven main types including:

1. Crisphead lettuce (*Lactuca sativa* var. capitata L. *nidus jaggeri*)
2. Butterhead lettuce (*Lactuca sativa* var. capitata L. *nidus tenerrima*)
3. Romaine or cos lettuce (*Lactuca sativa* var. *longifolia* Lam., var. *romana*)
4. Leaf or cutting lettuce (*Lactuca sativa* var. *acephala* Alef., syn. var. *secalina* Alef., *syn*. var. *crispa* L.) (Mou, 2008; De Vries, 1997; de Vries and van Raamsdonk, 1994).

2.3. Environmental requirements

Lettuce is commonly grown in the early spring and late autumn when the light intensity is low and the air temperature is cool. However, in certain places, it is cultivated and harvested throughout the year, either grown in open fields or in greenhouses (Default et al., 2006). The ideal temperature ranges between 7/16 °C to 21 °C night/day (Jayalath et al., 2017; Sanders, 2001). The plant can be grown in different soil types; however, it is well improved in moist, well-drained loose and light soil with a pH between 6.0 and 7.0 (Walker, 2019). Unlike other vegetables, lettuce can withstand a light frost and moderate shade. Lettuce is the most popular produced leafy crop in a hydroponic system. Because of its rapid growth and short life cycle, the plant requires a high quantity of nourishment, particularly nitrogen, which must be supplied on a weekly basis. The plant grows well in greenhouse and open field. Light intensity is an important environmental factor that influences plant development and production. The ideal light intensity for early autumn and late spring production is between 500-600 µmol m^{-2} s^{-1}, but the optimal shade light value for winter greenhouse lettuce production is 400-600 µmol m^{-2} s^{-1} (Fu et al., 2012).

In many situations, growing lettuce in greater or lower optimal weather conditions causes certain diseases or abiotic stress such as tipburn, which results in calcium shortage in the top meristem tissues (Frantz et al., 2004). When the temperature rises over 24 °C, the plant loses its quality through bolting or seed stalk, bitterness, and tipburn (Jenni, 2005; Nuez and Prohens, 2008; Ryder, 1999).

Figure 2. Types of lettuce
Source: Author's own, Debrecen (2019)

2.4. Factors affecting quality, yield and growth of lettuce

Lettuce is influenced by preharvest genetic variables, production technology (irrigation and fertilization), growing conditions (open field or greenhouse), physiological maturity stage, and postharvest procedures, as well as their interactions (Chiesa et al., 2009; Kader, 2001). Understanding the variables that influence fresh products is critical to enhance product quality and extend shelf life (Toivonen and Brummell, 2008). In terms of fresh product quality, both pre- and post-harvest activities are intertwined, and each can impact the other (Arah et al., 2015). The improvement of fresh product quality is a major global focus (Kopta et al., 2018). Consumers judge lettuce quality mostly by its appearance, which must be brightened in colour, clean, and devoid of browning, bug, rot, and mechanical damage in order to be purchased and counted as the best quality (Kader, 2013; Mampholo et al., 2016a; Watkins and Nock, 2012).

2.4.1. Genetic factors

Lettuce is a member of the most genetically diverse families, having 23,000 to 30,000 species (Bayer and Starr, 1998). The genetic component is the most important factor in determining the type of the plant's development and contents. Plants of the same variety have varied physiological and morphological characteristics. The “Great Lakes” cultivar, for example, is naturally greener in colour, accumulate more nitrate content, and produce fewer leaves than other kinds such as “May King” and “Kobak” (Yaseen and Takácsné-Hájos, 2021a). Some plants or even spices are more susceptible or resistant to some types of disease and stress, for example, head lettuce is more resistant for tip-burn disease than Romaine lettuce (Richard, 2016).

2.4.2. Growing methods

There are several factors can influence vegetable quality during the growing season. Preharvest variables involve a variety of parameters ranging from genetic materials to physiological maturity at harvest (María et al., 2008). Many breeding programs aim to produce better and improve postharvest technology proficiency (Nicola et al., 2009). The cultivar selection is increasingly important for physiological activities like enzymatic browning in fresh cut leaf lettuce tissues (Gil, 2016). García et al. (2005) have presented that some lettuce cultivars start producing jasmonic acid just after cutting which can induce browning and loose of fresh leaf quality.

Handling is the actual activity that occurs between preharvest and postharvest of fresh products, beginning with the last step of preharvest and ending with the first stage of postharvest procedures. Vegetable quality is determined after harvest, thus it is critical to retain the quality until it reaches the consumers (Bachmann and Earles, 2000). Because damaged or bruised fresh products are more sensitive to postharvest infection than the consequences of quality losses, handling is the most essential aspect in preserving fruits and vegetables from mechanical injury -scrapes, bruises, abrasions (Bachmann and Earles, 2000; de Oliveira et al., 2013). Vegetable quality deteriorates when plant tissue is injured during harvesting process, which promotes physiological and biochemical activity in the damaged tissues and shortens the product's shelf life (Hruschka, 1971; Kaufman and Lutze, 1954). Another handling technique to consider is picking or harvesting the product at the appropriate time. In addition, overmature or immature fresh products (Romaine lettuce) have a lower storage duration (shelf life) than properly mature harvested product (Tudela et al., 2013; Wilson et al., 1995).

2.4.2.1. Greenhouse lettuce production

Greenhouses are basically built to provide a suitable climate or climate protection for crops grown in severe weather conditions and to enable season extension to many crops (Lamont et al., 2002; Reeve and Drost, 2012). The world's ever-growing human population and increasing demand for food have compelled it to consider new methods and technologies for food production. Climate change and the loss of agricultural land have put the world's food supply in jeopardy. As a result, crop output for environmental preservation is rising year after year. Indoor farming is the only viable option for increasing food production for the future. Greenhouse plant production has been increasingly essential and widespread over the previous few decades across all continents. Thus, Asia, Europe, and North America have expanded indoor farming by 42%, 30%, and 21%, respectively (Pennisi et al., 2019). Other methods, such as a hydroponic system, which is practiced in greenhouse system, are now available for growing vegetables. Lettuce is among the most common leafy vegetable which is cultivated in this method. Vertical farming has become popular in recent years as a method of producing vegetables in soilless culture inside with artificial lighting, thus lettuce is the leading vegetable grown in this technic (Voutsinos et al., 2021).

2.4.2.2. Open-field lettuce production

In many countries, lettuce is known as a year-round harvested vegetable; however, this varies depending on the zones and places. In some regions, for example, if the environment is unfavourable throughout the early spring and summer months, the plant is at risk of tip burn, bolting, and leaf bitterness (Simonne et al., 2002; Zhao and Carey, 2009). The day/night temperature is the main factor affecting the quality and yield of lettuce. The optimal temperature for lettuce growing is 18.5 °C, above this temperature flower initiation occurs between 21 and 27 °C this will decrease the marketable quality and yield (Maynard and Hochmuth, 1997; Wallace et al., 2012).
It is obvious that the production system in open fields is more difficult to regulate and more prone to contamination since these fields are exposed to many contamination sources. The risks provided by livestock and wild animals vary according to the prevalence, incidence, and quantity of pathogen carriage in the animal hosts, as well as the level of interaction between the animals and the lettuce crop production field. Farmers nowadays choose to produce lettuce under high plastic houses rather than open fields due to a variety of environment factors such as high temperatures, hail, wind, and blowing dust (Wallace et al., 2012).

2.4.3. Environmental factors

In general, environmental variables are divided into two categories: biotic factors (Cultivars, disease, insects, and any living creature) which are the most influential in crop loss and damage, and abiotic factors, as water stress, nutrient deficiency, cold, chilling, freezing, high light intensity, extreme weather condition, physiological issues, salt stress, and high herbicide dosages, or any non-living organisms (Richard, 2016).
In most situations, plants are exposed to both biotic and abiotic stimuli at the same time,

or in other cases, two or more factors. When growing in an open field, biotic and abiotic variables, such as heat and pathogen infection, heat and drought, and/or insect and bacterium, interact (Pandey et al., 2017; Suzuki et al., 2014). These combination factors are causing more severe harm to the plants than a single component, especially when the combination consists entirely of biotics such as fungus, bacteria, and viruses (Pandey et al., 2017). In this section we separately discuss the effect of biotic and abiotic factors on lettuce production.

2.4.3.1. Biotic factors

Cultivars in the field are anticipated to be attacked by a variety of biotic agents such as fungus, viruses, bacteria, nematodes, and herbivore pests (Atkinson and Urwin, 2012). In leafy vegetables, improper abiotic variables such as humidity or soil condition promote biotic factors to occur and become more damaging; for example, lack of regular watering in lettuce leads to an increase in some soil pathogens and disease issues (Avramov et al., 2018).

The most common lettuce disease are as follows:

Lettuce mosaic virus (LMV), fungi *Rhizoctonia solani*, white mould fungal disease *Sclerotinia spp*., lettuce powdery mildew (*Golovinomyces cichoracearum*), and *Septoria spp.* are the most frequent lettuce pathogens (George, 1999), lettuce downy mildew (*Bremia lactucae*), lettuce anthracnose (*Microdochium panattonianum*), lettuce grey mould fungus disease (*Botrytis cinereal*), damping-off of lettuce (*Pythium spp*.) (Lynch et al., 1991).

Also, there are some important and most common lettuce insects which are lettuce aphids Myzus (*Myzus persicae*) (Alvarez et al., 2006), Nasonovia (*Nasonovia ribisnigri*) (van Helden and Tjallingii, 1993), and lettuce root aphid (*Pemphigus bursarius* L.) (Lebeda et al., 2007; Reinink, 1999; de Vries and van Raamsdonk, 1994).

2.4.3.2. Abiotic factors

Environmental factors influence both the morphological and internal quality parameters of vegetables (Gruda, 2005). The growing season can also influence the nutritional content of lettuce. According to Mou (2005), lettuce cultivated in the spring has a greater carotenoid content than lettuce grown in autumn season. Siong and Lam (1992) discovered that the *β*-carotene concentration of lettuce varies depending on the growth season, with the level being greater in the fall than in the summer. Temperatures above 30 °C and darkness reduce anthocyanin, carotenoids, and chlorophyll b concentrations in lettuce (Chon et al., 2012; Gazula et al., 2005; Mou, 2005). Because high temperatures increase the risk of serious physiological issues in lettuce such as tip burn, leaf bitterness, and bolting, resulting in quality and yield losses, they always determine the growing time of lettuce (Simonne et al., 2002; Zhao and Carey, 2009).

Light duration, light type, wavelength, and light density all have an impact on chlorophyll concentration, chloroplasts, photosynthesis, and certain bioactive chemicals, as well as mineral content in lettuce and other leafy vegetables (Davis, 2015; Hoenecke et al., 1992; Wang et al., 2015). Some quality indicators, such as mineral content and disease rate, are affected by the type of light used in lettuce growth. Lettuce cultivated with a far-red filter light has a greater calcium content, a less bitter flavour, less tipburn symptoms, and a lighter green leaf colour (Kleemann, 2004).

The combination of stress may not necessarily have a negative impact on the plant, but it does enhance the plant's resistance to certain plant diseases (Kaddour and Fuller, 2004; Pandey et al., 2017; Puckette et al., 2008; Rivero et al., 2014). The most frequent disease caused by a combination of calcium shortage and night-time temperature is tip-burn in lettuce or blossom end rot in tomatoes (Richard, 2016).

2.4.3.3. Postharvest practices

The duration of storage is one of the most significant criteria since it influences the sensory quality of perishable vegetables. Leafy vegetables contain a high quantity of water, which is why they are highly perishable and will remain alive even after harvesting, therefore to sustain life they use reserves components such as sugar and starch to breathe and acquire energy to stay alive (Chitarra and Chitarra, 2005; de Oliveira et al., 2013). The storage environment (temperature, relative humidity, light, and packaging) and the development of microorganisms define the shelf-life length of leafy vegetables (Agüero et al., 2011). Lettuce (*Lactuca sativa* L.) quality and shelf-life are adversely impacted by storage conditions with relative humidity (RH %) below optimum (95-98 %), since low humidity reduces water content in the tissue via increased transpiration rate (Herppich et al., 1999). High temperature, on the other hand, is another influencing factor that lowers lettuce quality (Bolin and Huxsoll, 1991), whereas low temperature extends lettuce shelf-life and prevents browning of fresh cut greens through reduced respiration (Deza-Durand and Petersen, 2011). According to Ferrante and Maggiore (2007), Lamb's lettuce (*Valerianella locusta*) began to lose chlorophyll and carotenoids at the day of eight when stored in a dark storage at 4 °C or 10 °C. According to sensory evaluation, the “Lollo Rosso” variety begins to lose its physical features and scent on the seventh day of storage at 5 °C (Allende et al., 2004). The quality of lettuce is also affected by how it is stored; for example, fresh-cut lettuce loses quality faster than keeping the entire head (without cutting), owing to the high respiration rate of fresh-cut lettuce (Cefola et al., 2014; Deza-Durand and Petersen, 2011), this might be owing to the increased respiration rate in fresh-cut lettuce or maybe to the faster attack of microbes in the storage environment (Farber, 1991). Cutting or injuring tissues increases surface area per unit volume, respiration rate, wound induced ethelyn production, and mixed enzyme and other compounds, resulting in a loss of product quality (Beaulieu, 2006; Toivonen and DeEll, 2002; Watada and Qi, 1999). According to Mitcham et al. (1996), the uniformity of the fresh product has an influence on the quality losses in the stored and transported items; malformed products damage more quickly than uniform products.

2.5. Quality parameters in fresh leafy vegetables

Quality is the grouping of characteristics, attributes and properties of the product that gives a value as a human food (Kader, 2001). Quality can be defined as a combination of agronomic qualities (yield, size, pest and disease resistance), organoleptic features (shape, colour, and firmness), nutritional value and vitamin content (Drobek et al., 2019). It is also defined by the international organization of standardization (ISO) as "the total of all features, traits, and attributes of a product or commodity that are targeted at meeting the specified or inferred consumer criteria" (ISO 8402, 1989). The inherent components of vegetable quality include nutritional, hygienic, sanitary, organoleptic, and technological functions (Gaviola, 1996). Several methods for determining customer preference for fruit and vegetable quality are provided, some of which are more accurate than others.

The major characters in fruit and vegetable quality evaluation are colour and appearance, flavour (taste and fragrance), texture, and nutritional content (Barrett et al., 2010).

Physical appearance of the product, which is related to sensory quality, is one of the most influential factors in customer purchasing decisions (Heimdal et al., 1995; Kader, 2002a; Kramer, 1965). In most circumstances, customers are unable to taste the product before purchasing. As a result, while purchasing fresh products, the flavour, fragrance, texture, sweetness, and other qualities might be characterised as hidden attributes. While the recurrent customer purchase of fresh fruits and vegetables is based on hidden qualities rather than outward appearances (Beaulieu, 2006; Waldron et al., 2003).

Before purchasing fresh products, consumers utilize all of their senses, including smell, taste (if feasible), touch and sight to choose whether to purchase or refuse the product (Abbott, 1999). Academically, several test techniques for evaluating food quality are provided, such as sensory evaluation and the use of equipment (Barrett et al., 2010). Each algorithm is applied for a unique purpose and on a different product. The 9-point hedonic scale is the most often used product assessment measure (Lawless and Heymann, 1998; Meilgaard et al., 1999). Due to having some data analytical issues of the previous method, 5-point willingness to purchase (Moskowitz et al., 2006) and the 3-point acceptability scale have been provided (Dubost et al., 2003).

In recent years, the non-destructive quality measurement is more emphasised and extremely used for fresh product quality evaluation (Abbott, 1999). Instrument quality evaluation was first described by Kramer (1965) then by Kader (2002b). The improved quality measurement method list is described in (Table 1).

The physical appearance and price of fresh products are the primary purchasing factors, while recurrent purchases are more closely connected to the product's taste, flavour, texture, and nutrient content (Mitcham et al., 1996). Product uniformity is another key quality criteria for fresh items and customer attractiveness to purchasing fresh products (Abbott, 1999). The major qualitative qualities of leafy greens are their fresh look, crisp texture, pleasant flavour, and lack of discoloration (Cantwell and Suslow, 2002).

Physiological factors and bioactive substances in the leaves and/or head affect lettuce quality. Many elements have been examined to determine the differences in lettuce quality. The method of growing may have an impact on various salad quality metrics. The quality of lettuce varies depending on whether it is grown organically or conventionally, and whether it is grown indoors or outdoors. For example, Kelly and Bateman (2010) discovered that the nitrate level of organic lettuce was much lower than that of conventional farming.

Table 1. Instrumental methods for determination of vegetable quality

Quality Attribute	**Vegetables**	**Objective method of measurement**
Appearance		
Size	Sieve size, drained weight	Dimensions (scales, screens, sizing rings, micrometers, etc.), weight and volume
Shape	Straightness	Dimension ratios, displacement, angles, diagrams and models
Wholeness	Cracked pieces	Counts, percent whole, photographs, models
Gloss	-	Gloss meters, goniophotometers, wax platelets
Consistency	Consistency	Consistometers, viscometers, flow meters, spread meters
Defects	Blemishes, bruises, spots, extraneous matter	Photographs, drawings, models, scoring systems, computer-aided visual techniques
Colour	Colour	Colour charts, dictionaries, reflectance and transmittance colorimeters, pigment extraction and spectrophotometers
Texture	Texture, firmness, grit, character, mealiness	Tenderometers, texture analysers – compression, shearing, extrusion meters, analysis of solids, moisture, grit, fibre
Aroma	Aroma	Gas chromatograph, enzymes
Flavour	Flavour, sweet, sour, salty, bitter	Hydrometer, refractometer, pH meter, determination of acidity and sugars, sodium chloride, enzymes, amines, bitter alkaloids or glucosides
Nutritional value	Vitamin A, B, C, E, polyphenols, carotenoids, glucosinolates	HPLC and spectrophotometric methods

Source: Barrett et al. (2010)

2.5.1. Nutritional value of lettuce

Lettuce nutritious value is still underestimated (Kim, et al., 2016). Water makes up the majority of lettuce's composition, accounting for approximately 95 % of the product (Mou, 2005; USDA, 2015). Lettuce is counted as a rich vegetable for bioactive compounds such as vitamin A (carotenoids, β-carotene), vitamin C (ascorbic acid), vitamin E (α- and γ-tocopherols), phenolic compounds, flavonoids and minerals like Na, Mg and Fe (Kim, et al., 2016). Many studies have been conducted to demonstrate the advantages or importance of vegetable consumption in the prevention of chronic diseases such as cancer, age-related functional losses, and cardiovascular disease (Hancock and Viola, 2005; Morris et al., 2006; Pavia et al., 2006). This is because vegetables contain a wide range of macronutrients, bioactive chemicals, and micronutrients that the human body requires on a daily basis (Kris-Etherton et al., 2002; Soetan et al., 2007). The nutritional content of lettuces, like that of any other product, varies depending on genetic, growing method, environment, and genotype variables, as well as their interaction (Baslam et al., 2013; Mou, 2012b).

Lettuce is a diet-friendly leafy vegetable due to its high nutritional content and low caloric value (10 kcal [60 kJ] 100 g^{-1}, FW) (Niederwieser, 2001). It has a high dietary fibre content (1.1 g 100 g^{-1}) that aids in food digestion and, as a result, promotes overall body health (Mampholo et al., 2016a). Mineral content and bioactive chemicals differ depending on lettuce genotype and leaf colour. Darker leaf colours (green leaf, red leaf, and romaine lettuce) have more Ca, Fe, Mn, Se, K, and β-carotene (Bunning et al., 2010). Fresh-cut or ready to eat vegetable quality and nutritional value is defined by its contents as minerals, antioxidant compounds, vitamins (Spinardi and Ferrante, 2012).

Leaf and Romaine lettuce, for example, have more nutritional content than Crisphead lettuce (Mou, 2009), the mineral content of baby green Romaine lettuce is higher than that of red leaf “Lollo Rosso”. The amount of Na is about 0.5 mg g^{-1} FW (10 mg g^{-1} DW) in baby green lettuce and 0.05–0.3 mg g^{-1} FW (1.0–6.0 mg g^{-1} DW) in red leaf “Lollo Rosso” (Fallovo et al., 2009; Neocleous et al., 2014). Baby green leaf lettuce contains around (0.3 mg g^{-1} FW or 6 mg g^{-1} DW) which is greater than in baby Romaine lettuce (0.12- 0.17 mg g^{-1} FW or 2.4- 3.3 mg g^{-1} DW) (Neocleous et al., 2014; Santos et al., 2014). Kim et al. (2016) has summarised the lettuce consumption based on the plant varieties (Figure 3.). Thus, only some of the factors will be discussed.

Antioxidants are compounds that play an important role in human protection against a variety of diseases and infections, including blood sugar and cholesterol control, immune system support, and antibacterial, antifungal, anticarcinogenic, antithrombotic, and anti-inflammatory properties in the human body (Bub et al., 2003; Vasanthi et al., 2012).

Figure 3. Summary of nutritional value of lettuce
Source: Kim et al. (2016)

Phytochemicals are being detected in many vegetable crops as a result of environmental and genetic variables. Plants defend themselves against minor environmental stress such as high and low temperatures, heat, and insect assaults by generating phytochemicals as a defence mechanism (Oh et al., 2009). Natural bioactive compounds and antioxidants, also known as phytochemicals, can be found in vegetables and fruits in an ideal combination of vitamins, polyphenols, carotenoids, flavonoids, steroidal saponins, organosulfur compounds, and vitamins, all of which are natural antioxidants (Figure 4) (González-Aguilar et al., 2008; Vasanthi et al., 2012). Plants having a high amount of bioactive chemicals are considered to be high marketability and consumer quality preference. Thus, by exposing the plants to short-term environmental stress, we may improve lettuce quality (Oh et al., 2009; Pérez-López et al., 2015).

Some quality parameters cannot be identified by the shape and appearance of the plant; they can appear the same on the outside, but there is a significant variation on the inside. Nitrate is found in nature in many different sources as water, food, cheese, air, and plants (Gassara et al., 2016). Nitrogen is essential for plant growth and development, resulting in a greater number of agricultural products, mostly plants. Nitrogen deficiency, for example, reduces lettuce quality and production (Broadley et al., 2000).

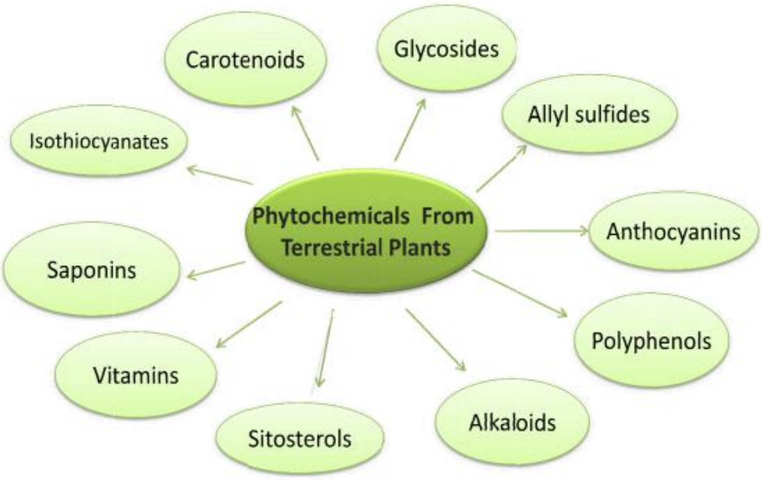

Figure 4. Phytochemicals from plants
Source: Vasanthi et al. (2012)

Today, nitrogen is the most commonly used fertilizer in the world, and demand is growing year after year (FAO, 2019). Excess nitrate, on the other hand, causes a variety of severe problems, including diabetes, Alzheimer's, and Parkinson's disease (De La Monte et al., 2009). Other health problems such as cancerogenic, gastric cancer, colon cancer, esophageal cancer, and other tumours may be linked to the conversion of nitrate in ingested goods to nitrite by salivary bacterial conversion (Tiso and Schechter, 2015), which subsequently transforms to nitrosamines in the stomach under acidic conditions (Bedale et al., 2016; Park et al., 2015). Around 5 to 7% of the nitrate ingested is transformed to nitrite in the oral cavity by bacteria in the mouth, which is subsequently converted to nitric oxide in the stomach and systemically absorbed (Bryan and Ivy., 2015; Gaston et al., 1994; Ma et al., 2018) (Figure 5). Leafy vegetables (lettuce, spinach, rocket, Swiss chard, celery, and parsley) are known as the primary nitrate-accumulating vegetables since the main plant component for nitrogen use is the leaf, and these plant groups exclusively produce leaves and no fruit (Colla et al., 2018a; Maynard et al., 1976; Santamaria, 2006).

The challenges in managing mineral nutrition and chemical protection in lettuce plants are related to the plant's short vegetative period, high levels of nitrate, and pesticide residue (Skovgaard et al., 2017). Nitrate levels in fresh lettuce leaves range from 26 to 2568 mg kg -1, depending on growth circumstances (primarily light availability), lettuce genotype, and nitrogen fertilizer application rate (Beninni et al., 2002; Cometti et al., 2011). The maximum allowable nitrate level in lettuce leaves varies depending on the country regulations and the growing season (spring and autumn). In Europe, the maximum nitrate in winter farming varies from 3,500 to 4,500 mg N-NO_3^- kg^{-1} fresh weight, whereas it is around 2,500 mg kg^{-1} in summer cropping (Europe, 2009).

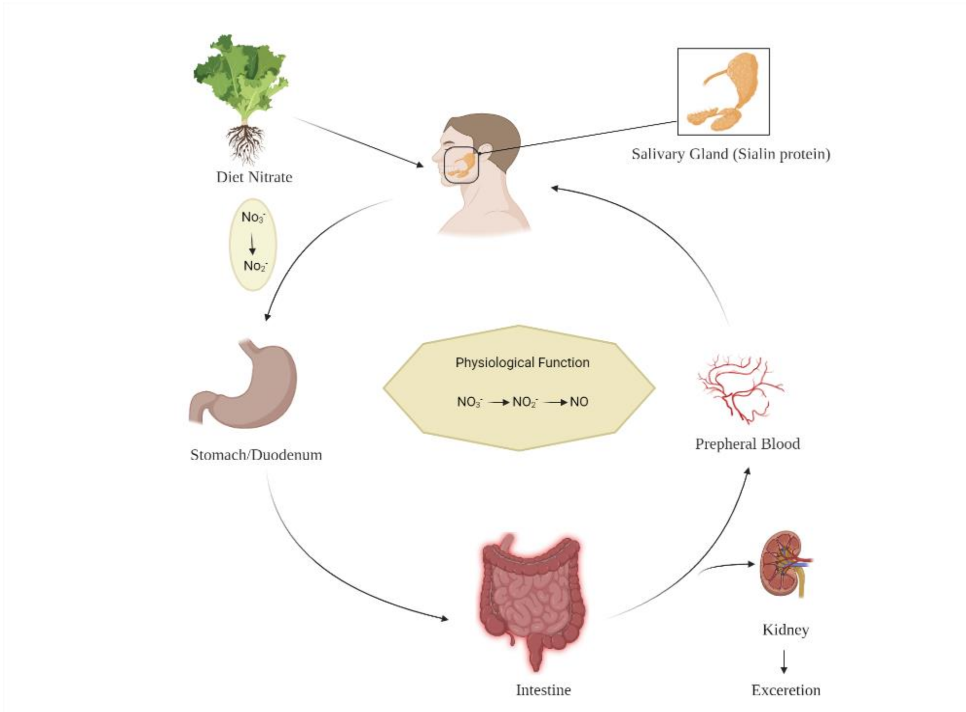

Figure 5. Circulation of nitrate in the body
Source: Author’s own (created in Biorender.com)

2.5.2. Physical parameters of lettuce

Physical characteristics are the primary quality criteria used by customers to choose a product in markets. The market price and consumer attractions are rely on some physical parameters as colour, shape, size, taste and texture (Mampholo et al., 2016b).

2.5.2.1. Leaf colour

Today, lettuce is available in a variety of colours (green, yellow, and red), shapes (leafy and head form), and sizes (baby and shred lettuce) (Martínez-Sánchez et al., 2012).

According to Bunning et al. (2010), the deeper the lettuce colour, the higher the amount of vitamin A, niacin, riboflavin, thiamine, Ca, Fe, K, Mn, Se, and β-carotene.

One of the most important quality parameters in lettuce quality is colour. The colour in plants comes from natural pigments for example: green (fat soluble chlorophylls), red, yellow and orange (carotenoids), yellow (flavonoids), red (betalains), red and blue (water soluble anthocyanins), and enzymatic and non-enzymatic browning (polyphenol oxidase) reactions may produce the formation of water soluble brown, grey and black coloured pigments (Barrett et al., 2010). Chlorophyll pigments provide plants with their green colour. Many factors, such as physical damage and storage duration, influence plant pigment colour deterioration in lettuce, and as a result, the product loses quality (Cantwell, 1996). Chlorophyll and anthocyanins, for example, are quickly destroyed by heat and acid; flavonoids and carotenoids are susceptible to oxidation and light; and betalains are also heat sensitive (Clydesdale and Francis, 1976).

2.5.2.2. Head structure

Lettuce head structure is one of the most important quality parameters to consider when purchasing the product. The morphogenesis of lettuce leaves illustrates the creation of heads in some lettuce cultivars, such as butterhead lettuce (Bensink, 1971). Head structure also has a direct relationship to the nutritional content of the product. A research study shows that head forming indicates the nutritional value of the lettuce. Opened head forming contain greater nutrient value as vitamin A and C, β-carotenoids, iron and calcium than closed head forming, for example, crisp head lettuce (contain lower nutrient content than Romaine lettuce (Mou and Ryder, 2002). The most obvious factors affecting head structure is the genetic factors followed by environmental factor such as soil and night air temperature as well as light intensity. A lack of head formation occur when temperatures in the root zone is less than 14 °C during the early stage of the growth phase, followed by greater temperatures later in the developing period (Maaswinkel and Welles, 1987).

2.5.2.3. Head weight

Lettuce head weight has a direct correlation to the marketable value. The genetic factor plays the most important role in determining head weight amongst lettuce cultivars (Tudela et al., 2013). For example, the "Great Lakes" variety produces a larger head than the "May King" and "Kobak" varieties. Many environmental and agronomic factors influence head weight while attached to the plant during plant growth, such as maturity stage at harvest, soil properties, abiotic and biotic factors (Gent, 2017; Tudela et al., 2013). Throughout the postharvest process, the most critical consequence in lettuce plants is head weight loss. This is because lettuce is a perishable produce that quickly loses moisture after being cut from the plant. The proportion of produce lost is determined by the storage environment, duration, maturity stage of lettuce at harvest, and method of storage (cut or uncut) (Agüero et al., 2008; Kang et al., 2008).

2.5.2.4. Root formation

Lettuce has a very shallow root system that grows in only 30 cm of top soil. As a result, if we want the best growth and yield, this section of the soil must be fertile and well-drained. The main factor influencing root formation is physicochemical soil properties, particularly soil pH (Inoue et al., 2000). Lettuce root is well developed in sandy loam soil with slightly acidic and neutral pH between 5.0-6.5 (Roosta, 2011). The water quality and irrigation duration are another factor can influence the root formation in lettuce. The plant is very sensitive to saline water and excessive irrigation or by water shortages (Urbano et al., 2017).

2.6. Integrated lettuce production

The rapid population increase has drowned a great attention of scientists to find alternative way of providing food to the humanity, while we all aware of the risks of unwanted practicing conventional farming to our living planet. Kirchmann and Bergström (2008) believe that without having conventional farming the humanity will face starvation and organic farming cannot be the only way of feeding the world. Nevertheless, Force,

(2008) reported that per capita production in organic farming could fill the food for today's inhabitants which can sustain the current human population. There is a misunderstanding among the farmers about organic farming for the production cost and inputs, so that it is complicated to explain and compare the profits between the conventional and organic agricultural production practices (Engindeniz and Tuzel, 2006). Vegetables in general have short life spam, so that, they require high amount of fertilizer during their growth season. Some of the vegetables (leafy vegetables) need more nitrogen fertilizer than others. Therefore, to improve soil fertility mainly soil nitrogen and sustainable production of the soil, organic system can be the best choice (Adediran et al., 2004; Gong et al., 2011). Microelements are essential for crop growth and development. The nutrient deficiency occurs in conventional grown crops frequently, this is because most of the chemical fertilizers do not contain all the essential elements (Adediran et al., 2004; Wong et al., 1999). Marinari et al. (2006) have found that soil under organic management contains higher nutrients, microbiological activities and available phosphorous and other biomass and enzymatic availability.

2.6.1. Plant care

2.6.1.1. Lettuce production requirements

Lettuce is known for all year-round vegetable; this is based on the locations and growing methods. Lettuce is typically grown from seeds. The seeds are first sown in trays for about one month till they reach to 3-4 true leaves then transplanted to the permanent location. The major important factor during lettuce growing cycle is regular irrigation. Both under-irrigation and over-irrigation have a negative influence on the crop performance which resulting in qualitative and quantitative loss of vegetable yield (Chen et al., 2019; Dukes et al., 2010). Lettuce vegetable requires frequent irrigation, especially when they begin to produce a head; any uneven watering reduces production and quality (Avramov et al., 2018). Plants' capacity to absorb water and nutrients in the soil is reduced when irrigation water includes excessive amounts of salt. Lettuce plants are susceptible to water stress and water quality; high electrical conductivity (EC) levels (4.6 mS cm^{-1}) in saline water reduce head weight by 51% and leaf number by 28%; nevertheless, high salinity in water reduces nitrate concentration in lettuce leaves (Miceli et al., 2003) because lettuce is a moderately salt tolerant vegetable. Drought, heat, and their combination are the most influential environmental variables restricting agricultural development and production across the world (Mittler, 2006; Pandey et al., 2017; Prasad et al., 2011; Rizhsky et al., 2002; Vile et al., 2012). Because water can be a source of microbial contamination, water quality is critical in lettuce irrigation. *Escherichia coli* is a major problem in lettuce production, with some data indicating that water was the cause of contamination of iceberg lettuce in Sweden (Soderstrom et al., 2005) and bagged spinach in the United States (Jay et al., 2007). Depending on the growing method (open field or greenhouse), different irrigation systems such as furrow irrigation, micro-sprinkler irrigation, plastic film mulching irrigation, and drip irrigation are used in lettuce production. Among the irrigation methods, the mulch drip irrigation methods could dramatically promote root growth in the upper soil profile, however the combination of plastic film mulching-micro-sprinkler irrigation with frequent and light irrigations is recommended for improvement of lettuce growth and yield (Chen et al., 2019).

2.6.1.2. Fertilization requirements

In the last few decades, chemical fertilizers have significantly boosted agricultural yields and food nutrition (Fageria, 2009; Wang et al., 2008). Excess fertilizer application, on the other hand, can have negative environmental consequences on water quality through leaching, and run off (Heckman, 2007; Heckman et al., 2003). Because lettuce has a short life cycle, it requires a lot of fertilizer throughout its growing season especially nitrogen (N). Some plants (leafy vegetables) require more nitrogen fertilizer than others. As a result, an organic system may be the greatest option for improving soil fertility, particularly soil nitrogen, and for ensuring long-term soil productivity (Adediran et al., 2004; Gong et al., 2011). Nutrient insufficiency is common in conventionally produced crops since most chemical fertilizers do not contain all of the necessary components (Adediran et al., 2004; Wong et al., 1999). Marinari et al. (2006) discovered that organically managed soil includes more nutrients, microbiological activity, accessible phosphorous, and other biomass and enzymatic availability. Lettuce requires more nitrogen than any other mineral nutrient. There are several factors that influence nitrogen fertilizer application to lettuce plants, including the growing season, plant growth stage, soil chemical content (soil fertility), lettuce cultivars, and climatic conditions. According to Breschini and Hartz (2002), iceberg lettuce requires an average N fertilization of 130 kg ha^{-1}, but romaine lettuce requires less, around 107 kg ha^{-1}. Imbalanced fertilization reduces lettuce quality because high nitrogen levels cause nitrite accumulation in fresh leaves where boosts postharvest decade (Hoque et al., 2010; Sugar et al., 1992), whereas low nitrogen levels reduce yield and leaf quality.

So that, the subsoil fertility is essential for leafy vegetables particularly lettuce, because lettuce produces very shallow roots and N uptake occurs in the top 30 cm of soil which can be called lettuce root zone (Bottoms et al., 2012; Jackson, 1995). The nitrogen rates utilized by the collaborating farmers were clearly greater than what was required to attain optimum lettuce production and quality. Despite a number of studies indicating that optimum lettuce yields may be reached with 100-150 kg ha^{-1} N rates, farmers are hesitant to utilize such conservative N rates (Breschini and Hartz, 2002).

To achieve excellent quality and extended shelf life, an appropriate major nutritional components such as nitrogen (N), phosphorus (P), and potassium (K) are required during lettuce cultivation (Hoque et al., 2010). However, most of research studies on lettuce production have concentrated on nitrogen fertilizer, although other minerals would have a role in lettuce yield and quality improvements. Unlike other leafy vegetables, lettuce cultivation needs the addition of nutrients such as phosphorus, potassium, calcium, and silicon. Under most situations, an appropriate phosphorus level is required to improve lettuce quality and marketable yields. Phosphorus (P) is essential to a variety of plant processes such as photosynthesis, energy transmission, respiration, and cell division (Esmail et al., 2019).

The amount of P and K depends on the soil's nutrient availability, plant variety, soil temperature, and planting season. Johnstone et al. (2005) proposed adding 4% more P to early grown lettuce than summer conditions, resulting in a range of 55 to 65 kg ha^{-1} for the highest marketable output. Whereas, Hoque et al. (2010) have provided 67 kg ha^{-1} of K to get the best lettuce quality and shelf life extension together with N and P. Silicon (Si) and calcium (Ca) are two elements that influence lettuce yield and quality. Some research has discovered a modest relationship between lettuce yield and soil Ca (Soundy and Smith, 1992). Although Si is not required for plant metabolism, it has been found to alleviate P deficiency and improve plant resistance to pathogens and fungi (Hoque et al., 2010). Chemical-free or organic lettuce cultivation is becoming a key issue among producers and consumers. Most organic resources, such as manure, compost, and humic acid, are recognized for use in organic farming systems to promote soil fertility and plant development. However, there is a list of additional fertilizers (both synthetic and non-synthetic) that may be utilized in organic lettuce production systems (Table 2) (Hollyer et al., 2013).

Table 2. Naturally occurring (Nonsynthetic) and synthetic materials allowed as fertilizers and soil amendments for the commercial organic production of produce (as per label restrictions)

Non-synthetic/naturally occurring materials	Synthetic materials (Manufactured and/or mined)
Animal manure	Aquatic plants (alkali extracted)
Blood meal	Boron
Bone and meat meal	Fish products (liquid, pH, adjusted with acid)
Calcium carbonate/limestone (mined)	Humic acid (alkali extracted)
Calcium chloride	Iron phosphate (molluscicide use only)
Compost: animal manure or plant-based	Iron sulphate
Decomposing crop residue	Lignin sulphate
Feather meal	Micronutrients (such as zinc sulphate)
Fish meal/shrimp	Magnesium sulphate
Fulvic acid	Newspaper
Guano (mined)	Sulphur (elemental)
Gypsum (mined)	Sulphurous acid
Humates / Leonardite (mined)	Vitamin B_1, C, E
Peat	-
Potassium magnesium sulphate, potassium sulphate (mined)	-
Rock phosphate (mined)	-
Seaweed/kelp	-
Worm castings	-

Source: OMRI (2020) and WSDA (2019)

Nitrogen fertilizer has the greatest impact on lettuce yield and growth, as well as other chemical contents such as nitrate concentration, bioactive compounds as well as physical parameters. Based on the suggested nitrogen fertilizer for leafy vegetables as stated by Liu et al., (2014), we administered the inorganic fertilizer FERTICARE 24-8-16+ 3.7 MgO+ microelements to our plants in the quantity of 200 kg ha^{-1}. However, due to poor light in the autumn season and significant nitrate accumulation, we applied the same quantity of 200 kg ha^{-1} high potassium fertilizer from the second half of the growth cycle of OMEX12-4-24+ME as recommended by Barickman et al. (2016).

2.6.1.3. Plant protection of Lettuce

Crop losses are defined as a decrease in the quantity and quality of plant output (Zadoks and Schein, 1979). Crop loss can occur in the field before harvest (pre-harvest) or after harvest (post-harvest), and it can happen in both stages (Oerke, 2006). Crop losses are often measured in kg ha^{-1}, financial loss ha and/or percent loss units (Sharma et al., 2017). Pests are responsible for a loss of 20-40% of global agricultural efficiency (Kennedy, 2008).

Oerke (2006) stated that, in addition to disease and insects, the principal hazardous species that cause agricultural losses are weeds, pathogens, and animal pests. Weeds, in general, are the leading loss for crop yield reduction at about (34%), while pests and infections are less important (losses of 18 and 16 percent). According to Schoonhoven et al. (2005), insects harm more than 15% of crops each year.

2.6.1.3.1. Organic lettuce protection

In the organic agricultural system, there is a misconception about the usage of "pesticide-free" or "fully-natural" ingredients. True, all products used in organic farming must be ecologically benign and pose no harm to human health. Farmers in the organic farming system are supposed to use cultural methods such as crop rotation, cultivar selection, and physical barriers to protect their crops; however, if these methods fail, they are allowed to use a variety of synthetic chemicals, botanical extracts, minerals, soaps, bacteria, and clays as pesticides under specific regulations (Avery, 2007; Hollyer et al., 2013).
Many scientists agree that organic agriculture is the most appropriate factor to protect the environment, human health, climate and product quality from the residue of chemical usages (Bengtsson et al., 2005; Crowder et al., 2010; Röös et al., 2018). However, others believe that organic farming cannot address the environmental concern (Kirchmann et al., 2008). Organic fertilizer or organic matter is rich for micronutrients, so that the physical,

chemical and biological properties of the soil can be improved through practicing a proper organic farming system (Albiach et al., 2001; Bassouny and Chen, 2015). Organic farming also reduces the environmental hazards comes from intensive agriculture through recycling the waste to the form of commercial fertilizer (Pant et al., 2004). In terms of nitrate content (NO_3^-) and pesticide residue, organically produced vegetables are healthier and contain far less nitrate than conversional vegetables (Woese et al., 1997). Worthington (2001) reported that organically grown fruits and vegetables contain higher nutrient value, in terms of vitamin C, iron (Fe), magnesium (Mg^{2+}), and phosphorus (P) than conventional crops. Besides, organic system can also improve crop yield under stress conditions. Rady et al. (2016) has found that wheat yield was improved and heavy metals was reduced by the use of bio-fertilizer and mineral N fertilizers under salinity stress grown condition (ECe = 7.84 dS m^{-1}).

There is a list of approved items that may be used in organic crop protection and are not highly hazardous. Except as specified by the United States Department of Agriculture's National Organic Program (NOP) rule 7 CFR 205, the majority of chemical pesticides are prohibited from usage (CFR., 2013) and European Pesticide regulation (EC) No. 1107/2009 (EPRS, 2017). According to the NOP national list, prohibited or extremely prohibited and non-prohibited pesticides are listed in §205.600 through §205.606, and Subpart G §205.602. Subpart G §205.601 contains those pesticide that can be used in organic farming system including; Alcohols, Copper sulphate, Chlorine materials, Hydrogen peroxide, Ozone gas for use as an irrigation system cleaner only, Soap-based algicide/demossers, Sodium carbonate peroxyhydrate. On the other hand, the Subpart G §205.602 list provides the extremely prohibited pesticides uses in organic farming which includes a list of Nonsynthetic substances as; Arsenic, Ash from manure burning, Calcium chloride, Lead salts, Potassium chloride, sodium fluoaluminate (mined), sodium nitrate, strychnine, and tobacco dust (nicotine sulphate). Hollyer et al. (2013) mentioned that those products are produced either from natural sources as chitin and neem or not natural sources which are made in manufactures such products like hydrogen peroxide, copper oxide, copper oxychloride, and peracetic acid, as it is shown in (Table 3).

In Europe, there are some regulations like regulation (EC) No. 1107/2009 to use “Basic Substances” in organic crop protection (Villaverde et al., 2014). In the organic lettuce protection, the land preparation and soil physical properties can have a significant impact on lettuce growth. Tillage is the first step in soil preparation, and constant loosening is required during the vegetable growing season.

Table 3. Naturally occurring (non-synthetic) and synthetic materials that are allowed in pesticides for commercial organic produce production (as per label instructions)

Non-synthetic / naturally occurring	Synthetic (manufactured and/or mined)
Acetic acid (vinegar)	Ammonium carbonate (as bait)
Bacillus subtilis, Bacillus thuringiensis, and other allowed Bacillus bacteria	Ammonium soaps
Botanical extracts (e.g., garlic, chili, rosemary, mint, etc.).	Boric acid / orthoboric acid, and borax (disodium octaborate tetrahydrate / sodium tetraborate decahydrate)
Chitin (from the shells of crustaceans and other sources)	Chlorine materials
Citrus (extract)	Copper (fixed), copper hydroxide, copper oxychloride, copper sulphate (Bordeaux mixtures)
Ethanol (ethyl alcohol)	Ethylene
Gibberellic Acid	Ferric phosphate
Neem oil (Azadirachtin)	
Pseudomonas syringae, Pseudomonas fluorescens, and other allowed Pseudomonas bacteria	Hydrogen peroxide
Pyrethrins	Isopropyl alcohol
Soybean oil (and other vegetable oils)	Lime-sulphur
Saccharopolyspora spinosa (bacteria) (aka spinosad)	Oils (horticultural: crop, petroleum, mineral, and paraffinic based)
-	Peracetic acid
-	Pheromones
-	Soap (horticultural, sodium/ potassium salts + fatty acid)
-	Sulphur (elemental)

Source: OMRI (2020) and WSDA (2019)

Loosening the soil to depth (adding fertilizer) enhances water availability in both wet and dry circumstances, as well as improving water usage efficiency, largely through impacts on root activity (Carr and Dodds, 1983). Subsoil compactness occurs throughout the agricultural process, such as irrigation, weeding, fertilizing, and crop protection procedures, and leads to reduced root development and root activity, resulting in a loss of crop ultimate output.

Because lettuce plants have weak roots, weeding should be done with caution to avoid spreading lettuce. Weeding is usually the case. After 15 days, or as necessary (Amar, 2021).

2.6.1.3.2. Conventional lettuce protection

The issues of overuse pesticides have become a real threatened matter all over the world to the human beings and scientists. According to Koul et al. (2008) around 2.5 million tons of pesticides are used per year which costs $100 billion worldwide to the environment. In some developed countries, pesticide consumption reaches nearly 3000 g ha^{-1} (Khater, 2012). Nowadays, scientists are looking for new crop protection tools such as 'Green pesticides' to overcome the issue of chemical effects on human health and nature. Many concepts have been prepared for the use of environmentally friendly products to control pest problems. Plant extracts, hormones, toxins from organic origins are examples of those green inventions. Also, to manage pests, there have been some encouragements to use natural resources like extremely biodegradable synthetic and semisynthetic products in controlling pests (Dhaliwal and Koul, 2007; Koul, 2008).

Koul, (2008) strongly recommends the use of essential oils as a safe and viable green pesticide. The most commonly known essential oils for their pest control properties are thyme (*Thymus vulgaris*), vetiver (*Vetiveria zizanoides*), clove (*Eugenia caryophyllus*), lemon grass (*Cimbopogon winteriana*), rosemary (*Rosmarinus officinalis*) and Tasmanian blue gum (*Eucalyptus globulus*). Mulching on the other hand, is an effective way of protecting salads from the soil pathogens and disease. Many studied have shown the positive influence of covering the soil as it is called mulching in environmentally friendly protection in vegetables. Avramov et al. (2018) have mentioned that mulching not only protecting salads from pathogens, but also improves the microbiological activities of rhizosphere microorganisms, the amount of nutrient intake then improvement of both quality and quantity indicators in vegetable production. Mulching is also protecting the economic plant from the competition weeds (Harrington and Bedford, 2017), then improve the quality and yield of the vegetable (Gross and Kalra, 2002; Yordanova, 2018).

Planting resistant varieties can reduce the cost of production and protecting the environment through the reduction in pesticide uses (Lu et al., 2011). Naturally, healthy plants with enough water and nutrient are able to compensate damages by some kind of attack by pathogens such as herbivory (Levine and Paige, 2004; Wise and Sacchi, 1996). Planting a tested variety to disease resistance is the key success in organic lettuce production, this will be evaluated in a controlled area with a specific pathogen like artificial inoculation under a growth chamber (Křístková et al., 2008).

Addressing disease and soil fertility problems in conventional farming system is based on a short-term target strategy for example use of pesticides to control the current pests in the field or nutrient solution whenever a nutrient deficiency appears in the plants; however, in organic farming system all the field issues are targeted for long-term approach for instance, nutrient cycling and conservation in crop rotation and weed, disease and pest control (Keatinge et al., 2001; Watson et al., 2002).

2.6.2. Plant biostimulants

Biostimulants are natural substances or materials derived from various living creatures, compounds, and plant extracts. This is applied directly to the plants or to the soil to stimulate the plants primarily for abiotic stress and to optimize production, quality, growth, and plant vigour while using a minimal amount (Yakhin et al., 2017). However, there are several meanings for the relatively new term "biostimulants" in various contexts. Plant biostimulants are defined by du Jardin (2012) as “substances or materials that, with the exception of nutrients and pesticides, have the capacity to modify physiological processes in plants in a way that provides potential benefits to growth, development, or stress response when applied to plants, seeds, or growing substrates in specific formulations.” Biostimulants made up of seaweed extract can improve the nutrient uptake in plant roots when it is added to the soil, this is through enhancing the soil structure and micronutrients in the soil which makes some changes in the root morphology and physiological characters (Halpern et al., 2015). Plants, in general, necessitate a variety of treatments from planting to harvest in terms of nutritional and protection requirements. Thus, plant biostimulants are used on the majority of plant species, including fruits and vegetables, for a variety of reasons. Because vegetables are more vulnerable to biotic and abiotic stress, they require more care and resources for cultivation during their life cycle than fruits and field crops. Biostimulants can play a significant role in reducing abiotic stress in vegetables (Bulgari et al., 2019).

The term of biostimulants was first described by Kauffman et al. (2007) where they classified into three main groups: Humic substances (HS), hormone containing products (HCP), and amino acid containing products (AACP). Biostimulants are considered eco-friendly products that play an essential role in both organic and conventional farming by decreasing the quantity of chemical fertilizers used to the plants, therefore lowering environmental pollution (Abou Chehade et al., 2018; Paradiković et al., 2011; Xu and Geelen, 2018). Botanicals, seaweeds, hydrolysates, beneficial bacteria and fungi, biopolymers and chitosan, inorganic chemicals, and nitrogen-rich substances are all sources of biostimulants in modern organic farming (Calvo et al., 2014; P. du Jardin, 2015). According to Rouphael et al. (2017), the combination of microbial-based biostimulants containing (*Rhizophagus intraradices*) and (*Trichoderma atroviride*) and the stress condition of the lettuce plant resulted in improved nutrient content in leaf tissue, chlorophyll content, and phytochemical activities of the plant Photosystem II (PSII). Many agents in Europe are in charge of registering items as biostimulants and biocontrol agents in organic farming under Regulation (EC) No 889/2008. The crops on which biostimulants are now being tested in Europe are mentioned in (Table 4).

Table 4. Biostimulants are currently applied in Europe (adapted from (EBIC, 2013)), with permission)

Vegetables and legume crops		**Other horticultural crops**
Broccoli	Onions	Ornamentals
Cabbage	Peppers	Nursery
Carrots	Lettuce	Turf
Cucumber	Squash	-
Aubergine	Strawberry	-
Garlic	Tomato	-
melons	Watermelon	-

2.6.2.1. Bistep (Ferbanat L)

Bistep is a type of humic acid that is manufactured as an organic material to promote plant development, shorten vegetative length, and aid in nutrient absorption by plants by increasing nutrient availability and improving soil structure (Cimrin and Yilmaz, 2005).

Humic acid promotes plant nutrient absorption as well as micronutrient availability and transport in plants (Böhme and Thi Lua, 1996). Many research have shown that humic compounds or plant biostimulants boost lettuce quality, growth and yield (Lucini et al., 2015; Russo and Berlyn, 1992; Smoleń et al., 2019; Yakhin et al., 2017). It can also enhance crop tolerance to abiotic stress, especially in salty soils, by utilizing irrigation water with high sodium chloride (NaCl) levels and decreasing transplant stress (Lucini et al., 2015; Qin and Leskovar, 2020).

2.6.2.2. Weeping willow (*Salix babylonica* L.) and benefits of its extract

Weeping willow, also known as *Salix babylonica*, is a deciduous tree that grows on nearly every continent. Babylonica spices is a tree native to arid regions of northern China, although it is now internationally recognized for its beauty in gardens and medicinal properties. The tree has a long history of being used as a pain reliever, and the most recent pharmaceutical medication that has been driven by it is aspirin (Alamgir, 2017; Fuster and Sweeny, 2011).

The plant contains many active ingredients as *salicin* (an alcoholic-glucoside of salicylic acid), essential mineral elements (N, P, K, Ca, B, Mn, Fe, Zn, and Mg), plant growth regulators or phytohormones *Indole 3-butyric acid* (IBA) and Salicylic acid (SA), high concentration of salicylate compounds and phenolics (Deniau et al., 2019; Mutlu-Durak and Kutman, 2021). Many studies have shown that willow bark and leaf extract improve seedling resistance to salt stress, can be utilized as a growth hormone for rooting development, and can speed up vegetative components in cutting propagation of lavender plant (soft wood) and chrysanthemum (semi-hard wood) (Mutlu-Durak and Kutman, 2021; Wise et al., 2020). It has also been discovered that the extract possesses natural fungicidal effects against *Botrytis cinerea* and *Penicillium expansum* (Andreu et al., 2018; Hussain et al., 2011). The extract of *Salix cortex* have also been applied as basic substance for the plant protection in *in vitro* tests against grapevine against downy mildew (*Plasmopara viticola*), decrease of leaf curl (*Taphrina deformans*) pathogen in peach tree, reduction of apple scab (*Venturia inaequalis*) diseases (Deniau et al., 2019).

The EU pesticide regulation, Regulation (EC) No 1107/2009, has recently authorised the Willow bark extract of the common plant willow for agricultural applications as a basic component having fungicidal properties (Deniau et al., 2019; Marchand, 2016). However, there have been few or no studies on the influence of willow bark extract on nutrient uptake and ion ratio in lettuce.

2.6.2.3. Moringa (*Moringa oleifera* Lam) and benefits of its extract

Moringa is a deciduous and fast-growing softwood tree native to the sub-Himalayan region of India, Pakistan, Bangladesh, and Afghanistan, where it grows along the Chenab River (Bhatnagar and Krishna, 2013; Fahey, 2017). It belongs to the Moringaceae family, which has just one genus and thirteen species. The tree is one of the most nutrient-dense plants ever discovered in nature, with high levels of macro and micronutrients such as potassium, calcium, magnesium, iron, total phenols, and proteins in the leaves (Hekmat et al., 2015; Yaseen and Takácsné-Hájos, 2020). It is very fast-growing tree and the most suitable environment is in tropical and subtropical areas where the tree can grow up to four meters in a year and the fully matured tree reaches the 6-15 meters (Price, 2007). The tree can survive in poor or less fertile soil and well adapted to drought (Anwar et al., 2007), and extreme weather condition with the temperature up to 48 ℃ for a short duration, however it can only tolerate cold temperatures ranging from (- 1) to 5 °C for a limited period of time (Tim and D'Aiuto, 2012). There is little information on growing moringa in cool climate zones, nevertheless based on our three years of experience on growing *Moringa oleifera* trees in Hungary, the tree can be grown well under environmental protection, primarily glasshouse conditions where the temperature does not fall below 20 ℃ during frost or cold winter. Nowadays, all the tree parts leaves, seeds, pods, flowers, brunches and roots (Figure 6) are used for medical and industrial purposes (Prasad and Elumalai, 2011; Stohs and Hartman, 2015). Many scientific studies show that moringa leaf extract and other components (seeds, roots, brunches) are a safe plant biostimulant that can increase crop quality, production, and stress tolerance in a variety of crops (El-mageed et al., 2017; El-saady and Omar, 2017; P. du Jardin, 2015; Matthew, 2016). The leaf extract contains high level of vitamin C, minerals, carbohydrates and many essential nutrients (Chukwuebuka, 2015), so that it is consumed in some poor nations where they suffer from malnutrition. Furthermore, the moringa tree contains around 40 natural antioxidants, the most prevalent of which are *kaempferol*, *quercetin*, *beta-sitosterol*, *zeatin*, and *caffeoylquinic acid* (Dhakar et al., 2011).

Figure 6. Parts of moringa (*Moringa oleifera* Lam.) tree
Source: Author's own (Debrecen, 2019)

There is very limited research on the role of moringa tree in plant protection, however some evidences show that it can be used as a safe antimicrobial property against some crop diseases. Ali et al. (2004) have demonstrated that moringa seed extract contain cytotoxicity which could have a positive role as antimicrobial property against bacterial growth of *Pseudomonas aeruginosa*, *Escherichia coli ATCC 13706*, *Bacillus stearothermophilus* and some other pathogens. Furthermore, *M. oleifera* oil extracts have been proven as promising biopesticides against *Aedes aegypti* instar larvae (Donli and Dauda, 2003; Ogbonna et al., 2014). Foliar spray of moringa leaf and seed extracts provides damping off in seedlings, plant immunity system enhancement against certain pests and diseases, and pathogen inhabitation of *in vitro* culture (Fahey, 2005; Makkar and Becker, 1996; Najar et al., 2011).

A number of research studies on the beneficial effects of moringa leaf extract on crop performance and yield enhancement are presented. Moringa leaf extract can improve nutrient quality and yield of tomatoes (Culver et al., 2012), physical and quality improvement of sweet peppers (*Capsicum annuum* L.) (Sowley et al., 2014), increasement of beans, cowpea and groundnut seed germination (Phiri and Mbewe, 2010), improvement of quality parameters of different lettuce cultivars (Yaseen and Takácsné-Hájos, 2021b), growth and productivity of some forage cereals as sorghum, pearl millet and rice under salt stress and dry weather condition (Abusuwar and Abohassan, 2017).

3.0. MATERIALS AND METHODS

3.1. Experimental design and plant materials

3.1.1. Spring experiment

3.1.1.1. Plant materials and experimental design

Lettuce seeds were sown under the optimum weather condition in plastic trays under glasshouse in February 2019-2021 at University of Debrecen, Farm and Regional Research Institute, Botanical and Exhibition Garden, Hungary. Three different cultivars "May King", "Kobak" and "Great Lakes" were chosen for this experiment as plant materials to be transplanted and grown under plastic house. One month after the seed sowing, the seedlings with 4 true leaves were transplanted under unheated plastic house (Figure 7) directly to the calcareous lowland chernozem soil which some chemical properties of the soil are shown in Table 5. The experiment was laid out based on factorial Randomized Complete Block Design (RCBD) experimental design with 3 replication and 20 seedlings per replication for each variety.

Figure 7. Spring experiment under plastic house
Source: Author's own (Debrecen, 2021)

Table 5. Soil chemical properties at the experiment site in spring under plastic house

Soil chemical content	Years		
	2019	**2020**	**2021**
Soil pH (KCl)	7.35	7.28	7.56
Organic carbon (humus content) ($m\ m^{-1}$) %	2.88	2.40	1.72
P_2O_5 ($mg\ kg^{-1}$)	3137	1483	1317
K_2O ($mg\ kg^{-1}$)	459	374.02	291
Mg ($mg\ kg^{-1}$)	584	378.67	240
Na ($mg\ kg^{-1}$)	69.7	78.64	60.6
S ($mg\ kg^{-1}$)	98.7	56.70	42.1
$CaCO_3$ ($m\ m^{-1}$) %	4.20	3.21	0.72
NO_3^- ($mg\ kg^{-1}$)	130	53.97	244
Zn ($mg\ kg^{-1}$)	35.85	20.60	11.0
Cu ($mg\ kg^{-1}$)	9.54	6.88	4.39
Mn ($mg\ kg^{-1}$)	35.0	75.49	90.6
Total water-soluble salts ($m\ m^{-1}$) %	0.10	0.05	0.003
Soil plasticity (K_A)	42.00	37.72	33.00

Source: Agricultural Laboratory Centre, University of Debrecen, 2019-2021

3.1.1.2. Climate condition of the spring experiment

The temperature, light and relative humidity was lower in the beginning of transplanting the seedlings in March, then it was gradually improved. However, some large variations were recorded during the growing months of the lettuce growing season (Table 6).

Table 6. The average weather condition and lettuce growing practices under plastic house of the **spring** experiments (Debrecen, 2019-2021)

Years	Months		
	March	April	May
	Temperature (°C) MEAN		
2019	8.9	11.5	13.6
2020	7.0	13.3	14.9
2021	10.6	14.3	20.3
	Relative Humidity (RH%) MEAN		
2019	62.1	63.5	77.8
2020	57.4	49.7	63.9
2021	78.2	56.2	39.2
	Light emission (W/m^2) MEAN		
2019	149.8	141.9	141.9
2020	136.0	185.8	172.0
2021	173.3	194.6	244.4
	Transplanting		Harvesting
2019	25th	-	16th
2020	18th	-	12th
2021	20th	-	11th

Source: Agricultural Laboratory Centre, University of Debrecen

3.1.2. Autumn experiment

3.1.2.1. Plant materials and experimental design

For the autumn experiment in September 2019-2021, seeds from the same plant varieties "May King," "Kobak," and "Great Lakes" were sown under glasshouse weather conditions. After the seedlings had four true leaves, they were transplanted directly into the glasshouse where the soil parameters listed in Table 7. The treatments [control and 6% Moringa leaf extract (MLE)] were applied once a week until harvest (Figure 8).

Table 7. Soil chemical properties at the experimental site in **autumn** seasons under glasshouse condition (Debrecen, 2019-2021)

Soil chemical content	**Years**		
	2019	**2020**	**2021**
Soil pH (KCl)	7.40	7.18	5.27
Organic carbon (humus content) ($m\ m^{-1}$) %	1.70	3.04	2.66
P_2O_5 ($mg\ kg^{-1}$)	236	314	788
K_2O ($mg\ kg^{-1}$)	177	381	499
Mg ($mg\ kg^{-1}$)	239	192	388
Na ($mg\ kg^{-1}$)	42	155	314
S ($mg\ kg^{-1}$)	50.40	11.70	262
$CaCO_3$ ($m\ m^{-1}$) %	3.03	1.62	<0.100
NO_3^- ($mg\ kg^{-1}$)	70.60	89.9	14.70
Zn ($mg\ kg^{-1}$)	1.90	3.27	9.52
Cu ($mg\ kg^{-1}$)	1.40	2.57	3.61
Mn ($mg\ kg^{-1}$)	22	11.40	60.70
Total water-soluble salts ($m\ m^{-1}$) %	0.11	0.04	0.04
Soil plasticity (K_A)	31	38	40

Source: Agricultural Laboratory Centre, University of Debrecen

Figure 8. Autumn experiment under glasshouse
Source: Author’s own (Debrecen, 2021)

3.1.2.2. Climate condition of the autumn experiment

We regulate the glasshouse temperature using priva smart climate control computer software at 25 °C for seed germination until the seeds germinated, then the trays were transplanted to a location with a lower temperature than the growth chamber (15 to 20 °C). When the seedlings had 3 to 4 true leaves, they were transplanted to the glasshouse's direct soil in early November, where the temperature was similar (15 to 20 °C) and the relative humidity was above 50% (Table 8).

Table 8. The average weather condition during the lettuce vegetation period under glasshouse of the **autumn** experiments (Debrecen, 2019-2021)

Years	**Months**		
	October	**November**	**December**
	Temperature (°C) MEAN		
2019	17.7	17.8	17.7
2020	21.1	18.9	16.5
2021	12.2	15.3	15.5
	Relative Humidity (RH%) MEAN		
2019	63.1	65.5	55.9
2020	54.3	53.8	63.9
2021	84.2	68.8	65.7
	Light emission (W/m^2) MEAN		
2019	133.54	59.97	52.57
2020	87.68	58.24	40.84
2021	119.78	74.68	56.83
	Transplanting		**Harvesting**
2019	17th	-	21st
2020	27th	-	26th
2021	21st	-	23rd

3.1.3. Moringa plantation

On 2nd November 2018, seeds of organic *Moringa oleifera* were bought from NATURES GIFT MORINGA Ltd. In the middle of November, the seeds were sown in a glasshouse for about two months at the temperature 25 °C (Figure 9). They were subsequently transported to a bigger container and relocated to a warmer environment with more sunshine while being attentively observed.

Figure 9. Moringa transplant production under glasshouse
Source: Author’s own (Debrecen, 2019)

The seedlings were transplanted to direct soil in April 2019 under a protected plastic house from extreme weather conditions, particularly sudden frost and cold nights (Figure 10). While the temperature in the plastic house, where the seedlings were slowly developing, ranged between 15 and 25 °C. The rapid growth of moringa seedlings began when the temperature rose above 25 °C from May to June. They grew to a height of three meters in just three months, from June to August.

Figure 10. Moringa tree grown under plastic house during Spring time
Source: Author's own (Debrecen, 2020)

Moringa oleifera trees grown in plastic houses were coated with a layer of dry soil and wheat straw before being wrapped in a plastic bag one meter above the ground to protect them from the cold winter season, which lasted from the end of October to the middle of April (Figure 11).

Figure 11. The protection method of moringa trees grown under plastic house during winter season
Source: Author's own (Debrecen, 2020)

However, the plants were unable to survive, particularly when the temperature dropped below -5 ℃ for an extended length of time (more than 10 hours), causing the plants to fall leaves and dry out their brunches (Figure 12).

Figure 12. Drying out the Moringa trees due to frost in winter time under plastic house
Source: Author’s own (Debrecen, 2021)

The only trees that survived were those cultivated in glasshouses where the temperature was regulated and they were protected from winter frosts without being covered with plastic bags and straw (Figure 13).

Figure 13. Moringa trees grown under glasshouse environmental condition
Source: Author’s own (Debrecen, 2020)

3.2. Moringa leaf extract preparation

3.2.1. Moringa leaf extract from fresh leaves

Fresh leaves (approximately 40 days old) were collected from the planted moringa trees under a glasshouse at the Agrar Campus, University of Debrecen, Hungary, during the end of August and beginning of September 2019-2021. The collected leaves were washed and left to drain the water. Following that, the leaves were removed and the leaf blade was kept in a refrigerator at 4 °C for 24 hours, as reported by Yaseen and Takácsné-Hájos (2020). After that, the stored leaves were ground with 1 L water 10 kg^{-1} fresh material using a kitchen blender, and the mixture was squeezed and passed through muslin cloth according to the technique developed by Foidl et al. (2001). To remove fine debris, the extract was centrifuged for 15 minutes at 8, 000 X g for 15 minutes. The extract was then diluted to 6% with distilled water to reach the required concentration for foliar spray application. The extract was then refrigerated at 4 °C until spraying for the autumn experiment of the same year (Figure 14).

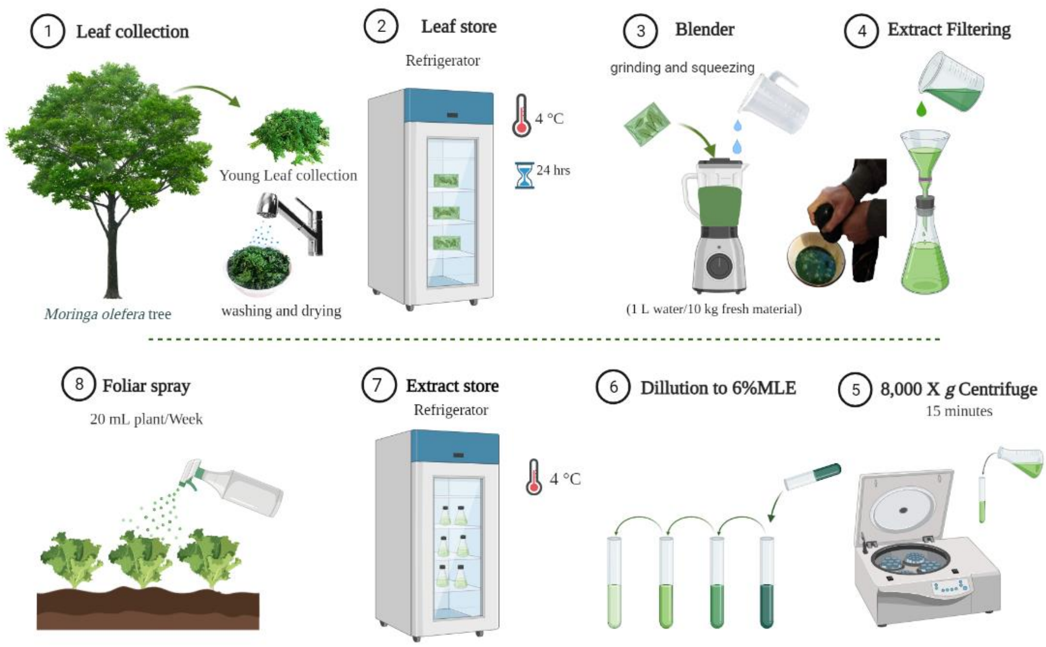

Figure 14. Moringa leaf extract preparation from fresh moringa leaves
Source: Author's own created in biorender.com (2021)

3.2.2. Moringa leaf extract from dried leaves

The extract was prepared using a method developed by Culver et al., (2012) based on Makkar and Becker's (1996) method. Fresh moringa leaves (less than 40 days old) were collected from a young tree grown at the University of Debrecen, Farm and Regional Research Institute in its Botanical and Exhibition Garden, Hungary. The leaves were washed and crushed to powder using a grinder after 72 hours of drying in a shaded area at room temperature (25-27 °C).

Afterward, 20 g of the leaf powder was mixed with 225 mL of 80 % ethanol and put aside for three hours before adding another 225 mL of 80 % ethanol continually until 625 mL of extract was obtained. The extract preparation detail is shown in Figure 15.

After homogenizing the extract, it was placed in 10 000 rpm ultra-turrax for 10 minutes before being 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 25 mL plant^{-1}.

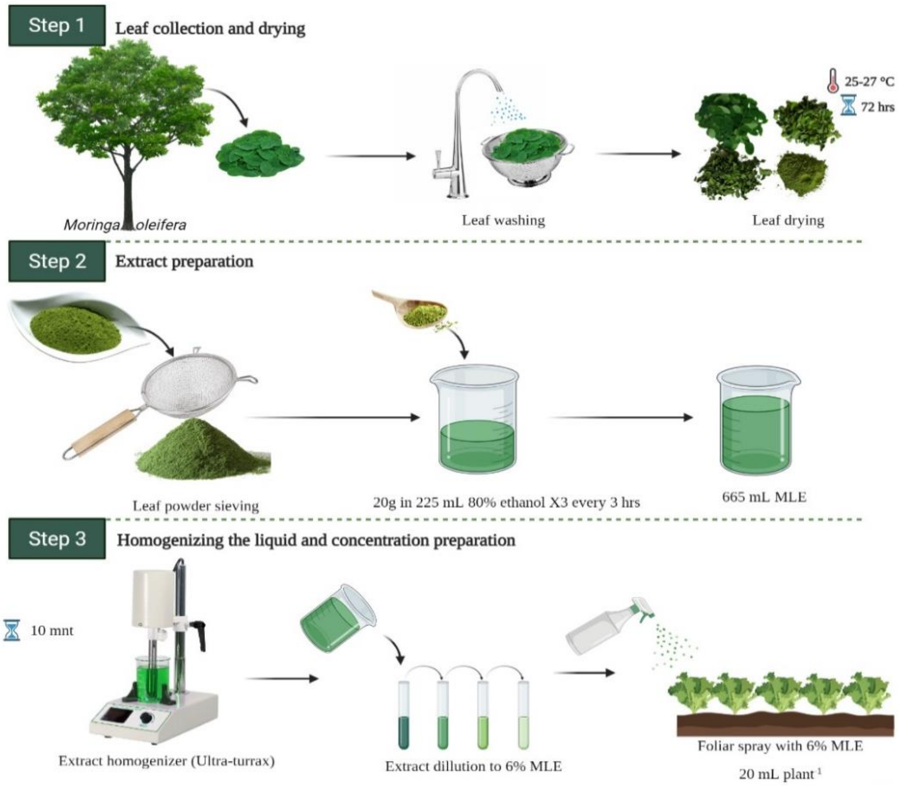

Figure 15. Moringa leaf extract preparation from dried leaf powder
Source: Author’s own created in biorender.com (2021)

Some chemical composition of moringa leaf extract (MLE) from fresh and dried leaves are shown in Table 9.

Table 9. Some chemical contents of the plant biostimulants used in the experiments (Debrecen, 2019)

Parameters	**Moringa leaf extract (MLE)**	
	Fresh (Autumn)	**Dry (Spring)**
pH	5.05	7.49
Nitrate (NO_3^-) (mg L^{-1})	0.18	1.17
Phosphorus (P_2O_5) (mg L^{-1})	100	51.40
Potassium oxide (K_2O) (mg L^{-1})	94.40	37.70
Magnesium (Mg) (mg L^{-1})	86.80	10.50
Manganese (Mn) (mg L^{-1})	0.94	0.99
Zinc (Zn) (mg L^{-1})	0.48	1.63
Iron (Fe) (mg L^{-1})	2.24	2.10
Sodium (Na) (mg L^{-1})	11.40	50.10
Boron (B) (mg L^{-1})	0.58	0.25
Sulphur (S) (mg L^{-1})	57.00	17.10
Calcium (Ca) (mg L^{-1})	326	154
Copper (Cu) (mg L^{-1})	0.32	0.51

3.3. Willow extract preparation

In February 2019-2021, young twigs (first-year) were collected from a mature weeping willow tree (*Salix babylonica*) growing on the Agrar Campus of the University of Debrecen, Hungary, when the plant was in the rest period. Martin and Stephens' (2008) technique was used to make the Willow bark extract. To obtain 3% Willow extract, 100 g of the twigs was cut into 2-3 cm pieces and placed in a jar which was filled with 400 mL of warm (80 °C) water and left for 24 hours (Figure 16). The mixture was continually stirred at 400 rpm (every 6 hours).

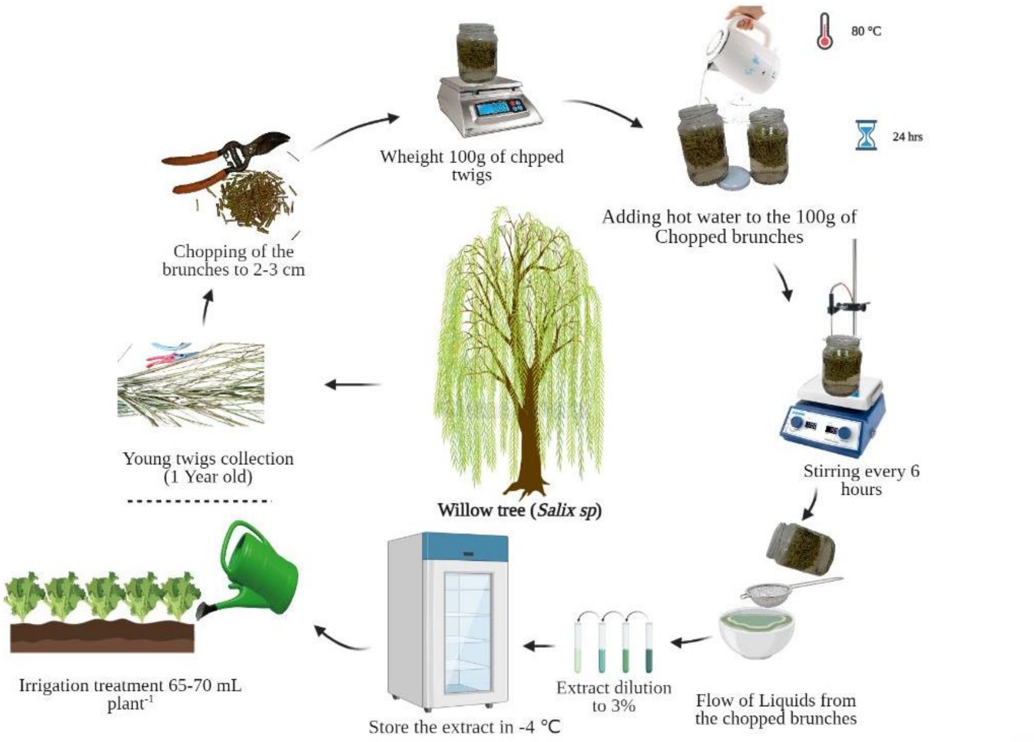

Figure 16. Willow bark extract preparation procedure
Source: Author's own created in biorender.com (2021)

The mixture was filtered through a stainless-steel sieve, then diluted to 3 % with distilled water, and the supernatants were stored in a fridge at (-4) °C for spring use. A sample extract was taken to the lab to measure some mineral contents (Table 10).

Table 10. Mineral content of the Willow bark extract (Debrecen, 2019)

Parameters	Willow extract
pH	7.2
Nitrate (NO_3^-) (mg L^{-1})	< 0.20
Phosphorus (P_2O_5) (mg L^{-1})	78.80
Potassium oxide (K_2O) (mg L^{-1})	235
Magnesium (Mg) (mg L^{-1})	6.02
Manganese (Mn) (mg L^{-1})	0.386
Zinc (Zn) (mg L^{-1})	0.069
Sodium (Na) (mg L^{-1})	14.7
Sulphur (SO_2) (mg L^{-1})	5.21
Copper (Cu) (mg L^{-1})	0.158

Source: Agricultural Laboratory Centre, University of Debrecen

3.4. The treatment with Ferbanat L (Bistep) and preparation description

Ferbanat L is an organic nano fertilizer solution which is produced in Hungary. It is allowed as "Bistep plant conditioner" in Hungary (Ördögh et al., 2019). As it was recommended by the company producer, the solution is prepared with the concentration of 0.05% and directly sprayed onto the plant leaves. The composition of biostimulant is shown in Table 11.

Table 11. Ferbanat L (Bistep) mineral content (Igazgatóság, 2011)

Parameters	Bistep
pH	7.40
Nitrate (NO_3^-) (mg L^{-1})	0.02
Phosphorus (P_2O_5) (mg L^{-1})	0.03
Potassium oxide (K_2O) (mg L^{-1})	0.30
Magnesium (Mg) (mg L^{-1})	0.02
Manganese (Mn) (mg L^{-1})	0.007
Zinc (Zn) (mg L^{-1})	0.008
Molybdenum (Mo) (mg L^{-1})	0.09
Organic material (%)	25.00
Iron (Fe) (mg L^{-1})	0.01
Boron (B) (mg L^{-1})	0.0002
Total number of germs (number cm^{-3})	0.8 10^7
Micro fungus (number cm^{-3})	1.0 x 10^2
Density (kg m^{-3})	1.01
Dry matter content (m/m %)	1.3
Organic matter content (m/m %) no.	25.0
Arsenic (As) (mg L^{-1})	10.0
Cadmium (Cd) (mg L^{-1})	2.0
Cobalt (Co) (mg L^{-1})	50.0
Chromium (Cr) (mg L^{-1})	100.0
Copper (Cu) (mg L^{-1})	100.0
Mercury (Hg) (mg L^{-1})	1.0
Nickel (Ni) (mg L^{-1})	50.0
Lead (Pb) (mg L^{-1})	100.0
Selenium (Se) (mg L^{-1})	5.0

3.5. Morphological descriptors of the tested varieties

Butterhead Lettuce “Május királya” (“May King”)

Heading: thin to relatively thick, medium size, delicate leaves with a clear midrib; leaf shape circular to transverse broad elliptic; no incised edge in general; head shape broad elliptic to transvers elliptic. This cultivar produces higher number of leaves between 30 to 40 leaves. It has too many wrinkles mainly inner leaf sides (Figure 17).

Figure 17. Butterhead Lettuce “Május királya” (May King) cultivar
Source: Author’s own (Debrecen, 2021)

Batavia type “Kobak”

open to strong heading; medium to thick, blistered leaves, often yellowish or medium green; leaf border with weak to strong undulation. This cultivar yields between 25 and 35 leaves. The inner leaves are pale yellow leaves, gradually becoming darker yellow as they progress to the middle and outer leaves, when the colour changes to yellowish to medium green (Figure 18).

Figure 18. Batavia type “Kobak” cultivar
Source: Author’s own (Debrecen, 2021)

Head lettuce “Great Lakes”

It has a crunchy big head size and thick and crispy leaves that are mainly green with a scarcely to very sharply cut leaf edge. It produces fewer leaves than the other two varieties, with about 15 to 25 larger leaves. The leaves are bending to create a ball-shaped circle around the inner head (Figure 19).

Figure 19. Iceberg lettuce “Great Lakes” cultivar
Source: Author’s own (Debrecen, 2021)

3.6. Biostimulant treatments
3.6.1. Spring plant treatments

These experiments were conducted on a continuous basis for three years, from spring 2019 to spring 2021, at the Agrar Campus of the University of Debrecen in Hungary. Three lettuce cultivars ("*May King", "Kobak", "Great Lakes*") were organised in a full randomized block design with three repetitions, with 20 plants chosen for each replication to be treated.

The following treatments were applied once every two weeks for four weeks throughout the growth season, beginning the first week after transplanting the seedlings.

- **Control (untreated plants)**: the plants were sprayed with distilled water.
- **Willow treatment (W)**: the plants were irrigated with willow bark extract with the concentration of 3% with the amount of 50-70 mL plant^{-1}
- **Bistep treatment (B)**: the plants were foliar sprayed onto the plant leaves after mixing with 2 drops of detergent with the concentration of 0.05% solution with the amount of 15-20 mL plant^{-1}.
- **The combination of willow bark extract (3%) and Bistep (0.05%) (W+B)**: the amount of 50-70 mL plant^{-1} (willow bark extract 3%) + 15-20 mL plant^{-1} of 0.05% Bistep.
- **Moringa leaf extract (MLE)**: The plants were foliar sprayed with moringa leaf extract 6 % (MLE) prepared in the leaf powder from moringa leaves stored in the end of August of the previous year from trees grown by ourselves, in the amount of 15-20 mL plant^{-1}.

Note: Because the moringa trees were not fully developed in 2019 to collect the leaves and prepare the extract for the treatment, the plants were only treated with willow and Bistep biostimulants in the spring 2019 trial.

3.6.2. Autumn plant treatments

For the autumn experiment from 2019 to 2021, we only applied moringa leaf extract (MLE), which we prepared from the fresh moringa leaves grown by ourselves. So that, similar to the spring experiment, three lettuce cultivars ("May King," "Kobak," and "Great

Lakes") were organised in a full randomized block design with three repetitions, with 20 plants chosen for each replication to be treated. However, the foliar application was carried out every ten days instead of two weeks as follows:

- **Control (untreated plants)**: the plants were sprayed with distilled water.
- **Moringa leaf extract (MLE)**: the plants were foliar sprayed with the moringa leaf extract 6% (MLE) prepared in the fresh leaves with the amount of 15-20 mL plant^{-1}.

3.7. Laboratory measurements

Macro and micro-nutrient analysis

Total dry matrix content was determined by drying the samples at 105 °C until mass constancy was reached (min. 4 h).

Microelements (Ca, K, Mg, Na, S, and P) were determined using the ICP-OES (iCAP 7400, Thermo Scientific) technique. From the properly prepared sample, 0.5 g was measured in a high-pressure Teflon bomb. 5 ml of distilled cc. HNO_3 and 3 ml of 30% H_2O_2 were added. It has been sealed and digested in Ethos Plus Microwave Digestion System (Milestone) applying a method (Application Note 076) of the manufacturer [3 min 85°C, 9 min 145°C, 4 min 200°C, 14 min 200 °C]. After cooling the Teflon bombs, the digested samples were diluted to 50 ml, homogenized and filtered (MN 640 W, Millipore) before element analysis. This was based on the method by (Krüger et al., 2014).

Determination of total polyphenol content

Total polyphenols in mg Gallic acid equivalent (GAE).100 g^{-1} fresh product were determined by Folin-Ciocalteu spectrophotometric method with gallic acid reference material based on the method by Meda et al., (2005).

Determination of vitamin C content

The sample was homogenized, and 10.0 to 20.0 g was weighed. Ascorbic acid was extracted from the weighed sample with oxalic acid solution and subsequently titrated with the 2,6 dichlorophenol-indophenol to salmon pink colour. Ascorbic acid content was calculated from the added volume (Ciancaglini et al., 2001).

Determination of nitrate

Frozen samples were homogenized and 40 g was measured. 35 g extraction buffer ([5% (v/v) HCl and 7.5% (v/v) ammonium, pH 9.6-9.7] and 325 g distilled water were added to the sample. The mixture was homogenized for 1 min and filtered through filter paper, and the filtrate was freshly analysed for the nitrate content. The general principle of the nitrate (NO_3^-) determination is using multichannel Continuous Flow Analyzer (CFA, FIASTAR 5000, Foss, Germany) on 540 nm after the reduction in copper coated cadmium column ($NO_3^- + 2^{e-}$ ® NO_2^-) to form diazo compound with colour-developing Griess reagent based on the method by (Kmecl et al., 2007).

3.8. Physical (morphological) parameters

The physical parameters of the lettuce cultivars were measured based on some standards which are used to evaluate or measure some quality parameters of lettuce as follows:

- Head structure: was evaluated based on the scale from 1 to 10, where 1 is for a highly loose head structure and 10 indicating a firm head structure.
- Closing of heads: was evaluated based on the scale from 1 to 10, where 1 is for very opened head and 10 for very closed or compacted heads.
- Head weight: was measured using a digital scale in (g $head^{-1}$).
- Root weight: after cutting the roots from very bottom of the lettuce heads and washing up with water, it was measured using a digital scale in (g $root^{-1}$).
- Head diameter: a measure tape (0-100 cm) was used to measure head dimeter in horizontal level (Figure 20).
- Internal stem size: after removing all the leaves, the internal stem size was measured using a 30 cm ruler (Figure 21).
- Number of leaves: was measured by counting outer, middle and inner leaves of each replication.

Figure 20. Lettuce head diameter measurement
Source: Author’s own (Debrecen, 2021)

Figure 21. Lettuce internal stem size measurement
Source: Author’s own (Debrecen, 2021)

3.9. Determination of SPAD and NDVI

A portable chlorophyll meter SPAD-502 (Brand: Konica Minolta, Japan) was used to measure the chlorophyll content and normalized difference vegetation index (NDVI) in lettuce cultivars using the technique developed by (Madeira et al., 2003). Chlorophyll content can be evaluated by non-destructive method as SPAD instrument instead of destructive method (Ali et al., 2021). Ten fully mature lettuce plants from each cultivar were randomly measured in three leaves each (middle leaves in three directions), and the average was used as a single SPAD vale for statistical analysis. The trimble GreenSeeker handheld crop sensor Model HSC-100 (Trimble Navigation Unlimited, Sunnyvale, CA), on the other hand, was utilized to measure the NDVI value at the same vegetable growth stage by scanning twenty plants in each variety and treatment at a height of about one meter above the ground. This instrument has an effective source of light that prevents sunlight from affecting readings (Bell, et al., 2013). The data was then recorded for each plant for examination and review (Figure 22).

Figure 22. SPAD and NDVI measurements in the experiment of autumn and spring (2019-2021).
Source: Author’s own (Debrecen, 2020)

3.10. Statistical analysis

The data from the three-year study's spring experiment (2019-2021) were subjected to statistical analysis using one-way analysis of variance (ANOVA) in SPSS (SPSS Inc., Chicago, IL, USA) computer program, version 25.0 (IBM Corp. Released, 2017). At a significance threshold of $p \geq 0.05$, the Duncan^{a,b} multiple range test was performed to validate the differences between the compared averages.

To assess if there were significant differences between the two groups (In the autumn experiments), the means of five samples were subjected to the independent sample *t-test* at a significance threshold of $p \geq 0.01$ for the glasshouse experiments.

4.0. RESULTS AND EVALUATIONS

4.1. Morphological parameters

The morphological parameters can be the second most important characteristics for fresh product purchasing among the low-income households after the product price (Webber et al., 2010) or it might be the first among medium or high income households. In our research studies, the most essential marketable quality criteria were separated into two categories: measured parameters using instruments and scaled parameters utilizing quality evaluation scaled procedures.

4.1.1. Effect of plant biostimulant on vegetative parameters of lettuce

The most important marketable parameters, which are among the first consumer attractions to purchase a new product, were evaluated. For example, head weight is not only important for purchasing the product, but it is also important for growers commercially. Other measured parameters are also important for growers and customers. However, some other parameters might have indirect connection to the quality parameters during the growing of the product as internal stem size and root weight. Though, the used plant biostimulants could significantly improve some of the measured parameters which are concerned by the growers and customers. Our three-year mean results show that there are no significant changes in lettuce head weight in plants treated with plant biostimulants. Plant biostimulants such as Willow bark extract (W), Bistep (B), and Willow bark extract + Bistep (W+B) did, however, slightly increase the head weight of several lettuce cultivars when compared to control plants. In contrast, a slight reduction in head weight was observed in lettuce treated with moringa leaf extract (% MLE) (Figure 23).

Significant differences were mostly seen between the growing years, with some biostimulants influencing better in cooler environments than in warmer. The best spring season result was obtained in 2019 (Appendix 1), when the temperature was cooler throughout the growth months of March, April, and May (Table 8).

On the other hand, the plant biostimulants had no significant influence on the head diameter and root weight (Figure 23). However, the internal stem size of the treated plants was enhanced by 6 % MLE, which might be because moringa leaf extract can influence better in warmer climate conditions than other plant biostimulants. This means that those biostimulants could improve the heat tolerance in the lettuce cultivars under specific weather conditions (Figure 23). These might be because those biostimulants contain some plant growth regulators or phytohormones *Indole 3-butyric acid* (IBA) and Salicylic acid (SA) mainly in willow bark extract (Deniau et al., 2019) and Bistep is a type of humic acid that can improve soil structure (Cimrin and Yilmaz, 2005).

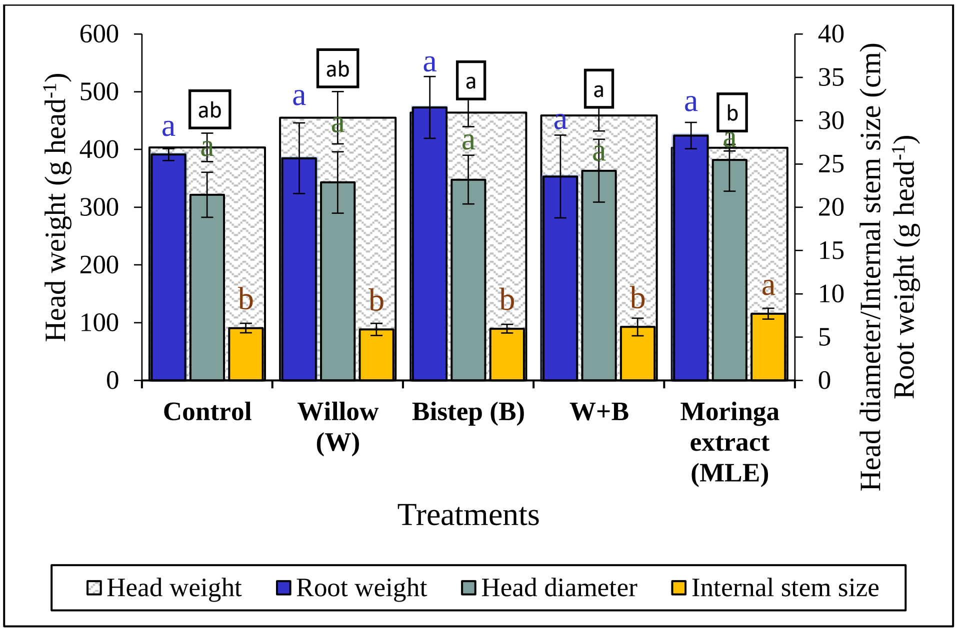

Figure 23. Effect of plant biostimulants on some measured vegetative parameters of lettuce in the mean of varieties in **spring** of 3 years (Debrecen, 2019-2021)

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 Duncan[a,b] Multiple Range Test

The combination of Willow bark extract+ Bistep (W+B) and Bistep could provide a superior outcome of physical parameter improvement for the majority of the evaluated parameters. A similar result has been provided by Paradiković et al. (2011) where they found a higher yield in mixing four commercial plant biostimulants (Radifarm®, Megafol®,Viva® and Benefit®) on different yellow pepper varieties.

We repeated similar experiments in the autumn using only 6% MLE to compare the effect of plant biostimulants on the measured morphological parameters. In general, the physical parameters measured in autumn were much lower than in spring. The mean results from autumn experiments show that a plant biostimulant of 6% MLE has a similar effect on the measured quality parameters (Figure 24). Some lettuce morphological parameters improved, including head weight, head diameter, internal stem size, and root weight. There were, however, no discernible differences. The only significant differences were found between growing years, with some results seen in 2019 and 2021 (Appendix 4). Our three-year mean of results shows that plant biostimulant of 6%MLE could improve the head weight of lettuce cultivars by 24%. This result agrees to the results by many research articles which show that plant moringa leaf extract can improve the final yield of many vegetables as growth, yield and nutritional quality enhancement by the application of MLE every two weeks on cabbage (Hoque et al., 2020), tomato yield (Bashir et al., 2014), cauliflower (Rana et al., 2019) and in many other vegetables shown in research papers.

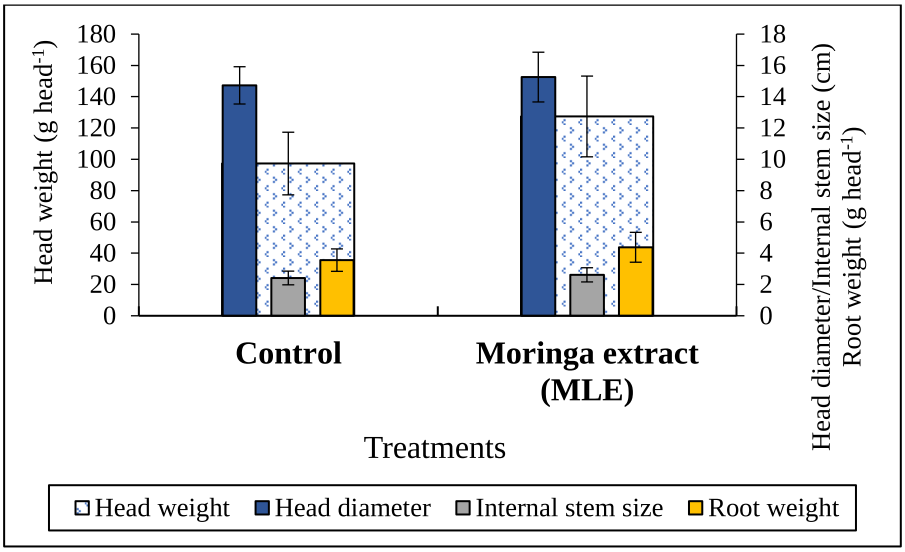

Figure 24. Effect of plant biostimulants on some measured vegetative parameters of lettuce in the mean of varieties in **autumn** of 3 years (Debrecen, 2019- 2021)

4.1.2. Effect of plant biostimulant on some scaled vegetative parameters of lettuce

The scaled method was used to measure head structure and head closure, as detailed in chapter of Materials and Methods. These features are essential for consumers purchasing products in markets. This physical attribute improves the appearance of the lettuce and entices customers purchases. Our mean results for spring seasons in Figure 25 demonstrate that all of the data indicate that the treated plants could have a better head structure, but only the treated plants with Willow bark extract (W) showed a significant improvement. Furthermore, the head closing or the compacting of the heads or head opening is also measured because they are also important for the consumer buying and retail packaging. Similar to the head structure, the head closing was positively influenced by plant biostimulant treatments for all three years. However, among the treatments, the significant results were performed for the lettuce varieties treated with Bistep, W+B and MLE, whereas no significant difference was seen in Willow (W) treatment (Figure 25). The growing years also had an effect on the two above-mentioned scaled parameters, where the biostimulants could all have a significant influence on the head structure in 2020, whereas, with the exception of willow bark extract, other biostimulants could significantly improve head closing (Appendix 2). We also noticed a wide range of reactions to plant biostimulants during our three-year study. Leaf number, on the other hand, was mostly impacted by genetic variations rather than plant biostimulants. There was a minor variation in leaf number, but it was not statistically significant. For example, the mean of growing years shows that plants treated with 6% MLE had the highest leaf number (40.44 leaves plant^{-1}), whereas MLE could significantly increase the number of leaves in 2020 (46.6 leaves plant^{-1}), and the combination of Willow + Bistep (W+B) produced the highest number of leaves in 2021 (38.5 leaves plant^{-1}) (Appendix 2).

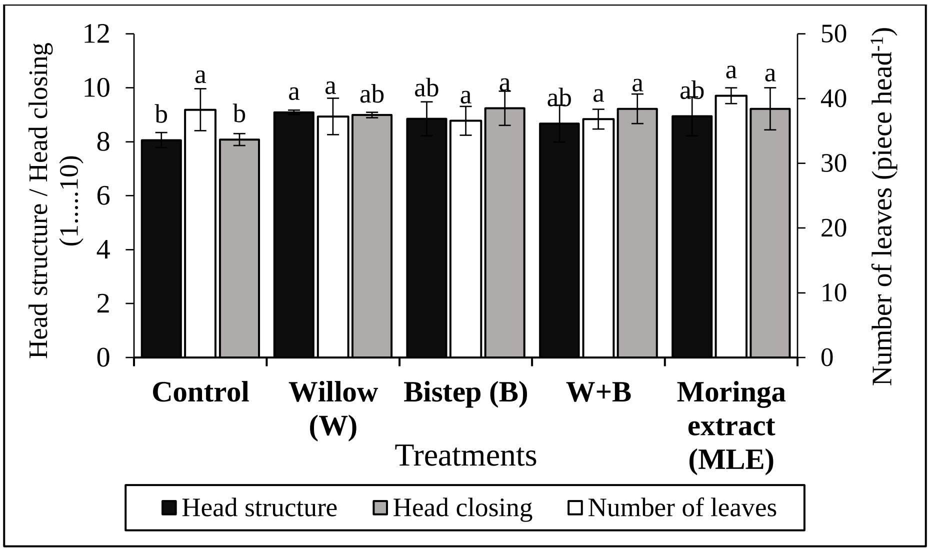

Figure 25. Effect of plant biostimulants on some scaled vegetative parameters of lettuce in the mean of varieties in **spring**time, (Debrecen, 2019-2021)

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 Duncan[a,b] Multiple Range Test

The mean results of the autumn growing season, on the other hand, show no significant improvement in scaled morphological parameters and leaf number (Figure 26). The head structure and number of leaves of lettuce cultivars treated with moringa leaf extract (6% MLE) show a slight improvement, while head closure is similar between treated and untreated plants.

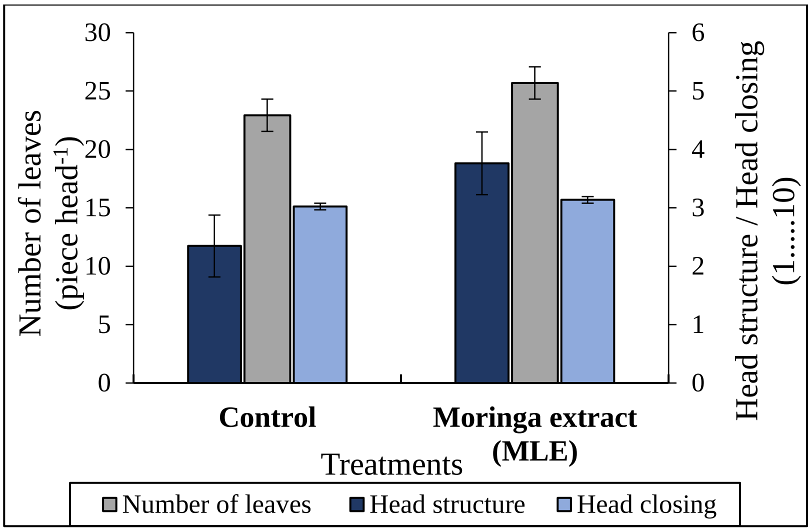

Figure 26. Effect of plant biostimulants on some scaled vegetative parameters of lettuce in the mean of varieties in **autumn** season, (Debrecen, 2019-2021)

4.1.3. Effect plant varieties on some measured vegetative parameters

Plant varieties are distinguished primarily by their physical characteristics. We evaluated three different lettuce cultivars with different genotypes in our research studies: Butterhead Lettuce "May King," Batavia type "Kobak," and Iceberg type "Great Lakes" cultivars because each one has different vegetative characteristics. Plant phenotypes can be influenced by both genetic and environmental factors, as well as their interactions (Fasahat et al., 2015; Mou, 2009).

Figure 27 indicates that throughout the growth seasons, the "Great Lakes" variety produced significantly higher head weight (g head^{-1}), although no significant variations were identified between the "May King" and "Kobak" cultivars. On the other hand, there were no significant differences in head diameter amongst the three lettuce cultivars; the only significant differences were between the growing years of 2019 and 2020, with the "May King" variety having the biggest head diameter in 2020 at 31.25 cm head^{-1} (Appendix 3).

There was also a significant difference in internal stem size (cm head^{-1}) among cultivars, which was mostly related to the number of leaves. Plants with more leaves, such as "Kobak" have a longer internal stem size than other cultivars since the leaves in lettuce vegetable are closely tied to the stem.

In contrast, no substantial variation in root weight was identified across the examined cultivars; the only changes were attributed to growing years. For example, in 2020, the most root weight was measured in the "May King" variety, while in 2021, it was in the "May King" and "Kobak" cultivars, which might be due to lower relative humidity in the soil (Appendix 3).

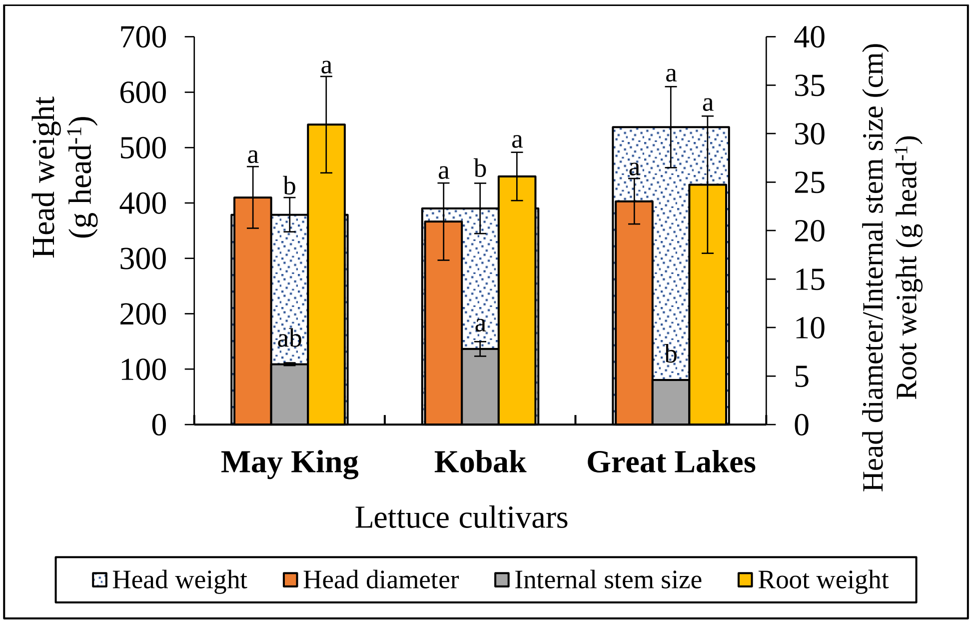

Figure 27. Effect of lettuce genotype on some measured vegetative parameters of lettuce in the mean of treatments in **spring** season, (Debrecen, 2019-2021)

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 Duncan[a,b] Multiple Range Test

In contrast, the means of growing years show that lettuce cultivars grown in autumn seasons had no statistically significant differences in the measured morphological parameters (Figure 28). However, changes were mostly observed over time based on environmental factors, particularly light intensity. For example, the significantly largest head weight was measured in the growing year of 2021, mostly in the "Great Lakes" variety, which could be attributed to higher light emission in the late growing months of

November and December (Table 10). Similar results were observed for the other measured physical parameters which can be seen in Appendix 6.

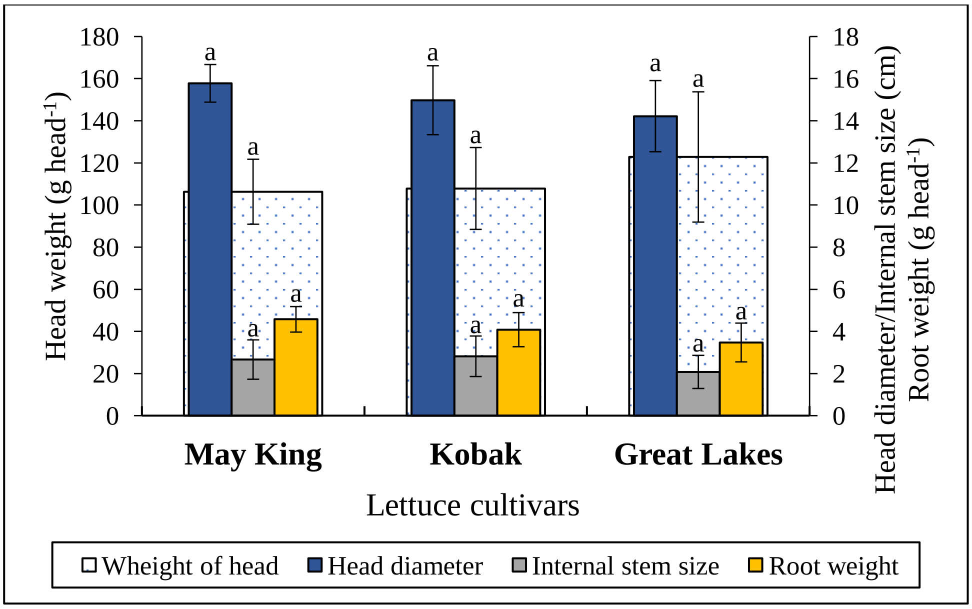

Figure 28. Effect of lettuce genotype on some measured vegetative parameters of lettuce in the mean of treatments in **autumn** season, (Debrecen, 2019- 2021)

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 Duncan[a,b] Multiple Range Test

4.1.4. Effect of lettuce varieties on some scaled vegetative parameters of lettuce

The phenotypic appearance in lettuce is determined by environmental (E) and genetic (G) factors, as well as their interactions (Burns, et al., 2011). The exterior forms or head development of lettuce in the same cultivars are clearly distinguishable. Some produce well-formed heads, known as Crisphead as in "Great Lakes", while others produce unformed or mostly divided leaves, known as Butterheads as "May Kings" or Batavia types as "Kobak" varieties. In the spring seasons, our mean result shows that the genotypes had the same result for head structure and head closure (Figure 29). However, there were significant differences between growing years, with the "May Kings" and "Kobak" varieties producing significantly better head structure and head closing compared to "Great Lakes," while the "Kobak" variety produced significantly poorer head structure and head closing in 2021 (Appendix 4).

The number of leaves, on the other hand, was perhaps the most strongly influenced morphological parameter by genotype differences. The "Kobak" lettuce variety produces a significantly higher number of leaves, followed by "May Kings," and the "Great Lakes" lettuce variety had the lowest number of leaves.

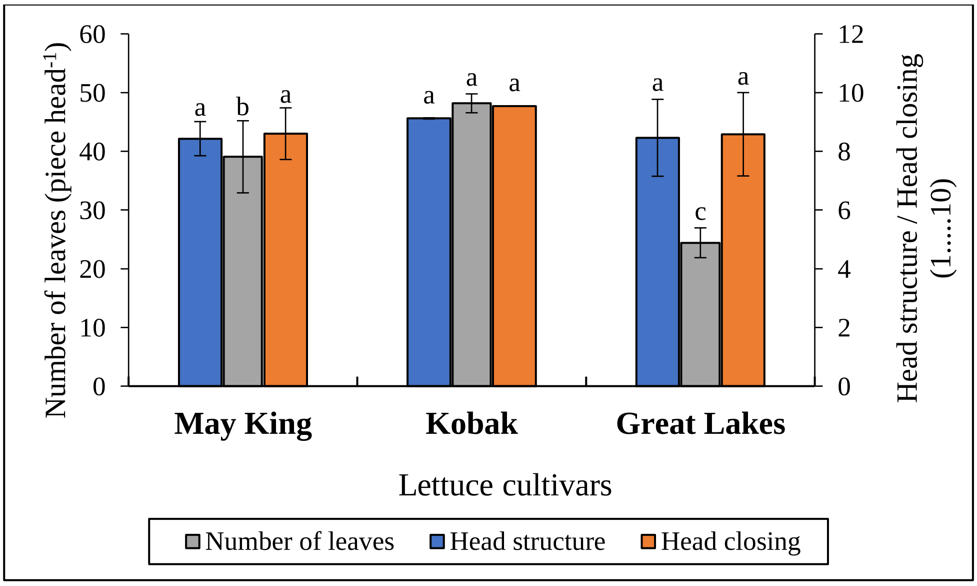

Figure 29. Effect of lettuce genotype on some scaled vegetative parameters of lettuce in the mean of treatments in **spring** season, (Debrecen, 2019- 2021)

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 Duncan[a,b] Multiple Range Test

Despite the fact that genetic factors dominated the number of leaves. There was a strong connection between the number of leaves and the internal stem size. For example, the "Great Lakes" cultivar has a larger head size but fewer leaves with shorter internal stem size than the leafy cultivars "May King" and "Kobak" (Figure 30).

The same lettuce cultivars grown in autumn season had a similar result on the head structure, head closing and number of leaves as it was grown in spring season. The mean results show the only significant variation in the number of leaves, whereas no significant differences were recorded for the head structure and head closing (Figure 31). However, the significant better head structure and head closing was seen in the growing years of 2019 and 2020 where the glasshouse temperature was lower than in 2021 (Appendix 8).

Figure 30. Lettuce varieties (a. "May King". b. "Kobak" and c. "Great Lakes") of the research experiments in spring season.
Source: Author's own (Debrecen, 2021)

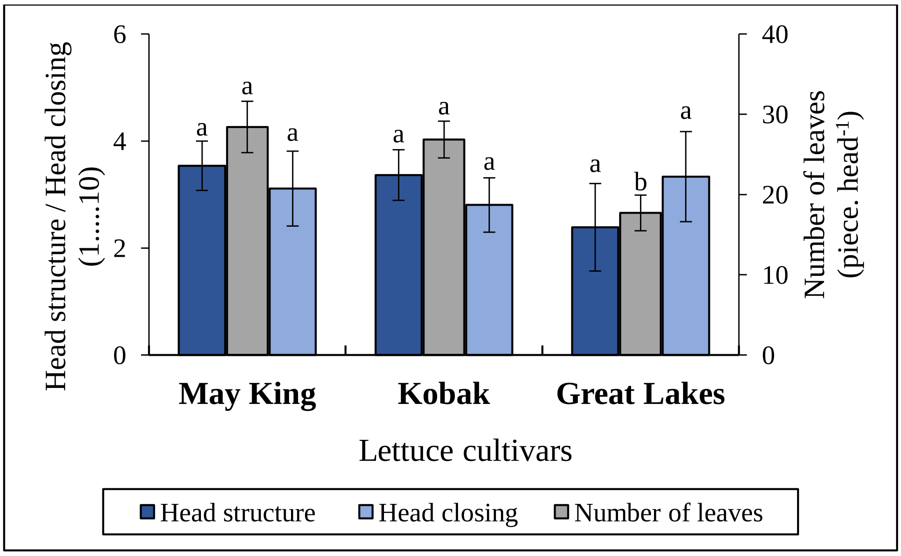

Figure 31. Effect of lettuce genotype on some scaled vegetative parameters of lettuce in the mean of treatments in autumn season, (Debrecen, 2019-2021)

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 Duncan[a,b] Multiple Range Test

4.1.5. The interaction effect of plant biostimulant and variety on some measured vegetative parameters of lettuce

The major influence factors on some measured vegetative parameters were the interaction of genetic, growing years and treatments. As a result, while producing vegetables for commercial reasons, it is critical to examine these aspects and their interactions.

Head weight is the most essential quality metric in the commercial world because it determines the product's final yield (Fonseca, 2006). The "Great Lakes" variety had the highest head weight in all three years of the spring experiment, 2019-2021, followed by "Kobak" and "May King." The "Great Lakes" variety was the most influenced by the interplay of the factors, while "May King" was the least influenced variety, followed by "Kobak," according to the results of the three-year tests (Figure 32). The mean of years indicates that the interaction of biostimulant treatments of Willow bark extract (W) and W+B, and the "Great Lakes" variety had significantly increased head weight by 32 % and W+B by 25 % compared to untreated lettuce plants, but no significant differences was measured for Bistep and 6% MLE. On the other hand, "Kobak" and "May King" was least influenced by the interaction of treatments and genotypes.

The main differences were noticed among the growing years, where the highest head weight was in the growing years of 2019 and 2021 (Appendix 5), this might be because of the soil mineral content of $NO_3^-$was higher and soil pH were less acidic (Table 7). The interaction of plant biostimulants Willow (W) and W+B with the "Great Lakes" variety had a significant influence in improving head weight in all years 2019-2021, whereas 6% MLE and Willow (W) had the lowest. Bistep interacted favourably only with the "Great Lakes " variety.

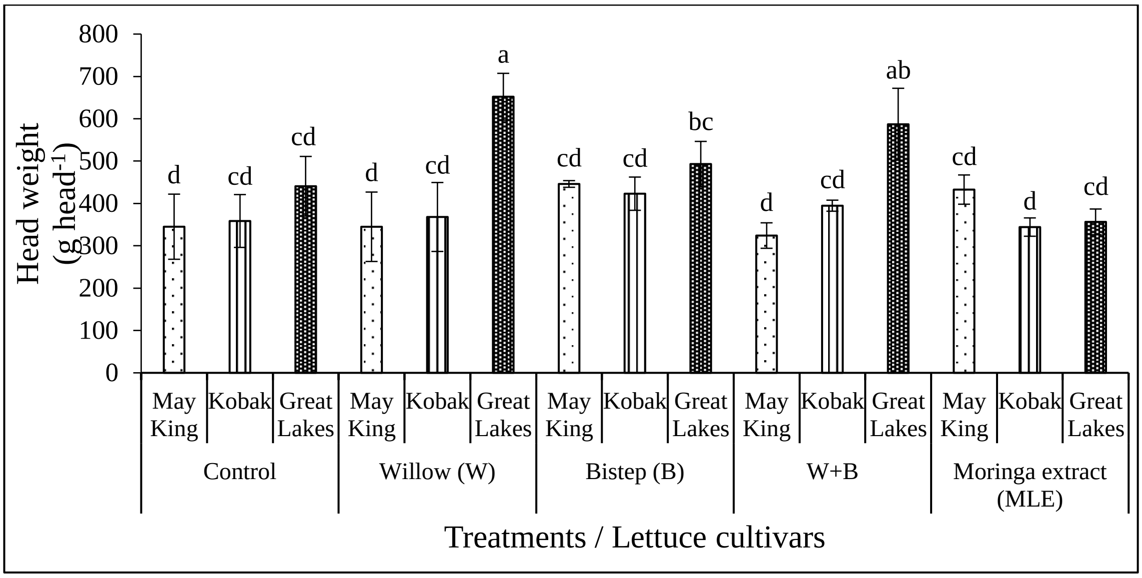

Figure 32. The interaction effect of plant biostimulant and variety on lettuce head weight grown in **spring** season, (Debrecen, 2019-2021)

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 Duncan[a,b] Multiple Range Test

The mean data from the autumn growing years (Figure 33), on the other hand, show no significant difference in the interaction of treated and untreated lettuce cultivars. It can be seen that the treated lettuce cultivars had a slight improvement in head weight, but there was no significant difference between them. The interaction of plant biostimulants with lettuce genotypes had a significant impact in the growing year of 2020 in the "Kobak" and "Great Lakes" varieties treated with 6% MLE, as well as "Great Lakes" treated with 6% MLE in 2021 (Appendix 6).

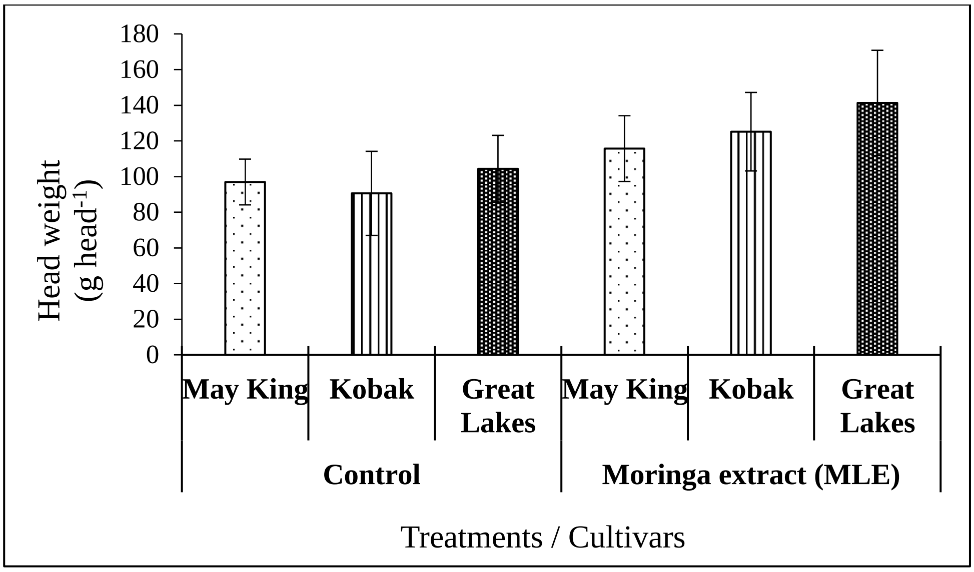

Figure 33. The interaction effect of plant biostimulant and variety on lettuce head weight grown in **autumn** season, (Debrecen, 2019-2021)

Because lettuce leaves are the most edible part of the plant, the number of leaves is considered one of the most important quality indicators in lettuce evaluation. This, however, is primarily influenced by genetic inheritance rather than environmental or interaction factors. For example, "Kobak" naturally produces more leaves than the other two varieties of "May King" and "Great Lakes" (Yaseen and Takácsné-Hájos, 2021a).

Figure 34 illustrates the interaction of plant biostimulants and lettuce genotypes, which influences the number of leaves. The average of the years shows that only the "Great Lakes" variety treated with 6% MLE had significantly more leaves than the control.

Moreover, head diameter, or the plant's horizontal extension, reflects the morphology and form of the lettuce's head. The head diameter improved somewhat as a result of the interaction impact of the parameters. The sole significant change in the spring growing season was seen in the "May King" genotype after treatment with 6% MLE (Figure 34). Significant variations occurred mostly throughout the growing years, which might be attributed to how each treatment and variety reacted to distinct weather conditions. For example, in 2019, the major significant difference was discovered for the "Great Lakes" variety treated with Bistep; however, in 2020, the "May King" variety treated with Bistep and 6% MLE resulted in a substantial largest head expansion compared to the control. The only significant change in 2021 was for the "May King" genotype treated with W+

B. As a result, the "May King" variety was among the most effected by the combination of the variables (Appendix 5).

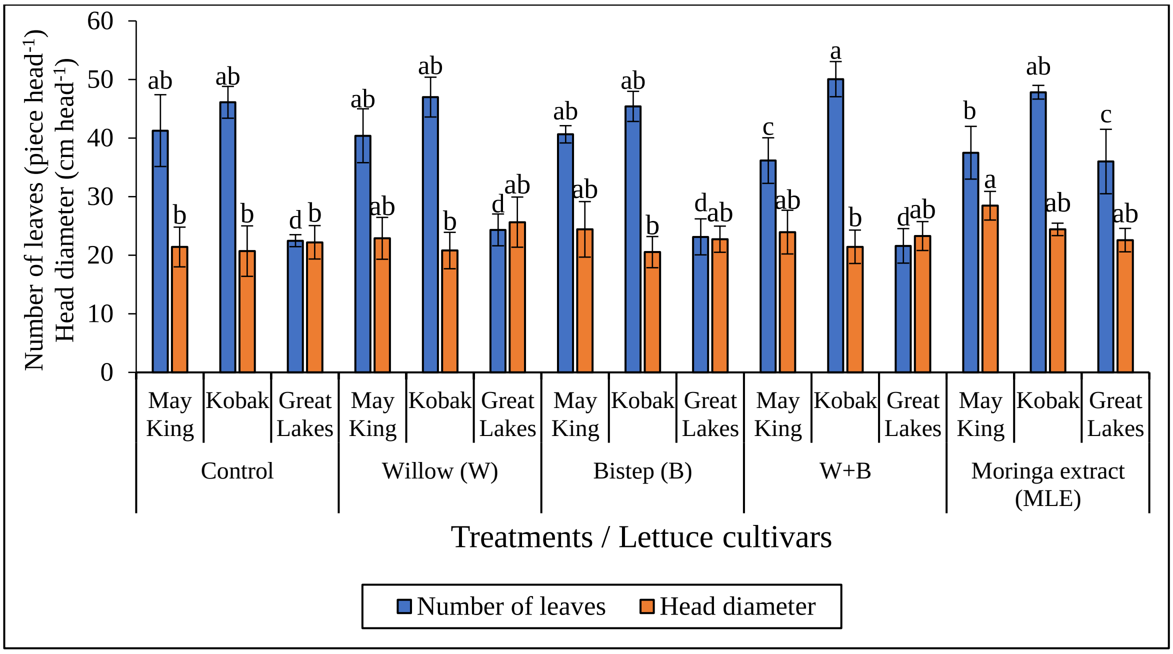

Figure 34. The interaction effect of plant biostimulant and variety on number of leaves and head diameter of lettuce grown in **spring** season, (Debrecen, 2019-2021)

Means within the same column followed by the same letter(s) are not significantly different at the probability level of (*P≤0.05*) according to Duncan[a,b] Multiple Range Test

Lettuce is known as a cool-season vegetable because it grows well in areas where the sunlight is low and the temperature is steadily decreasing, which is commonly grown in the autumn season. However, growing lettuce in autumn seasons is known for high nitrate accumulation and lower yield value compared to those grown in spring season (Urlić et al., 2017). Our three-year mean data show no significant difference for interaction of plant biostimulants with the lettuce grown in autumn season in improving number of leaves and head diameter (Figure 35). The only significant differences in the number of leaves and head diameter were between growing years, where the interaction of 6% MLE with "May King" having a significantly higher number of leaves in all three grown years (Appendix 8). The "Kobak" variety treated with 6% MLE had a significantly higher number of leaves and head diameter in the growing year of 2020 (Appendix 6), whereas the highest leaf number in the "Great Lakes" variety was recorded in the season of 2021.

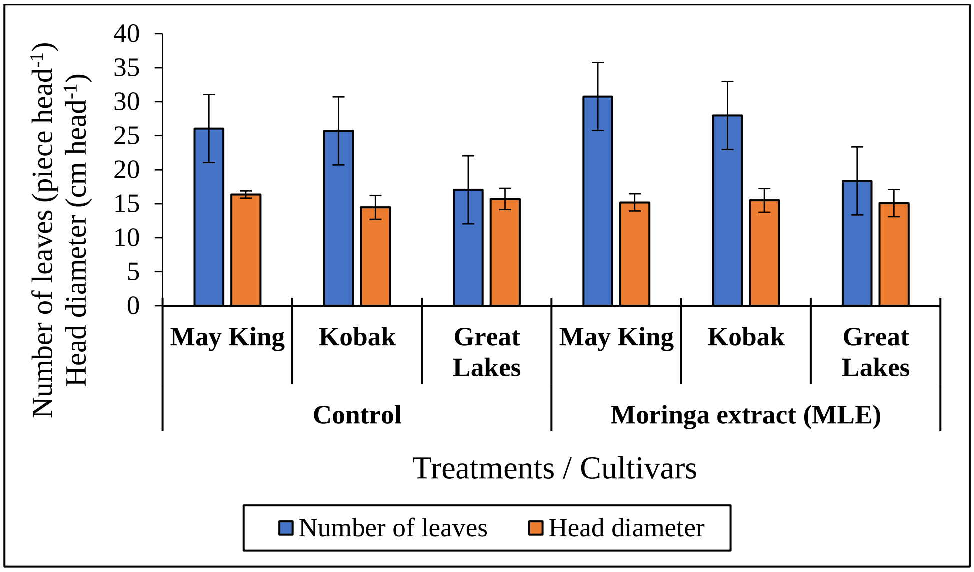

Figure 35. The interaction effect of plant biostimulant (6% MLE) and varieties on number of leaves and head diameter of lettuce grown in **autumn** season, (Debrecen, 2019-2021)

The internal stem growth of lettuce vegetables varies depending on the number of leaves and environmental factors, most notably temperature. For example, the "Great Lakes" type naturally produces less leaves ranging from 18 to 28 leaves, resulting in the smallest internal stem size when compared to the other two tested varieties. This might also be because the "Great Lakes" variety is a day-neutral variety in terms of bolting or blooming needs (Ryder, 1979). The reduction of the rapid growing in internal stem size in lettuce has a significant economic benefit because it delays maturity, bolting, and seed release (Yaseen and Takácsné-Hájos, 2021b). According to our findings, cultivars with more leaves had longer internal stem diameters; nevertheless, the interaction variables also had an impact on lettuce stem growth. Figure 36 shows that "May King" variety treated with Willow (W) significantly reduced the internal stem size growth, whereas "Great Lakes" sprayed with 6% MLE had significant improvement of internal stem size in spring season. In this case, this biostimulant of 6%MLE may be beneficial for lettuce varieties in which the stem is the primary edible component.

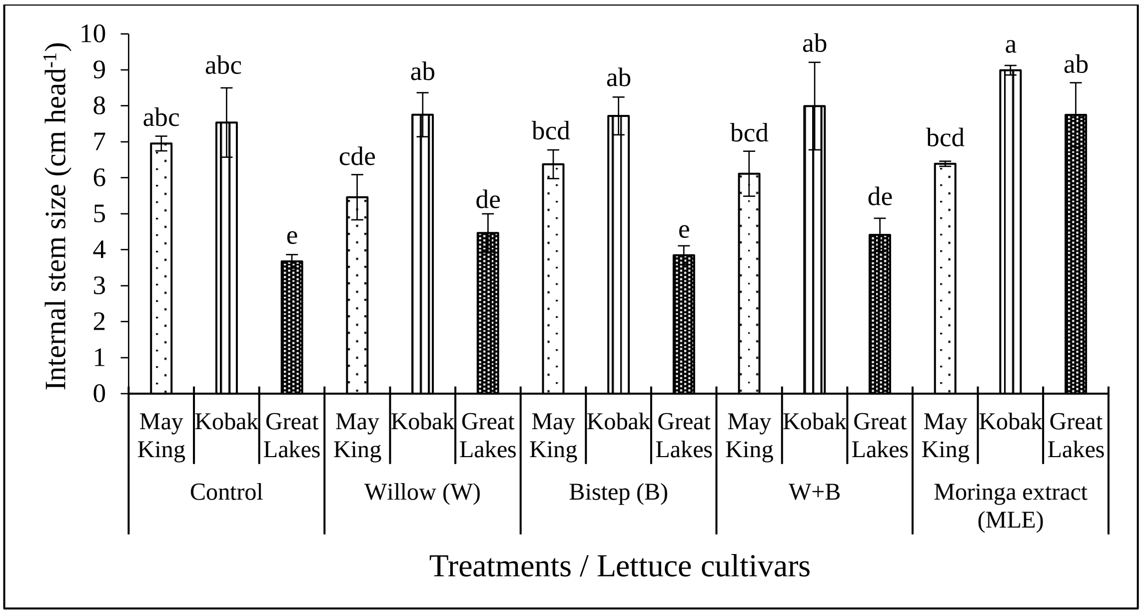

Figure 36. The interaction effect of plant biostimulant and variety on internal stem size of lettuce grown in **spring** season, (Debrecen, 2019-2021)

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 Duncan[a,b] Multiple Range Test

It is worthwhile to compare the use of 6% MLE to lettuce varieties grown in the autumn season to those grown in the spring season for the internal stem size. As illustrated in Figure 37, no significant improvement was observed for the interaction of 6% MLE with lettuce cultivars grown in the autumn season. This is critical because increasing lettuce internal stem size causes the plant to blossom fast or bolt, which is known as a negative quality trait in the lettuce and *Brassica* family (Hao et al., 2018; Yaseen and Takácsné-Hájos, 2021b; Yaseen and Takácsné Hájos, 2021c). This could be because moringa leaf extract can influence better in cooler weather conditions in autumn than in warmer weather conditions in spring.

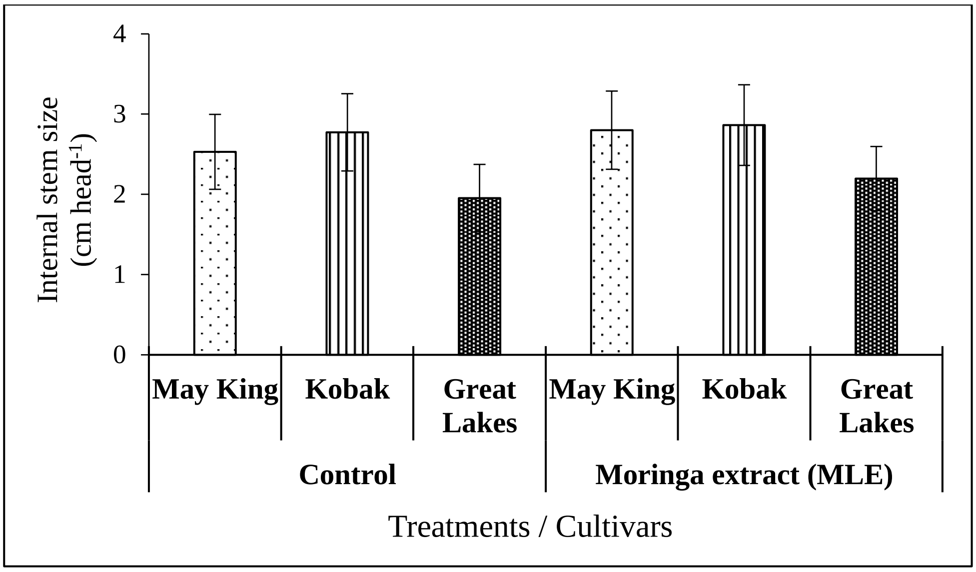

Figure 37. The interaction effect of plant biostimulant and variety on internal stem size of lettuce grown in **autumn** season, (Debrecen, 2019-2021)

Lettuce produces an extremely shallow root system that does not reach more than 0.6 m long (Thorup-Kristensen, 2006). Plants with greater root forming can absorb more nutrients from the soil (Burns, 1980). In our research experiments, the root weight was varied among the lettuce cultivars (Figure 38). Similar to the previous measured vegetative parameters, root weight was slightly influenced by the interaction factors. The significant difference was recorded for the plants treated with 6% MLE in "May King" variety, whereas other varieties had least influenced by the interactions. The root weight changes were also varied among the growing years. In 2019, the heaviest root weight was recorded for the "Great Lakes" treated with Willow bark extract at 40.20 g head^{-1}. However, in 2020, the significantly greatest root weight was found in the "May King" variety treated with Bistep and MLE at 50.78 and 50.12 g head^{-1} respectively. No significantly differences were found for the experiment of 2021, however the greatest root weight was in the "Kobak" variety treated with Bistep at 37.06 g head^{-1} in 2021 (Appendix 5). Our results show that "Kobak" variety positively reacted to Bistep and Willow bark extract + Bistep (W+B) treatments, whereas "May King" with Willow bark extract (W) and moringa leaf extract (MLE) and "Great Lakes" with Willow, Bistep and MLE.

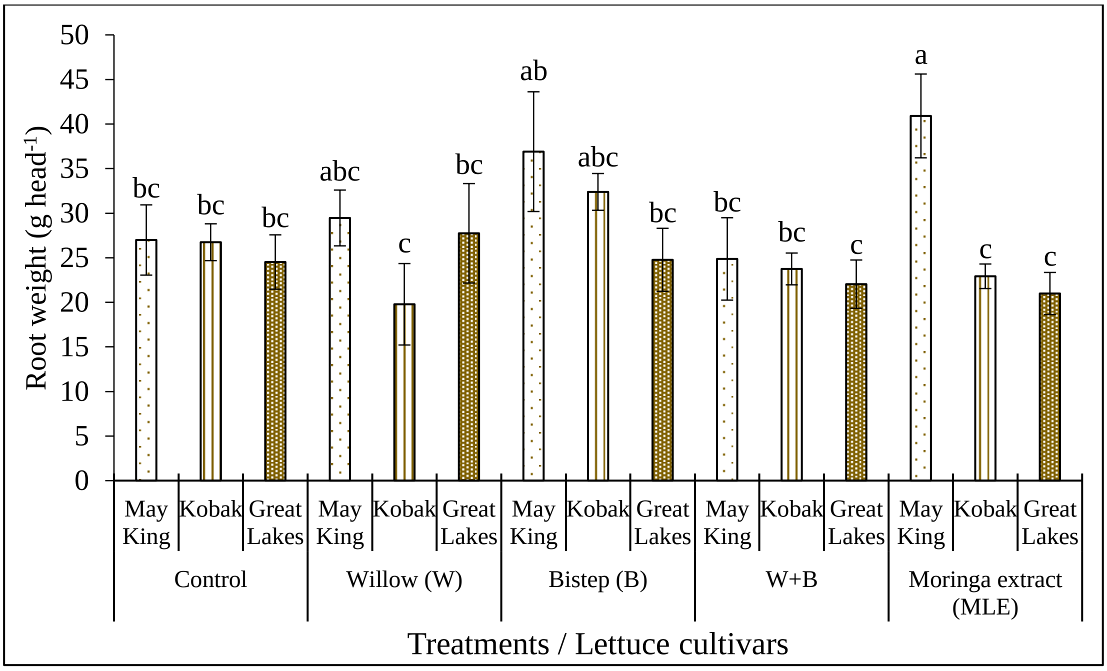

Figure 38. The interaction effect of plant biostimulant and variety on internal stem size of lettuce grown in **spring** season, (Debrecen, 2019-2021)

Means within the same column followed by the same letter(s) are not significantly different at the probability level of (*P≤0.05*) according to Duncan[a,b] Multiple Range Test

In autumn experiment, a similar result was obtained when the root weight was influenced by the interaction of a plant biostimulant that included 6% MLE and a lettuce genotype Figure 39.

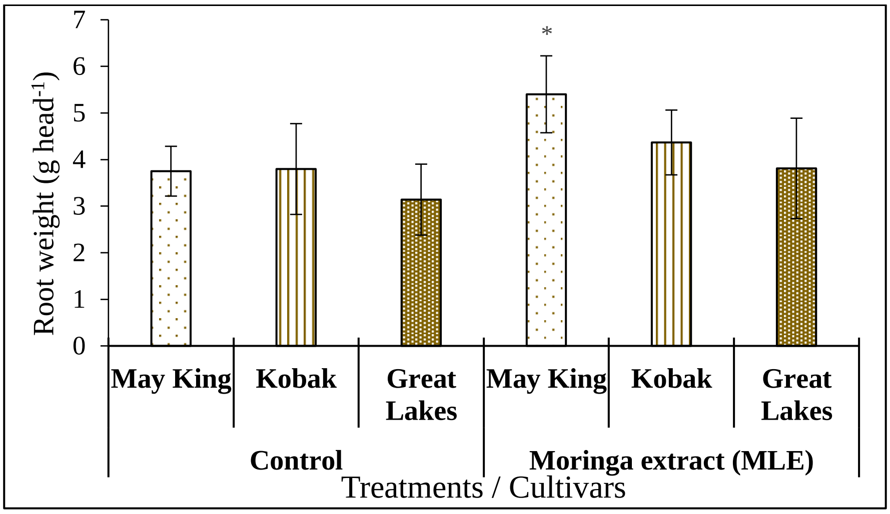

Figure 39. The interaction effect of plant biostimulant and variety on lettuce root weight grown in **autumn** season, (Debrecen, 2019-2021)

The mean results of the autumn experiment show that the only significant improvement in lettuce root weight was in the May King variety treated with 6% MLE. The root weight of the other varieties did not improve significantly.
In general, plant biostimulants (6% MLE) had a minor influence on morphological characteristics in our three-year study. This might be because the minimum light emission was higher in 2020 and 2021 than in 2019. A lack of light or poor light quality can inhibit the growth and development of most plant species (Bula et al., 1991; Gruda, 2005; Yorio et al., 2001).

4.1.6. The interaction effect of plant biostimulant, variety and growing season on some scaled vegetative parameters of lettuce

Head structure is an important quality indication that can attract customers to buy the product while also demonstrating the plant's healthiness (Schreiner et al., 2013). Our findings indicate that the combination of lettuce cultivars, growth seasons, and treatments had the least significant effect on head structure (Figure 40). The main distinctions were between the growing years. In 2019, "May King" treated with Willow bark extract and "Kobak" treated with Bistep had the best head structure when compared to the control.

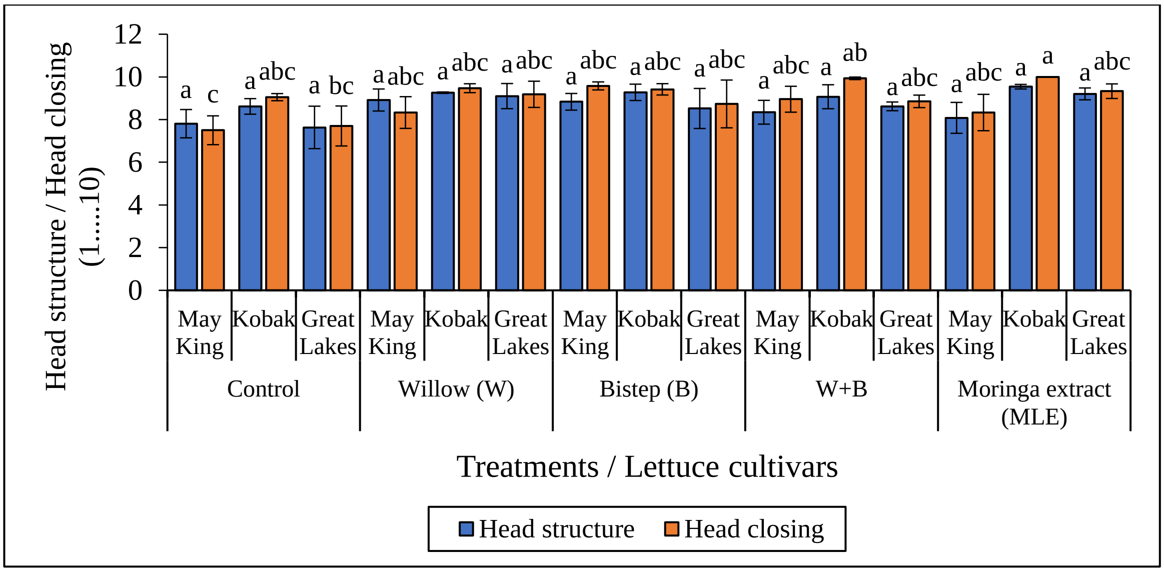

Figure 40. The interaction effect of plant biostimulant and variety on head structure and head closing of lettuce grown in **spring** season, (Debrecen, 2019-2021)

Means within the same column followed by the same letter(s) are not significantly different at the probability level of (*P*≤0.05) according to Duncan[a,b] Multiple Range Test

However, in the 2020 experiment, plants sprayed with 6% MLE had significantly improved head structure in all three lettuce varieties. However, in the seasons of 2020 and 2021, the "Kobak" variety reacted favourably to the W+B treatment (Appendix 7).

The enclosure of lettuce leaves within a head structure is referred to as head closing. It shows how the leaves condense or adhere to one another to create the head. A tighter head closure enhances the appearance of the lettuce's physical parameters. However, Mou and Ryder (2002) revealed that head closure or head shape is also connected to the nutritional value of the lettuce plant.

Figure 40 displays the same finding for head closure as impacted by interaction variables such as head structure. The interaction of the above parameters resulted in no substantial improvement in head closing, according to the means of the factors. In the Appendix 7 it is clearly shown that in 2019, the "May King" variety produced significantly greater head closure when treated with Willow bark extract, whereas the "Kobak" variety responded positively to Bistep foliar spray. The key differences between "May King" and "Kobak" were revealed in 2020, when the cultivars achieved the best head closure when Willow, Bistep, and a combination of W+B treatments were applied. However, in 2020, the interaction of moringa leaf extract (MLE) was the most impactful factor on all three varieties. The only significant variation seen in 2021 was in the "May King" cultivar treated with Bistep. These studies indicate that MLE spraying works best in increasing heat tolerance in lettuce plants, whereas Willow and Bistep perform better in cooler and moister conditions.

Scaled vegetative metrics are key quality criteria that are commercially regarded for marketing reasons. It is obvious that lettuce varieties vary in terms of head shape; some are known as Crisphead, while others are leaf or Romaine types. Aside from the importance of the head structure as a physical parameter for consumers to purchase the product, the head structure and head closure are two linked factors that can impact the nutrient value in lettuce. Crisphead lettuce that has been artificially opened contains less vitamins and minerals than closed lettuce (Mou and Ryder, 2002). However, well-structured heads in lettuce are much considered to be purchased because it gives a better appearance.

Lettuce grown in autumn season recorded a slightly different result for the scaled parameters. The means of our data in Figure 41 reveals that the interaction of

"May King" and "Kobak" types treated with 6% MLE produced a much superior head shape than untreated or control, however the "Great Lakes" variety showed no significant changes. When compared to untreated lettuce cultivars, plants treated with moringa leaf extract produced better head structure. On the contrary, there were no significant variations across lettuce cultivars; nevertheless, there were variances between growing years, which might be related to environmental variables, particularly temperature differences (Appendix 4).

Head closure is the process by which the leaves join to the primary head to make a better shape; this will result in a better head structure if the leaves are well attached to each other. Three years of data showed that except for “Kobak” there was no significant difference between the interactions and treatments, as well as between lettuce cultivars.

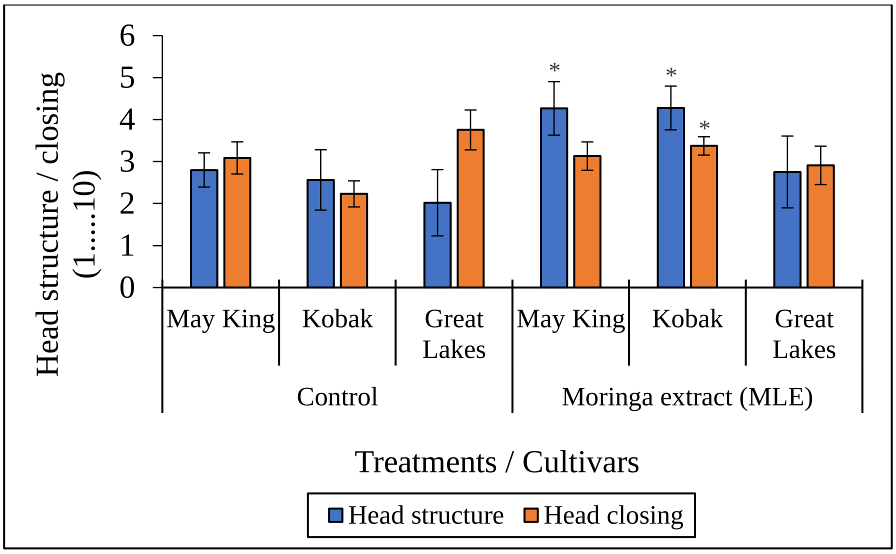

Figure 41. The interaction effect of plant biostimulant and variety on head structure and head closing of lettuce grown in **autumn** season, (Debrecen, 2019- 2021)

4.2. Mineral element content

4.2.1. The interaction effect of plant biostimulants and growing years on macronutrient content in three lettuce cultivars

Lettuce seems to be an essential part of the human diet. However, when compared to other green vegetables such as spinach, the plant is nutritionally underestimated (Kim, et al., 2016). It is essential to increase the mineral content of the plant using environmentally friendly biostimulants without raising the nitrate content of the leaves. As shown in Table 12, foliar spray and irrigation treatment of the applied plant biostimulants considerably boosted various macronutrients in lettuce cultivars.

Table 12. Macronutrient content in lettuce in related to plant biostimulants

Treatments	**Cultivars**			**Years**			**Mean of years**
	May King	**Kobak**	**Great Lakes**	**2019**	**2020**	**2021**	
Potassium (mg kg^{-1} DW)							
Control	1352.33^{a}	1390.00^{a}	1485.15^{a}	1124.00^{c}	1772.47^{a}	1331.00^{a}	1409.16^{a}
Bistep	1106.85^{b}	1006.34^{b}	1137.85^{b}	997.35^{d}	1318.30^{d}	935.38^{b}	1083.70^{d}
Willow	1164.23^{ab}	1374.52^{a}	1302.46^{b}	1303.37^{a}	1579.45^{b}	958.38^{b}	1280.41^{b}
W+B	1186.32^{ab}	1232.85^{a}	1176.84^{b}	1230.85^{b}	1421.66^{c}	943.50^{b}	1198.67^{c}
Calcium (mg kg^{-1} DW)							
Control	419.74^{b}	422.00^{bc}	491.75^{ab}	500.33^{b}	492.16^{b}	314.00^{d}	444.48^{b}
Bistep	411.77^{b}	399.59^{c}	476.85^{ab}	468.90^{c}	441.33^{c}	378.00^{c}	429.40^{b}
Willow	410.74^{b}	458.74^{b}	463.73^{b}	517.00^{a}	400.46^{d}	415.75^{b}	444.40^{b}
W+B	521.59^{a}	540.10^{a}	505.76^{a}	506.83^{ab}	573.53^{a}	487.08^{a}	522.48^{a}
Phosphorus (mg kg^{-1} DW)							
Control	269.66^{b}	281.33^{ab}	262.03^{ab}	240.17^{ab}	292.00^{bc}	280.86^{ab}	271.01^{b}
Bistep	261.29^{b}	258.68^{b}	270.52^{ab}	227.64^{b}	300.00^{b}	262.85^{b}	263.51^{b}
Willow	259.66^{b}	278.50^{ab}	260.02^{b}	255.51^{a}	275.33^{c}	267.34^{b}	266.06^{b}
W+B	298.33^{a}	299.00^{a}	287.67^{a}	246.01^{ab}	338.33^{a}	300.66^{a}	295.01^{a}
Magnesium (mg kg^{-1} DW)							
Control	231.52^{b}	228.81^{c}	251.00^{b}	279.77^{b}	246.66^{c}	184.88^{d}	237.12^{d}
Bistep	252.66^{b}	256.69^{b}	270.80^{b}	268.33^{b}	273.53^{b}	238.30^{c}	260.05^{c}
Willow	282.13^{a}	307.50^{a}	310.06^{a}	306.33^{a}	322.86^{a}	270.51^{b}	299.90^{b}
W+B	301.97^{a}	315.53^{a}	307.50^{a}	307.66^{a}	283.68^{b}	333.66^{a}	308.33^{a}
Sodium (mg kg^{-1} DW)							
Control	72.94^{c}	78.45^{c}	91.33^{c}	106.82^{c}	80.36^{d}	55.54^{c}	80.91^{d}
Bistep	100.75^{b}	104.55^{b}	116.50^{b}	76.96^{d}	140.36^{c}	104.48^{ab}	107.26^{c}
Willow	123.23^{ab}	152.67^{a}	141.82^{a}	132.72^{a}	176.77^{a}	108.23^{a}	139.24^{a}
W+B	131.67^{a}	125.44^{b}	127.38^{b}	121.66^{b}	163.22^{b}	99.61^{b}	128.16^{b}
Sulphur (mg kg^{-1} DW)							
Control	94.75^{b}	113.93^{a}	105.19^{a}	109.17^{c}	118.07^{b}	86.64^{a}	104.63^{b}
Bistep	89.17^{b}	88.67^{b}	106.76^{a}	92.19^{d}	117.16^{b}	75.25^{b}	94.87^{d}
Willow	88.00^{b}	105.94^{ab}	109.65^{a}	127.55^{a}	104.62^{c}	71.44^{b}	101.20^{c}
W+B	118.28^{a}	112.32^{a}	108.98^{a}	115.85^{b}	138.22^{a}	85.51^{a}	113.19^{a}

Means within the same column followed by the same letter(s) are not significantly different ($P \leq 0.05$) according to Duncan[a,b] test Multiple Range Test

Willow bark extract and the combination of W+B were the most efficient biostimulants in enhancing nutritional content in lettuce cultivars when compared to untreated plants and Bistep. A significant improvement is shown in the combination of W+B significantly enhanced calcium (Ca) content in "May King" and "Kobak" over a period of years. Plants treated with Willow bark extract and W+ B, on the other hand, had considerably higher levels of magnesium (Mg), sodium (Na), and phosphorus (P).

In contrast, no significant effects were observed for the potassium concentration in lettuce genotypes for Willow (W) and the combination of W+B treatments. Despite the fact that the Bistep biostimulant reduced K accumulation in all three cultivars. Willow bark extract significantly improved the macronutrients Ca, K, and P in colder seasons (2019) compared to warmer seasons (2020 and 2021). During the seasons, however, higher levels of Na and Mg were discovered in lettuce treated with Willow extracts. In addition to this, Willow bark extract+ Bistep (W+B) significantly raised Ca, Mg, P, and S throughout the grown years, while Willow bark extract (W) also significantly enhanced Na in lettuce cultivars. This might be because Willow bark extract (W) was applied to the plant via irrigation, allowing the roots to absorb more nutrients from the soil, and the extract itself is high in minerals (Table 12).

The results from mean of years show that the combined treatment of W+B significantly increased most of the measured macronutrients, with calcium, magnesium, phosphorus, and sulphur being the key macronutrients. The treatment of W+B, on the other hand, dramatically lowered potassium levels. Willow bark extract, on the other hand, considerably improved sodium levels which might be due to its high sodium content (14.7 mg L^{-1}).

4.2.2. Effect of lettuce cultivars and growing years on macronutrient content in lettuce in the mean of treatments

Macronutrients, micronutrients and bioactive compounds in fresh vegetables have a positive correlation to reduce the risks of many chronic disease in the human body (Kris-Etherton et al., 2002; Soetan et al., 2007). Our research studies demonstrate that *Lactuca sativa*'s macronutrient content was determined by genetic and environmental factors throughout lettuce growth (Table 13). Calcium and magnesium levels were greater in the 2019 growing season, when air temperatures were milder in the latter growth months of

April and May, while sodium, potassium, phosphorus and sulphur content were higher in the 2020 growing year, which was warm and somewhat moist. In contrast, lettuce cultivars performed poorly during the warmer and drier season of 2021, when temperatures were high in March, April, and May and the environment was slightly dry. The chemical properties of the soil did not appear to have any discernible effect on the mineral content of the plants. Among the lettuce cultivars, the "Great Lakes" variety had the highest macronutrient content, followed by the "Kobak" and "May King" varieties.

Table 13. Macronutrient content in lettuce in related to growing season and cultivars (Debrecen, 2019-2021)

Year and Cultivar	**Ca**	**Mg**	**Na**	**K**	**P**	**S**
	(mg kg^{-1} DW)					
Year						
2019	498.26^{a}	290.52^{a}	109.54^{c}	1163.89^{c}	242.33^{c}	111.19^{b}
2020	476.87^{b}	281.68^{ab}	140.183^{a}	1522.97^{a}	301.41^{a}	119.52^{a}
2021	405.46^{c}	256.84^{c}	91.96^{d}	1042.06^{d}	277.93^{b}	79.71^{d}
Cultivar						
May King	440.96^{c}	267.07^{c}	107.151^{c}	1202.43^{c}	272.23^{ab}	97.55^{c}
Kobak	455.10^{b}	277.13^{b}	115.28^{b}	1250.93^{b}	279.38^{a}	105.21^{b}
Great Lakes	484.52^{a}	284.84^{a}	119.26^{a}	1275.57^{a}	270.06^{b}	107.65^{a}

Means within the same column followed by the same letter(s) are not significantly different ($P \leq 0.05$) according to Duncan [a,b] test Multiple Range Test

4.2.3. The interaction effect of plant biostimulants and growing years on micronutrient content in three different lettuce cultivars

Many physiological activities in plant cells, including enzymatic processes, respiration, photosynthesis protein stability, and redox transition elements, are influenced by micronutrients (Salih, 2021).

The results in the Table 14 demonstrate that similar to the macronutrient, the micronutrient was significantly influenced by the combination treatment of plant biostimulants and the growing years.

Iron content was clearly influenced by biostimulant treatments. Willow bark extract+ Bistep (W+B) for example, recorded a significantly greater iron content compared to control. The only significant difference for zinc content was found in the "May King" lettuce variety treated with W+ B treatments at 2.84 mg kg^{-1} DW. Compared to the control plants, there was a significantly higher manganese content in the "May King" and "Kobak" treated with W+ B, manganese was also higher in the "Great Lakes" variety however, no significant difference was recorded. On the other hand, foliar spray of Bistep significantly reduced the manganese content in the lettuce cultivars.

Table 14. Micro nutrient content in lettuce in related to plant biostimulants

Treatments	Cultivars			Years			Mean
	May King	Kobak	Great Lakes	2019	2020	2021	
Iron (mg kg^{-1} DW)							
Control	21.55^{b}	22.48^{b}	25.75^{b}	18.17^{ab}	37.93^{b}	13.68^{b}	23.25^{b}
Bistep	20.24^{b}	19.50^{b}	21.11^{b}	16.47^{b}	29.65^{c}	14.72^{b}	20.28^{c}
Willow	23.78^{ab}	23.46^{b}	23.90^{b}	18.52^{a}	37.55^{b}	15.07^{b}	23.71^{b}
W+B	34.07^{a}	37.39^{a}	37.31^{a}	18.14^{ab}	68.46^{a}	22.16^{a}	36.26^{a}
Zinc (mg kg^{-1} DW)							
Control	2.694^{b}	2.882^{a}	2.915^{a}	2.723^{ab}	2.843^{bc}	2.922^{a}	2.833^{ab}
Bistep	2.605^{b}	2.765^{a}	2.735^{a}	2.566^{b}	2.754^{c}	2.786^{b}	2.700^{c}
Willow	2.594^{b}	3.104^{a}	2.768^{a}	2.834^{a}	2.935^{ab}	2.696^{b}	2.822^{b}
W+B	2.840^{a}	2.928^{a}	2.957^{a}	2.720^{ab}	3.022^{a}	2.992^{a}	2.900^{a}
Manganese (mg kg^{-1} DW)							
Control	1.490^{bc}	1.753^{b}	1.929^{a}	1.735^{a}	1.582^{c}	1.854^{c}	1.724^{c}
Bistep	1.412^{c}	1.274^{c}	1.472^{b}	1.303^{c}	0.978^{d}	1.877^{bc}	1.385^{d}
Willow	1.652^{b}	1.720^{b}	1.973^{a}	1.653^{b}	1.732^{b}	1.960^{b}	1.783^{b}
W+B	2.105^{a}	2.510^{a}	2.073^{a}	1.751^{a}	2.561^{a}	2.377^{a}	2.230^{a}
Copper (mg kg^{-1} DW)							
Control	0.727^{a}	0.740^{ab}	0.903^{a}	0.706^{ab}	0.892^{b}	0.772^{a}	0.790^{b}
Bistep	0.730^{a}	0.669^{b}	0.978^{a}	0.704^{ab}	1.000^{a}	0.674^{b}	0.792^{b}
Willow	0.741^{a}	0.747^{ab}	0.974^{a}	0.736^{a}	1.027^{a}	0.698^{b}	0.821^{a}
W+B	0.719^{a}	0.787^{a}	0.945^{a}	0.684^{b}	1.021^{a}	0.746^{a}	0.817^{a}

Means within the same column followed by the same letter(s) are not significantly different ($P \leq 0.05$) according to Duncan [a,b] test Multiple Range Test

However, in the mean of years, there was no significant difference in the copper content in the lettuce varieties treated with the plant biostimulants. A similar result was found on the broccoli treated with biostimulants under drought weather conditions on micro and macronutrient content by Kałużewicz et al. (2018).

In addition, the treatments, lettuce genotypes, and growing seasons all had an effect on iron content, which is one of the most important micronutrients in leafy vegetables.

As shown in Table 14, W+B could greatly increase the iron content of all lettuce cultivars. Furthermore, among the lettuce cultivars, the "Great Lakes" variety had more iron than the "May King" and "Kobak" cultivars.

In the mean of cultivars, there was no substantial variation in zinc and copper content in lettuce types in 2019, although plant biostimulants could greatly enhance copper content in 2020. W+B considerably increased manganese and zinc content in 2020 and 2021, which might be attributed to warmer weather and greater light in the late season of April and May before harvesting (Table 14). The results of the growing years demonstrate that Willow and W+B have the greatest influence on the micronutrient content of lettuce cultivars, whereas Bistep had the least impact on the micronutrient content.

4.2.4. Effect of lettuce cultivars and growing years on micronutrient content in lettuce in the mean of treatments

The key element impacting nutrient absorption and content in lettuce leaves was the weather or growing season. Table 15 shows that in the mean of cultivars, copper, zinc and iron elements were much higher in the 2020 trial at 0.985, 2.888 and 43.40 mg kg^{-1} respectively, while manganese significantly greater in 2021.

Table 15. Micronutrient content in lettuce in related to growing season and cultivars

Year and Cultivar	**Cu**	**Mn**	**Zn**	**Fe**
	(mg kg^{-1} DW)			
Year				
2019	0.708^{d}	1.724^{c}	2.711^{c}	17.83^{c}
2020	0.985^{a}	1.386^{d}	2.888^{a}	43.40^{a}
2021	0.723^{c}	1.782^{b}	2.849^{ab}	16.41^{d}
Mean	$\mathbf{0.805^{b}}$	$\mathbf{2.230^{a}}$	$\mathbf{2.813^{b}}$	$\mathbf{25.88^{b}}$
Cultivar				
May King	0.729^{b}	1.665^{c}	2.683^{c}	24.91^{c}
Kobak	0.736^{b}	1.814^{b}	2.920^{a}	25.70^{b}
Great Lakes	0.950^{a}	1.862^{a}	2.844^{b}	27.02^{a}

Means within the same column followed by the same letter(s) are not significantly different (P≤0.05) according to Duncan [a,b] test Multiple Range Test

Furthermore, the micronutrient content was varied among the lettuce cultivars. The "Great Lakes" variety contained significantly greater copper, manganese and iron content than "May King" and "Kobak" varieties, whereas higher zinc content was found in the "Kobak" variety (Table 15).

4.3. Bioactive compounds

Lettuce is commonly eaten fresh, which allows the plant to retain bioactive chemicals as it enters the human body. Various studies have demonstrated that lettuce has many healing benefit due to its richness in bioactive compounds that function as anti-inflammatory, cholesterol-lowering, and anti-diabetic agents in the human body (Kim, et al., 2016). Total polyphenol and vitamin C were assessed as the two most essential quality parameters in lettuce vegetables. Nitrate content was also tested because there are several regulations defining nitrate level in leafy vegetables, and it is regarded as one of the most important elements that can directly impact human health.

4.3.1. The effect of plant biostimulants on total polyphenol, vitamin C and nitrate content in lettuce leaves in the mean of cultivars

Phenolic chemicals are a form of secondary metabolite derived from plants that serve significant physiological activities throughout the plant's life cycle. Phenolics are produced under both favourable and suboptimal environments. Under abiotic stress circumstances, plants demonstrate enhanced synthesis of polyphenols such as phenolic acids and flavonoids, which aid the plant in coping with environmental restrictions (Sharma et al., 2019). The results in Table 16 show that the impact of plant biostimulants on total polyphenols in lettuce cultivars varied depending on the growing years and treatments. The results reveal that plants treated with Willow bark extract + Bistep (W+B) considerably increased total polyphenols, although no significant difference was found for other plant biostimulants compared to the control. Furthermore, the mean of treatments indicates a significantly higher total polyphenol in the year 2019. This might be due to the fact that the temperature in 2019 was a bit lower throughout the growing season than in 2020 and 2021.

Table 16. Effect of plant biostimulants on evaluation of bioactive compound in the mean of variety grown in spring season (Debrecen 2019-2021)

Treatments	Bioactive compounds											
	Total polyphenols (mg GAE $100g^{-1}$ FW)				Vitamin C (mg 100 g^{-1} FW)				Nitrate (mg kg^{-1})			
	2019	**2020**	**2021**	**Mean**	**2019**	**2020**	**2021**	**Mean**	**2019**	**2020**	**2021**	**Mean**
Control	55.85^{b}	57.48^{a}	60.15^{a}	57.82^{b}	4.87^{b}	4.68^{bc}	7.37^{b}	5.64^{c}	488.21^{b}	682.66^{b}	812.88^{b}	661.25^{b}
Willow	59.41^{b}	50.54^{b}	55.53^{ab}	55.16^{b}	5.29^{b}	5.27^{a}	8.11^{a}	6.23^{ab}	692.15^{a}	862.00^{a}	850.33^{a}	801.49^{a}
Bistep	55.60^{b}	57.44^{a}	52.73^{bc}	55.26^{b}	5.44^{b}	5.09^{ab}	6.94^{bc}	5.82^{bc}	527.81^{b}	604.00^{c}	815.88^{b}	649.23^{b}
W+B	80.64^{a}	52.46^{b}	57.09^{ab}	64.40^{a}	7.31^{a}	5.37^{a}	7.02^{bc}	6.57^{a}	536.50^{b}	659.00^{b}	786.55^{b}	660.68^{b}
MLE	-	60.88^{a}	51.73^{c}	56.31^{b}	-	4.55^{c}	6.71^{c}	5.63^{c}	-	680.32^{b}	810.11^{b}	745.21^{b}
Mean	62.87^{a}	55.76^{b}	55.45^{b}	-	5.72^{b}	4.99^{b}	7.23^{a}	-	561.17^{c}	697.59^{b}	815.15^{a}	-

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 Duncan [a,b] Multiple Range Test
FW: Fresh weight
W+B: Willow+Bistep

In 2019, the only significant difference was discovered for the combination of plant biostimulants of Willow bark extract + Bistep (W+B), whereas in 2020 a significantly reduction of total polyphenol was measured in the plants treated with Willow bark extract + Bistep (W+B) and moringa leaf extract (MLE) plant biostimulants. This might be because these biostimulants can reduce heat stress in warmer weather conditions (Latif and Mohamed, 2016; Nayanakantha et al., 2019). The means show that the most influenced biostimulant on the total polyphenol improvement was Willow bark extract+ Bistep (W+B). This might be because the combination of the treatments could provide all the necessary elements for the plant's physiological metabolisms.

Our result mean of years shows that in the mean of varieties all the plant biostimulants could improve the vitamin C in lettuce leaves. However, compared to the control or untreated lettuce cultivars Willow bark extract (W) and Willow bark extract + Bistep (W+B) could significantly increase vitamin C, whereas no significant differences were recorded for the Bistep and MLE plant biostimulants. Total polyphenols and vitamin C had a negative connection, with an increase in total polyphenols lowering vitamin C levels in lettuce leaves.

In the year of 2019, only cultivars treated with Willow bark extract + Bistep (W+B) had substantially higher vitamin C content at 7.31 mg 100 g^{-1} FW, but in 2020 plant biostimulants of Willow and Willow bark extract + Bistep (W+B) significantly enhanced vitamin C content in lettuce leaves by 11% and 13%, respectively. The maximum vitamin C content was found in plants treated with Willow bark extract during the 2021 growing season, at 8.11 mg 100 g^{-1} FW. Our findings correspond with the findings by Schmidt et al. (2010) that the antioxidant content in Kale (*Brassica oleracea* var. *sabellica*) vegetable was most impacted and improved by climatic conditions, particularly cold temperatures from October to December.

According to our results, *Lactuca sativa* treated with Willow bark extract (W) significantly accumulates more nitrate content in their leaves throughout a three-year study. Among the plant biostimulants, Bistep (B) was the only biostimulant which could reduce nitrate level in spring growing season by around (-)13%.

In the mean of treatments, a significant greater nitrate level was measured in the growing year of 2021, while the light emission and temperature was higher than in 2020 and 2019. This might be because Willow bark extract (W) contains more nitrogen than the Bistep plant biostimulants at <0.20 mg L^{-1} in Willow, but only 0.02 mg L^{-1} in Bistep (Table 6), this will provide the plant to absorb and retain more nitrogen in its leaves. Drobek et al. (2019) have found a similar result on the corn treated with the combination of fertilizer (300 kg N ha^{-1} + 120 kg K ha^{-1}) and protein hydrolysate biostimulant of chicken feathers (7.2 L ha^{-1}) where a higher nitrogen level was provided to the plants.

4.3.2. The effect of lettuce genotypes on total polyphenol, vitamin C and nitrate content in lettuce leaves

Based on our results in Table 17 the interaction among the lettuce varieties, the total polyphenol content in lettuce cultivars ranged from 51.95 to 64.30 mg GAE $100g^{-1}$ FW. This value was more affected by the plant variety and growing season. In general, "Kobak" variety contains higher total polyphenol content than two other varieties. This might be because "Kobak" variety contains more anthocyanin and less chlorophyll than "May King" and "Great Lakes". According to Liu et al. (2007) there is a considerable difference in polyphenol content between red and green lettuce cultivars, red lettuce containing more polyphenol than green lettuce.

Vitamin C, also known as antioxidants, is one of the related health benefits of vegetable and fruit product consumption. It inhibits the oxidation process in cells, with plant variety, species, and weather conditions influencing the vitamin C concentration in each plant spice during the growth period (Korus, 2011). Vitamin C levels in lettuce genotypes were found to be comparable. Among lettuce cultivars, "Kobak" has higher vitamin C than "May King" and "Great Lakes" in 2020. The greatest outcome for vitamin C was obtained in 2021, when light emission was substantially greater than in the previous two seasons.

Nitrate level of our three evaluated lettuce cultivars ranged from 546.75 to 821.46 mg kg^{-1} depending on the varieties and growing years.

Our findings showed that there was no significant variation in nitrate concentration amongst lettuce cultivars; nevertheless, nitrate accumulation varied depending on the growth years in spring growing season. The 2019 season had the lowest nitrate level, followed by 2020 and 2021.

Table 17. Effect of plant cultivars on bioactive compound content in three growing seasons of spring 2019-2021 in the mean of treatments.

Varieties	Bioactive compounds											
	Total polyphenols (mg GAE $100g^{-1}$ FW)				Vitamin C (mg 100 g^{-1} FW)				Nitrate (mg kg^{-1})			
	2019	2020	2021	Mean	2019	2020	2021	Mean	2019	2020	2021	Mean
May King	61.10^{a}	51.95^{b}	56.36^{a}	56.47^{a}	5.74^{a}	4.47^{b}	7.30^{ab}	5.83^{a}	561.81^{a}	745.60^{a}	815.53^{a}	707.64^{a}
Kobak	63.22^{a}	61.96^{a}	55.51^{a}	60.23^{a}	5.65^{a}	5.76^{a}	6.97^{b}	6.12^{a}	546.75^{a}	727.00^{ab}	808.46^{a}	694.07^{a}
Great Lakes	64.30^{a}	53.37^{b}	54.48^{a}	57.38^{a}	5.79^{a}	4.75^{b}	7.42^{a}	5.98^{a}	574.95^{a}	719.00^{b}	821.46^{a}	705.13^{a}

Means within the same column followed by the same letter(s) are not significantly different at the probability level of ($P \geq 0.05$) according to Duncan ^{a,b} Multiple Range Test

FW: Fresh weight

This might be related to the fact that the soil of the season 2021 includes more NO_3^- than the soil of the growing seasons 2019 and 2020, as well as the significantly variable weather conditions seen in the growing season of 2020, such as temperature and light emission.

It is supported with the fact, that lettuce grown in low light intensity and duration mostly in autumn season contain greater nitrate than in the season where more light emission is available especially in spring season (Escobar-Gutierrez et al., 2016).

4.3.3. The interaction effect of lettuce genotype, year of experiments and plant biostimulants on total polyphenol, vitamin C and nitrate content in lettuce leaves

Polyphenol is found in lettuce leaves in various forms such as *chlorogenic acid*, *3-O-glucoside* of *kaempferol*, *quercetin-3-O-glucoside*, and *Dicaffeoyl tartaric acid* (Rouphael et al., 2012). The growing environment (light and temperature) and genetic characteristics are the two primary factors that might have a substantial impact on the quantity of bioactive chemicals in lettuce plants (Riga et al., 2019; Sytar et al., 2018). During our three-year study, the means show that the interaction of plant biostimulants, plant genotype, and growth season had a varied impact on the total polyphenol compounds in lettuce leaves. This might be because plant biostimulants lower plant stress under unfavourable environmental condition, while polyphenol increases in the plant as reaction to adverse weather or abiotic stress conditions (Sharma et al., 2019).

The mean interaction of the evaluated factors shows a slight improvement in some lettuce varieties some plant biostimulants (Table 19). For example, Willow and (W+B) could work positively with "May King" and "Kobak" varieties for the total polyphenol improvement. Whereas MLE performed better with the "Kobak" variety only. No significant improvement was shown for the interaction of the Bistep with the lettuce cultivars. The most noticeable improvements occurred during the growing years. In 2019, lettuce treated with (W+ B) showed significantly greater total polyphenol content; however, in 2021, just a 9% increase in total polyphenol content in the "May King" variety was seen.

Table 18. Interaction effect of plant biostimulants, lettuce cultivars and growing years on bioactive compounds of lettuce grown in **spring** of 3 years (Debrecen, 2019-2021)

Treatments	Varieties	Bioactive compounds: Total polyphenols (mg GAE 100g^{-1} FW)				Vitamin C (mg 100 g^{-1} FW)				Nitrate (mg kg^{-1})			
		2019	2020	2021	Mean	2019	2020	2021	Mean	2019	2020	2021	Mean
Control	***May King***	54.53^{b}	53.71^{bc}	58.46^{bc}	55.56^{ab}	4.88^{c}	4.65^{de}	7.19^{bc}	5.57^{a}	500.39^{a}	660.00^{gh}	716.00^{g}	625.46^{c}
	Kobak	55.74^{b}	60.96^{bc}	60.19^{ab}	58.96^{ab}	4.82^{c}	5.10^{cd}	6.93^{cd}	5.61^{a}	478.00^{c}	950.00^{c}	953.00^{a}	793.66^{abc}
	Great L.	57.28^{b}	57.77^{bc}	61.81^{ab}	58.95^{ab}	4.93^{c}	4.30^{de}	7.99^{ab}	5.74^{a}	486.26^{c}	438.00^{j}	769.66^{efg}	564.64^{c}
Willow	***May King***	55.92^{b}	43.54^{e}	52.18^{de}	50.54^{b}	5.45^{ab}	4.48^{de}	7.83^{ab}	5.92^{a}	690.00^{a}	1080.00^{a}	865.66^{bc}	878.55^{ab}
	Kobak	56.49^{b}	59.33^{bc}	59.83^{abc}	58.55^{ab}	5.29^{bc}	6.14^{b}	8.26^{a}	6.56^{a}	695.00^{a}	819.00^{e}	842.66^{bcd}	785.55^{abc}
	Great L.	65.83^{ab}	48.76^{de}	54.59^{bc}	56.39^{ab}	5.15^{c}	5.19^{bc}	8.26^{a}	6.20^{a}	691.47^{a}	687.00^{gh}	842.66^{bcd}	740.37^{abc}
Bistep	***May King***	51.59^{b}	53.67^{bc}	51.65^{de}	52.30^{ab}	5.24^{bc}	4.68^{de}	6.88^{cd}	5.60^{a}	492.16^{b}	744.00^{fg}	791.00^{def}	675.72^{bc}
	Kobak	58.26^{b}	62.48^{ab}	55.02^{bc}	58.58^{ab}	5.21^{bc}	5.58^{bc}	6.69^{de}	5.82^{a}	493.00^{b}	516.00^{i}	855.66^{bcd}	621.55^{c}
	Great L.	56.96^{b}	55.82^{bc}	51.52^{de}	54.76^{ab}	5.87^{ab}	5.02^{cd}	7.25^{bc}	6.04^{a}	598.29^{a}	552.00^{i}	801.00^{cde}	650.43^{bc}
W+B	***May King***	82.37^{a}	55.28^{bc}	64.56^{a}	67.40^{a}	7.40^{a}	4.34^{de}	7.34^{ab}	6.36^{a}	564.29^{a}	429.00^{j}	901.00^{ab}	631.43^{c}
	Kobak	82.40^{a}	57.80^{bc}	52.28^{cde}	64.16^{ab}	7.29^{ab}	7.08^{a}	6.42^{de}	6.93^{a}	521.00^{a}	649.00^{h}	627.00^{h}	599.00^{c}
	Great L.	77.16^{a}	43.30^{e}	54.43^{bc}	58.29^{ab}	7.24^{ab}	4.70^{cd}	7.31^{ab}	6.41^{a}	523.80^{a}	899.00^{d}	831.66^{cde}	751.48^{abc}
MLE	***May King***	-	52.55c^{d}	54.93^{bc}	53.30^{ab}	-	4.23^{e}	7.28^{bc}	5.75^{a}	-	815.00^{e}	804.00^{cd}	809.50^{ab}
	Kobak	-	68.87^{a}	50.23^{e}	59.55^{ab}	-	4.90^{cd}	6.56^{de}	5.73^{a}	-	701.00^{fg}	764.00^{fg}	732.50^{ab}
	Great L.	-	61.22^{ab}	50.04^{e}	55.63^{ab}	-	4.52^{de}	6.30^{e}	5.41^{a}	-	1023.00^{b}	862.66^{bc}	924.83^{a}

Means within the same column followed by the same letter(s) are not significantly different at the probability level of ($P \geq 0.05$) according to Duncan [a,b] Multiple Range Test

FW: Fresh weight

W+B: Willow+Bistep

In contrast, in the 2020 season, "Kobak" and "Great Lakes" reacted positively to the foliar application of 6% MLE, with the "Kobak" variety having the highest polyphenol content at 68.87 mg GAE 100g^{-1} FW, while no significant differences were observed in the other interaction of genetic, biostimulants for total polyphenol content. Vitamin C is one of the most important quality parameters in lettuce, which was least affected by lettuce varieties and treatments (Table 18). The most significant variations in vitamin C were driven by environmental factors or growing seasons. Vitamin C levels increased significantly in all three lettuce cultivars treated with W+B, but other treatments had no influence on vitamin C levels.

The response of lettuce to biostimulants was highly dependent on the growth years or environmental conditions throughout the growing period. Plants produced in 2021, for example, have higher vitamin C content than plants cultivated in 2020 and 2019. This might be because light and temperature were somewhat higher in 2021 than in 2020 and 2019. Fu et al. (2017) have demonstrated that high light intensity and low nitrogen availability lead to accumulate more vitamin C and reduces nitrate in leafy vegetables.

Lettuce is well-known for its capacity to collect nitrate in its leaves. Because nitrate accumulation in the lettuce plant is unclear feature, the interplay of genotype (G) and environment (E) must be clarified (Burns, et al., 2011). Comparing to the control plants, the effect of the interaction among the above factors resulted in some improvement and reduction of nitrate content. This might depend on the nitrate content in the biostimulants and the environmental factors as light emission in the growing period. Another reason might be due to the nitrate availability in the soil, where soil of the experiment in 2020 contained higher nitrate than two other growing years. McCall and Willumsen (1998) have explained that lettuce with greater nitrate application or soil with higher nitrate availability can accumulate more nitrate without improving the final yield. The means of the growing years show that the interaction of plant varieties to the biostimulants like Willow, W+B and MLE had a little improvement of the nitrate level where they contain greater nitrogen level as discussed before. Bistep on the other hand, reduced the nitrate accumulation in the lettuce leaves. However, no significant differences can be seen among the factors.

Similar to other quality assessments, the nitrate accumulation was varieties based on the environmental factors more than the interaction of the genetic, treatments and the cultivar differences (Table 19). In the 2019 experiment, no significant difference in nitrate reduction in lettuce cultivars was found; however, in 2020 and 2021, the greatest nitrate reduction was recorded in the "Kobak" variety treated with W+B and MLE by around (-) 40%, (-) 50%, (-) 30%, (-) 20%, followed by the "May King" variety by about (-) 55%, respectively (Table 19).

Cozzolino et al. (2020) discovered similar results, demonstrating that spraying lettuce foliar with biostimulants of a tropical plant extract (PE) could lower nitrate content in the leaves by 23%. This observation may be connected to the high amino acid content of the plant extract, which may impede the root system's ability to accept the nitric ion in the soil while being absorbed by the plant leaves. Many research, such as those on iceberg lettuce (Bulgari et al., 2015), spinach (Edward Kunicki et al., 2010), and greenhouse tomato quality evaluation (Colla et al., 2017), corroborate our findings.

Although lettuce grown in the autumn season has higher nitrate accumulation than lettuce grown in the spring season (Colla et al., 2018b), vitamin C and total polyphenols may be unaffected by the growing season. In this case, we repeated the experiments in the autumn season and in different surroundings (under glasshouse condition) to see how the growing season and MLE treatment affected the bioactive compounds and nitrate levels in the lettuce varieties. As it is shown in Table 19, the changes in total polyphenol, vitamin C, and nitrate content during the autumn season in relation to the plant biostimulant application of 6% MLE, lettuce genotypes, growing years, and their interactions.

The total polyphenol content in lettuce cultivars was less affected by the aforementioned factors, but the changes were most noticeable among growing years. The significant variation of the total polyphenols can be seen in the growing seasons of 2019 and 2020, whereas no changes were found in 2021. In the season of 2019, 6% MLE could significantly reduce the total polyphenol content, whereas in 2020 6%MLE could significantly improve the total polyphenol content. This could be because the temperature in 2019 fluctuated less and the plants were less stressed than in the 2020 season (Table 8). The interaction of plant biostimulants, lettuce cultivars, and growing years results in a slight increase in total polyphenols in plants treated with 6% MLE compared to untreated plants. The same was true for the vitamin C content. However, few differences were observed for the 2020 growing season. This is primarily due to the fact that the growing year of 2020 was significantly warmer than the other two years, 2019 and 2021.

Table 19. The interaction effect of plant varieties, treatments and growing years on total polyphenol, vitamin C and nitrate content in lettuce vegetable grown in **autumn** seasons of 3 years (Debrecen, 2019- 2021)

Varieties	Treatments	Bioactive compounds											
		Total polyphenols (mg GAE 100g^{-1} FW)				Vitamin C (mg 100 g^{-1} FW)				Nitrate (mg kg^{-1})			
		2019	2020	2021	Mean	2019	2020	2021	Mean	2019	2020	2021	Mean
Interaction between MLE foliar application and plant variety													
May King	Control	37.65	51.63	51.40	46.89	5.85	6.03	9.72	7.20	784.00*	439.00*	1281.00*	834.66*
	MLE	35.59	65.83	48.40	49.94	4.81	9.21*	8.85	7.62	692.00	379.00	964.00	678.33
Kobak	Control	37.95*	65.4	48.90	50.75	5.83	8.87	8.99	7.89	950.00*	642.00*	1046.00*	879.33*
	MLE	35.61	70.88	47.60	51.36	4.91	9.92	8.59	7.80	772.00	517.00	876.00	721.66
Great Lakes	Control	41.73*	55.69	39.60	45.67	8.61	10.30*	6.19	8.36	1180.00*	702.00*	986.00*	956.00*
	MLE	37.26	73.03*	37.50	49.26	8.30	7.78	5.63	7.23	820.00	489.00	840.00	716.00
In the mean of cultivars													
Control		39.11*	58.93	46.63	48.22	6.76	7.28	8.30	7.44	971.55*	594.33*	1104.33*	890.00*
MLE		36.15	69.91*	44.50	50.18	6.01	9.81*	7.69	7.83	761.00	461.66	893.33	705.00
In the mean of season													
May King		36.62^{b}	58.73^{b}	49.90^{a}	48.41^{a}	5.33^{b}	7.62^{a}	9.28^{a}	7.41^{a}	738.0^{b}	409.0^{b}	1122.50^{a}	756.50^{a}
Kobak		36.78^{b}	70.17^{a}	48.25^{a}	51.05^{a}	5.37^{b}	9.39^{a}	8.79^{a}	7.85^{a}	861.0^{ab}	579.5^{a}	961.00^{a}	800.50^{a}
Great Lakes		39.50^{a}	64.36^{ab}	38.55^{b}	47.46^{a}	8.46^{a}	8.62^{a}	5.91^{b}	7.80^{a}	1000.3^{a}	595.5^{a}	913.00^{a}	836.16^{a}

Means within the same column followed by the same letter(s) are not significantly different at the probability level of ($p \leq 0.01$) according to Duncan[a,b] Multiple Range Test

In general, the year 2020 had the highest total polyphenols and vitamin C content, while the year 2019 had the lowest. This could be due to environmental factors such as higher temperatures and lower light emission during the 2020 growing season.

In the autumn experiments we focused on moringa leaf extract (6% MLE) as a plant biostimulant that is safe, inexpensive, and easy to manufacture and apply to plants with the purpose of maximizing quality metrics, yield, and reducing nitrate content. Many research publications describe MLE as an efficient plant biostimulant for improving the quality parameters of many vegetables.

There is also an EU regulation No 1258/2011 for vegetable nitrate content for autumn or winter growing leafy vegetables, with maximum levels permitted for lettuce grown under cover at 5000 mg NO_3^- kg^{-1} and open air at 4000 mg NO_3^- kg^{-1} (Commission regulation, 2011). Thus, our three-year experiment was also repeated in the autumn season but this time under glasshouse weather conditions, where the environmental variable was computerised.

The nitrate content differed significantly in the autumn growing season. Foliar application of 6% MLE performed better in the cooler autumn growing season than in the warmer spring growing season. The mean interaction results show that there is a significant difference between treated and untreated lettuce cultivars. The use of 6% MLE has the potential to significantly reduce nitrate content by 21%. This reduction was modified based on the genotype reaction and the interaction of the factors with the biostimulant used. The interaction means of "Great Lakes" variety treated with 6% MLE showed the greatest reduction in nitrate content, followed by "May King" and "Kobak" for about 25%, 20%, and 15%, respectively. Despite the fact that the "Great Lakes" variety has a naturally higher nitrate content than "May King" and "Kobak," no significant differences were found among lettuce cultivars (Table 20).

Nitrate accumulation in lettuce cultivars can be influenced by growing years or environmental factors in the same season. For example, in 2019, the "Great Lakes" variety of Crisphead lettuce accumulated significantly more nitrate than the "May King" and "Kobak" varieties. Several studies have found that morphological differences between varieties are related to nitrate accumulation in lettuce, with Crisphead lettuce cultivars accumulating more nitrates than iceberg and romaine lettuce cultivars (EFSA, 2008; Santamaria et al., 1999).

4.4. Relative leaf chlorophyll content (SPAD) and normalized difference vegetation index NDVI

The SPAD 502 Plus Chlorophyll Meter (Konica Minolta®, Japan) is a portable diagnostic tool used to determine the relative leaf chlorophyl concentration in plant leaves in a non-destructive manner (Gianquinto et al., 2004). Measuring the chlorophyll content is beneficial since it is directly connected to the nitrogen or nitrate content of the lettuce leaves (Mendoza-Tafolla et al., 2019). Additionally, the normalized difference vegetation index (NDVI) value is also a non-destructive tool for detecting biotic stress in plants during image screening (Sandmann et al., 2018).

4.4.1. The effect of plant biostimulants on SPAD and NDVI value of lettuce

According to the results in Table 20, there is a negative connection between SPAD and NDVI. Biostimulants increase SPAD while decreasing NDVI and conversely. This indicates that plants with greater nitrogen and chlorophyll content will be subjected to more biotic stress than plants with lower nitrogen and chlorophyll content. This data supports the suggested assumption by Hendry and Price (1993) that a quick increase in total chlorophyll/carotenoids is a known plant stress response. The results reveal that when the plants were treated with biostimulants, there was no significant reduction in the SPAD value. Also, there was a little difference between treated and untreated plants with Bistep and MLE, however the NDVI value was increased. However, as compared to the control plants, Willow and W+B did not significantly improve SPAD and NDVI. Treatments have resulted in a considerable improvement during the course of the growing years. In the growing year of 2021, where there was a substantial light emission during the lettuce growing period, the SPAD value was much higher. Whereas the lowest NDVI value was shown to be significant in the growth year of 2020.

Table 20. Effect of plant biostimulants on the SPAD and NDVI value in different lettuce cultivars grown in **spring** seasons (Debrecen, 2019-2021)

Treatments	SPAD				NDVI			
	2019	2020	2021	Mean	2019	2020	2021	Mean
Control	29.86ab	34.05b	33.85a	32.58a	0.730a	0.590b	0.716abc	0.678a
Willow	28.34b	36.20a	32.04b	32.19a	0.713a	0.604b	0.710bc	0.675a
Bistep	26.23c	30.41c	33.95a	30.19a	0.726a	0.662a	0.744a	0.710a
W+B	31.64a	32.03c	33.95a	32.54a	0.708a	0.602b	0.737ab	0.682a
MLE	-	28.25d	33.84a	31.04a	-	0.700a	0.690c	0.695a
Mean	29.01b	32.18ab	33.52a	-	0.714a	0.631b	0.719a	-

Means within the same column followed by the same letter(s) are not significantly different (*P≤0.05*) according to Duncan[a,b] test Multiple Range Test

4.4.2. The effect of lettuce genotypes on SPAD and NDVI value of lettuce

The genetic differences had an extreme influence on the SPAD and NDVI value (Table 21). The SPAD and NDVI values were greatly altered by genetic diversity among plant cultivars since the "Great Lakes" variety is naturally greener in colour and generates a greater head size. The "Great Lakes" type has 42% more chlorophyll than the "Kobak" variety and 35% more chlorophyll than the "May King" variant in the mean of years. Furthermore, the mean of years indicate that "Great Lakes" variety has a 4% higher NDVI value than the "May King" variety and 11% higher NDVI value than the "Kobak" variety.

Table 21. Effect of plant cultivars on the SPAD and NDVI value in **spring** seasons (Debrecen, 2019-2021).

Cultivars	SPAD				NDVI			
	2019	2020	2021	Mean	2019	2020	2021	Mean
May King	23.07b	30.43b	29.21b	27.57b	0.706b	0.662a	0.722b	0.696a
Kobak	22.46b	25.09c	26.08c	24.54b	0.677c	0.584b	0.673c	0.644a
Great Lakes	41.53a	41.04a	45.25a	42.60a	0.775a	0.649a	0.763a	0.729a

Means within the same column followed by the same letter(s) are not significantly different (*P≤0.05*) according to Duncan[a,b] test Multiple Range Test

4.4.3. The interaction effect of lettuce genotype, growth season and plant biostimulants on SPAD and NDVI value of lettuce

The results in Table 22 reveal that the interaction of plant materials, genotypes, and growing seasons had a very limited influence on the chlorophyll content or SPAD value and normalized difference vegetation index. The means of years show that a significant improvement was only occurs for the "Kobak" variety treated with Willow bark extract. On the other hand, the 6% MLE treatment significantly reduced the SPAD value in "Great Lakes" variety. Other treatments or interactions show no significant changes.

Table 22. Interaction of plant variety, cultivar and growing years on SPAD and NDVI value in lettuce grown in spring seasons (Debrecen, 2019-2021).

Treatments	Varieties	SPAD value				NDVI value			
		2019	2020	2021	Mean	2019	2020	2021	Mean
Control	***May King***	24.27^{c}	29.36^{d}	28.66^{cde}	27.43^{cd}	0.73^{bc}	0.61^{cd}	0.72^{bc}	0.68^{abcd}
	Kobak	22.38^{c}	25.88^{e}	25.40^{g}	24.55^{d}	0.69^{cd}	0.58^{de}	0.69^{cd}	0.65^{bcd}
	Great Lakes	42.93^{a}	46.92^{a}	47.50^{a}	45.78^{a}	0.77^{ab}	0.57^{de}	0.74^{bc}	0.69^{abcd}
Willow	***May King***	24.04^{c}	40.22^{b}	28.73^{cde}	30.99^{c}	0.72^{bc}	0.58^{de}	0.72^{bc}	0.67^{bcd}
	Kobak	22.08^{c}	29.38^{d}	22.20^{h}	24.55^{d}	0.67^{de}	0.52^{e}	0.64^{de}	0.61^{cd}
	Great Lakes	38.92^{b}	39.00^{b}	45.20^{b}	41.04^{ab}	0.74^{bc}	0.70^{ab}	0.76^{ab}	0.73^{ab}
Bistep	***May King***	18.39^{e}	25.22^{f}	29.96^{cd}	24.52^{d}	0.69^{cd}	0.70^{ab}	0.71^{bc}	0.70^{abc}
	Kobak	20.56^{e}	25.48^{f}	27.20^{efg}	24.41^{d}	0.69^{cd}	0.57^{de}	0.71^{bc}	0.65^{bcd}
	Great Lakes	38.73^{b}	40.54^{b}	44.70^{b}	41.32^{ab}	0.79^{a}	0.71^{ab}	0.81^{a}	0.77^{a}
W+B	***May King***	25.57^{c}	30.30^{d}	30.46^{c}	28.77^{cd}	0.68^{de}	0.66^{bc}	0.72^{bc}	0.68^{abcd}
	Kobak	24.82^{c}	21.14^{g}	26.43^{fg}	24.13^{d}	0.65^{e}	0.62^{cd}	0.73^{bc}	0.66^{bcd}
	Great Lakes	44.52^{a}	44.66^{a}	44.76^{b}	44.64^{ab}	0.79^{a}	0.52^{e}	0.75^{ab}	0.68^{abcd}
MLE	***May King***	-	27.08^{e}	28.23^{def}	27.65^{cd}	-	0.75^{a}	0.73^{bc}	0.74^{ab}
	Kobak	-	23.58^{g}	29.20^{cde}	26.39^{cd}	-	0.62^{cd}	0.59^{e}	0.60^{d}
	Great Lakes	-	34.10^{c}	44.10^{b}	39.10^{b}	-	0.73^{ab}	0.74^{bc}	0.73^{ab}

Means within the same column followed by the same letter(s) are not significantly different at the probability level of ($P \geq 0.05$) according to Duncan ^{a,b} Multiple Range Test

Similar to other results, the changes were mostly seen among the growing years. The combination of plant types and treatments resulted in a significantly higher chlorophyll content in the growing season of 2021, whereas the lowest chlorophyll content was reported in the season of 2019. This is mostly because the environmental component (light emission) was greater in 2021 than in other seasons.

Among the factors, light is the main source of energy to the plants (Fukuda et al., 2008), which is captured by chlorophyll in the plant leaves during the photosynthesis process, so that any changes in the light (quantity, density, duration) lead to abnormality in the plant content. In the 2019 season, the interaction of W+B with "Great Lakes" variety generated the highest SPAD value of 44.52, while the "Kobak" variety treated with Bistep plant biostimulant yielded the lowest SPAD value of 20.56. Similarly, in the season 2020, the control and W+B treatments resulted in the maximum chlorophyll content at 46.92 and 44.66 in "Great Lakes," respectively. However, the "Kobak" variety treated with W+B resulted in the lowest chlorophyll contents at 21.14. In 2021, where the control "Great Lakes" variety had the greatest SPAD value, while the "Kobak" type treated with Willow bark extract had the lowest at 22.20.

The Normalized Difference Vegetation Index (NDVI), on the other hand, is the vegetative coverage of plants on the soil surface and indicates how the plants are responding to abiotic stress. According to our findings, the interplay of plant variety, cultivar, and growing year on NDVI value in lettuce has a very minimal affect. The combination of "Great Lakes" with Bistep plant biostimulants resulted in the only substantial improvement in NDVI; treatments of MLE on "May King" and "Great Lakes" types resulted in a small improvement. The variations were detected again among the growth years, with the "Great Lakes" variety treated with W+B yielding the best results at 0.79 in 2019. In comparison to the control plants, the "Great Lakes" variety treated with Willow and Bistep in 2020 and 2021, MLE only in 2020, had considerably higher NDVI (Table 23). This is mostly because the "Great Lakes" type is greener in colour and produces larger heads than the "May King" and "Kobak" variants (Yaseen and Takácsné-Hájos, 2021a). Plant biostimulants may also minimize biotic stress in lettuce and enhance plant tolerance to the growing environment (Patrick du Jardin, 2015; Zulfiqar et al., 2020). Similar experiments in the autumn season show that the SPAD value or chlorophyll content in lettuce leaves were significantly lower than in the spring season, but there was no difference in the NDVI value between the two seasons (Table 23). It is obvious that chlorophyll content is directly related to light emission, light quality, and duration (Fu et al., 2012). The light intensity in the growing location varies greatly between spring and autumn seasons; Tables 8 and 10 show a substantial difference in light emission in the experimental location during the lettuce growing season. There was also a significant difference in the duration of light between the two growing seasons.

Table 23. Interaction of plant variety, cultivar and growing years on SPAD and NDVI value in lettuce grown in the **autumn** seasons (Debrecen, 2019-2021).

Varieties	**Treatments**	**SPAD value**				**NDVI value**			
		2019	**2020**	**2021**	**Mean**	**2019**	**2020**	**2021**	**Mean**
Interaction between MLE foliar application and plant variety									
May King	Control	13.16	19.88	17.70	16.91	0.736	0.711	0.716	0.721
	MLE	17.56*	21.71	17.84	19.03	0.753	0.713	0.734	0.733
Kobak	Control	16.23	19.90	17.42	17.85	0.778	0.726	0.726	0.743
	MLE	23.90*	20.45	19.06*	21.13	0.793	0.703	0.748*	0.748
Great Lakes	Control	32.76	37.48	32.72	34.32	0.793	0.663	0.730	0.728
	MLE	40.03*	36.56	34.60*	37.06	0.750	0.676	0.810*	0.745
In the mean of cultivars									
Control		20.72	25.75	22.61	23.02	0.770	0.700	0.720	0.730
MLE		27.16	26.24	23.83	25.74	0.765	0.697	0.762*	0.740
In the mean of season									
May King		15.36^{b}	20.80^{b}	17.77^{b}	17.97^{b}	0.745^{b}	0.712^{a}	0.720^{b}	0.725^{a}
Kobak		20.06^{b}	20.17^{b}	18.24^{b}	19.49^{b}	0.786^{a}	0.715^{a}	0.732^{ab}	0.744^{a}
Great Lakes		36.40^{a}	37.02^{a}	33.66^{a}	35.69^{a}	0.771^{ab}	0.670^{b}	0.770^{a}	0.737^{a}

Means within the same column followed by the same letter(s) are not significantly different at the probability level of ($p \leq 0.01$) according to Duncan^{a,b} Multiple Range Test

The light duration in the experimental location was between 8 and 11.5 hours in the autumn growing season, and 11 to 15.5 hours in the spring growing season (Time and Date, 2022). A similar result to the spring growing season was detected for the influence of plant biostimulants, growing years, plant varieties and their interactions on the SPAD value. The means show that plant biostimulant of 6% MLE could slightly improve chlorophyll content, but no significant differences was recorded (Table 23). However, there was a significant difference between lettuce genotypes, with the greener lettuce cultivar "Great Lakes" containing significantly more chlorophyll than the two other varieties "May King" and "Kobak." Furthermore, among the growing years, the interaction effect of plant biostimulants, growing years, and lettuce cultivars was observed. The means, on the other hand, show a slight improvement in the SPAD value, but no statistically significant difference. The interaction factors had the greatest impact during the season, where there was a bit of a similarity from beginning to end with a better light emission from 133.54 to 52.57 (W m^{-2}), whereas the lowest chlorophyll content was detected in the colder growing year of 2021.

The above factors had less of an impact on the normalized difference vegetation index (NDVI) during the autumn season. The NDVI is a non-destructive method for detecting abiotic stress in plants (Sandmann et al., 2018). Our experiments in the autumn season were conducted in a fully controlled environment (glasshouse), so we expected less abiotic stress than growing lettuce in a plastic house in the spring season. Table 23 shows that the NDVI value in the autumn season was slightly higher than in the spring season. This could be due to the fact that there were less unfavourable factors inside the glasshouse, whereas lettuce grown in a plastic house was not fully protected.

Plant biostimulant (6% MLE) performed better in climates with higher light emission during the growing seasons of 2021, whereas the lowest NDVI values were recorded in climates with lower light emission in 2020. However, lettuce cultivars had no significant influence on NDVI, but the differences varied depending on the growing year. While, higher NDVI values were observed during the seasons of lower temperature and higher light intensity in 2021.

4.4.4. Correlation and regression analysis results

4.4.4.1. Correlation and regression analysis results of the spring experiments

The correlation analysis in Table 24 was applied to the spring experiments of three years' results to see if there was a link between the measured parameters. The nitrate content had significantly negative correlated with lettuce fresh head weight and total polyphenol content at p =0.05, with larger head weight containing less nitrate than smaller head weight and plants with higher total polyphenol content containing less nitrate. The correlation between head weight, SPAD, and NDVI, on the other hand, was significantly positive. Vitamin C and SPAD (chlorophyll content), on the other hand, had a positive correlation with nitrate and NDVI at $p = 0.01$ and $p = 0.05$, respectively.

Table 24. Correlation analysis for some physical and bioactive compounds of lettuce grown in **spring** seasons (Debrecen, 2019-2021)

Correlations for spring experiments						
	Head Weight	Total polyphenol	Vitamin C	Nitrate	SPAD	NDVI
Head Weight	1.00					
Total polyphenol	0.087	1.00				
Vitamin C	0.159	0.166	1.00			
Nitrate	-0.234**	-0.234**	0.181*	1.00		
SPAD	0.292**	-0.119	0.077	0.144	1.00	
NDVI	0.585**	0.062	0.231**	-0.047	0.274**	1.00

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

4.4.4.2. Correlation and regression analysis of the autumn experiments

During the autumn season, the correlation between some very important physical and bioactive compounds was different. The only statistically significant relationship discovered was one between total polyphenol and vitamin C content and NDVI value. Total polyphenol content was found to be significantly correlated with vitamin C but negatively correlated with nitrate content and NDVI value. Table 25 shows that increasing vitamin C content had a significant inverse correlation with NDVI value at $p = 0.01$.

Table 25. Correlation analysis for some physical and bioactive compounds of lettuce grown in **autumn** seasons (Debrecen, 2019-2021)

Correlations for autumn experiments						
	Head weight	Total polyphenol	Vitamin C	Nitrate	SPAD	NDVI
Head weight	1.00					
Total polyphenol	0.017	1.00				
Vitamin C	0.029	0.647**	1.00			
Nitrate	0.185	-0.541*	0.071	1.00		
SPAD	0.102	0.019	0.112	-0.047	1.00	
NDVI	0.21	-0.732**	-0.567*	0.444	-0.062	1.00

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

The results of the regression analysis were rather complex due to the evaluation of different varieties and different years. We attempted to demonstrate some links between the influence of biostimulants on various bioactive compounds in autumn and springtime using this analysis. Our various analyses revealed a genuine link between vitamin C content evaluation and rising spring temperatures. As can be seen, the biostimulant with the highest regression (R^2) was Vitamin C. Willow has an R^2 of 0.588, Bistep has an R^2 of 0.371, and Moringa leaf extract (MLE) has an R^2 of 0.342. (Figure 42, 43 and 44).
All of the applied plant biostimulants (Willow, Bistep and Moringa leaf extract) supported this trend. The regression analyses of other quality parameters were not shown or produced a strong result where these the regression analysis figures are shown in the appendix 9, I and II.

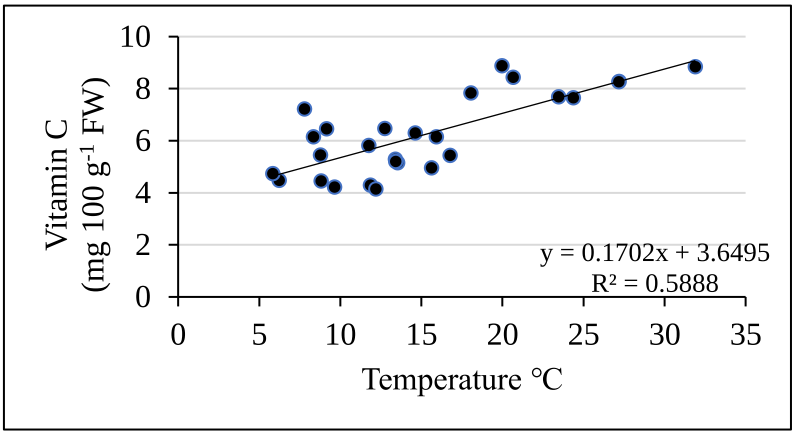

Figure 42. The results of the regression equation for vitamin C vs. temperature °C of lettuce growing in three years of experiments in the spring season treated with Willow bark extract (Debrecen, 2019-2021).

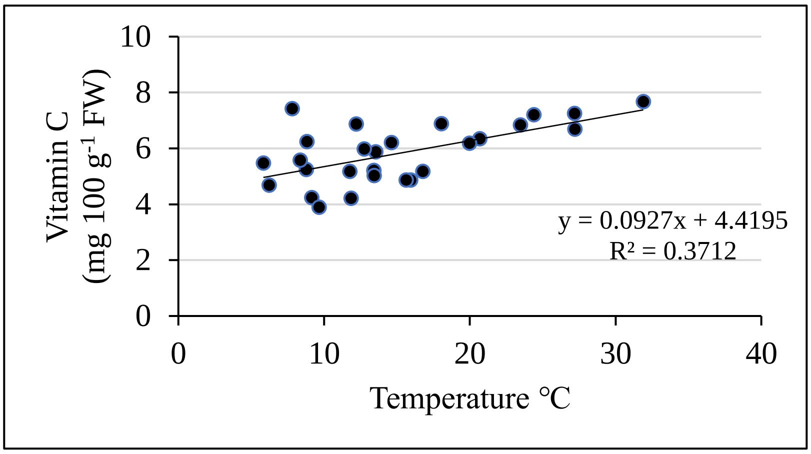

Figure 43. The results of the regression equation for vitamin C vs temperature °C of lettuce growing in three years of experiments in the spring season treated with Bistep plant biostimulant (Debrecen, 2019-2021).

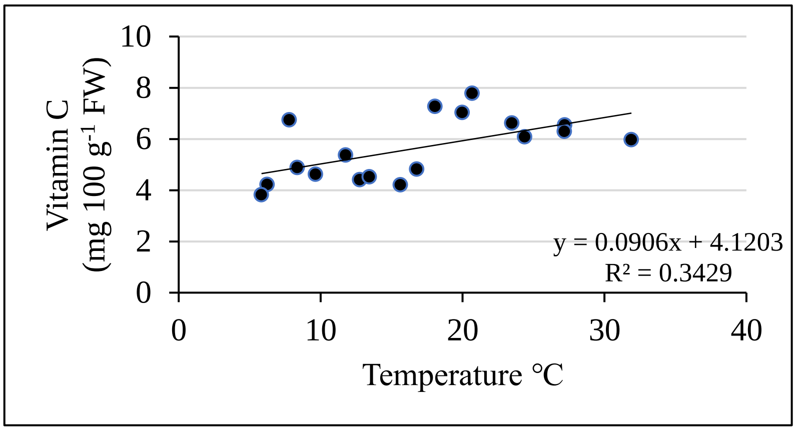

Figure 44. The results of the regression equation for vitamin C vs temperature °C of lettuce growing in three years of experiments in the spring season treated with Moringa leaf extract (MLE) (Debrecen, 2019-2021).

5.0. CONCLUSIONS, SUGGESTIONS

Our three-year experiment in the autumn and spring seasons demonstrates that plant biostimulants, rather than physical parameters, can improve nutritional value in lettuce cultivars. The growing environment, on the other hand, had a significant impact on the quality parameters. Biostimulants can improve lettuce quality in areas where the climate is cool and there is enough sunlight.

Because lettuce is a cool-season vegetable, the quality improved when the temperature was lower and the light emission was higher. As a result, while all physical parameters and bioactive compounds increased in the spring season of the three-year experiment, nitrate concentration in lettuce leaves increased significantly in the autumn growing season. The most noticeable changes occurred during the growing years within each season.

Nutrient availability in the soil can also affect the nutritional and physical quality of the grown plant. Soil with a high concentration of macro- and micro-nutrients, as well as a high level of humidity, can improve internal and external quality parameters.

Plant biostimulants, in general, can be a better alternative to reduce fertilizer inputs, improving mineral, physical, and bioactive compounds, and lowering nitrate content in lettuce vegetables without harmfully impacting the environment. However, in order to maximize the effectiveness of the used plant biostimulants, the method of application, environmental factors, genetics, and their interactions must all be considered. Our findings indicate that the measured parameters have some significant positive and negative correlations. Total polyphenols had a significant negative correlation with nitrate content in both growing seasons at p = 0.01 and 0.05, whereas other parameters such as head weight, vitamin C, SPAD index, and NDVI could have different results depending on the environmental factors. However, the regression analysis reveals that biostimulants could only improve vitamin C with temperature increases during the spring season.

Based on our results our suggestions will be:

1. Using a combination of biostimulants as Willow+ Bistep (W+ B) because when used alone, willow bark extract (W) has some negative effects such as increasing nitrate accumulation in lettuce leaves.

2. Moringa leaf extract (MLE) is widely recommended for use on lettuce grown in the autumn season because it can significantly reduce nitrate levels when the environment is cooler without affecting other quality parameters.

3. The "Great Lakes" variety significantly outperforms other lettuce cultivars in terms of quality parameters in autumn, but the only disadvantage is that it accumulates more nitrate, whereas "May King" and "Kobak" perform better in spring and accumulate less nitrate in autumn.

4. Lettuce grown in a glasshouse facing less biotic and abiotic stress, but the yield is much lower than in the spring season due to the low light emission.

5. To achieve a better result in a glasshouse for autumn lettuce production, we recommend establishing artificial light and extending daily light for at least 10 hours day/night lighting or/and producing lettuce in a glasshouse in the spring season as well.

6. Finally, we propose similar work in the autumn season using Willow bark extract (W), Bistep, and Willow+ Bistep (W+B) in a plastic- and glass-house. We also recommend doing more research on MLE in different dozes to see how it affects in both seasons.

6.0. NEW SCIENTIFIC RESULTS

1. Moringa leaf extract (6% MLE) was applied as a plant biostimulant which it could reduce nitrate content in lettuce cultivars grown under glasshouse in the autumn season by 20%, 15%, and 25%, depending on the lettuce cultivar. But this impact was less seen in spring season. This could be because moringa leaf extract has a stronger influence in lower temperature regime than warmer. Bistep (B) plant biostimulant could also reduce nitrate levels in lettuce grown under plastic house in the spring season by 13%. However, using Willow bark extract (W) in the spring season increased the nitrate content by around 20%.
2. Willow bark extract (W) can be used not only as a biofungicide, as many researchers recommend, but also as plant biostimulants to improve some physical quality parameters as head structure and head closing and head weight in “Great Lakes” variety, vitamin C and magnesium content.
3. Combining plant biostimulants could give better results in terms of improving some nutritional value in lettuce without affecting nitrate content than single applications. Willow + Bistep (W+B) could increase the nutritional value of lettuce in terms of macronutrient content of calcium, phosphorus, magnesium, sodium, sulphur, micronutrient content iron, manganese, and copper, and total polyphenols by about 10%.
4. Lettuce grown in the spring produced higher physical and nutritional value than lettuce grown in the autumn. This was due primarily to environmental factors. Light intensity and duration were higher in the spring than in the autumn, and lettuce is a light-sensitive vegetable. Our recorded metrological data showed that the daily light duration in the spring season was approximately (11 to 12 hours), but in the autumn season was at about (8 to 9 hours).
5. There was also a large variation among the lettuce cultivars. The "Great Lakes" variety produces larger head weight, greater mineral content (potassium, calcium, magnesium, sulphur, copper, manganese and iron) and chlorophyll content. In contrast, greater number of leaves, phosphorus content was found in "May King" and "Kobak" lettuce cultivars in spring and autumn seasons.
6. The interaction of the factors mentioned (genetic, climate, and growing seasons) had a greater impact on physical parameters than bioactive compounds and mineral

content. The interaction of Willow bark extract (W) and Willow + Bistep (W+ B) with the "Great Lakes" variety resulted in a significant improvement in head weight. The interaction of moringa leaf extract (MLE) with the "May King" variety led to a significant larger head diameter. The "Kobak" variety, when treated with moringa leaf extract (MLE), had better head closure than the other varieties.

7. According to the correlation analysis, total polyphenols had a negative correlation with nitrate content in both seasons, spring (-0.234**) and autumn (-0.541*). Only in the spring the head weight has a negative correlation with nitrate. The correlation analyses for the other measured parameters differed depending on the season. In the spring season, vitamin C had a significant positive correlation with NDVI (0.231**), but in the autumn season, it had a negative correlation (-0.567*).
8. Regression analysis reveals that plant bio-stimulants (Willow, Bistep, and Moringa leaf extract) could only improve vitamin C by raising the temperature in the spring season, with R^2 values of 0.588, 0.371, and 0.342, respectively.

7.0. PRACTICAL UTILIZATION OF RESULTS

1. Based on our three years of research in both seasons (spring and autumn), biostimulants can be more effective when applied to lettuce in a cooler environment with high humidity level.
2. Growing leafy vegetables, particularly lettuce, requires consideration of environmental (light emission and temperature) and plant varieties, so we recommend growing "May King" and "Kobak" varieties primarily in spring, while "Great Lakes" is best grown in autumn.
3. Willow bark extract (W) is a new biostimulant that can be applied to plants through irrigation once every two weeks at a concentration of 3% to improve other quality parameters in addition to acting as a fungicide and root enhancer.
4. It can be stated that plastic-houses and glasshouses are suitable for lettuce production, however lettuce grown in autumn season may require longer and much intensive light and this can be done with artificial lightening.
5. Our findings suggest that, in addition to the Bistep manufactured biostimulant, home-made biostimulants Willow (W) and moringa leaf extract (MLE) can be applied to lettuce as a safe, easy-to-prepare, and low-cost product with favourable results.
6. Finally, we recommend applying the Willow+ Bistep (W+ B) biostimulant combination to lettuce grown in the spring season in the amounts of 50-70 mL plant^{-1}+ 15-20 mL plant^{-1} in cooler weather conditions of early morning or late afternoon with the doors open to keep the temperature cool and proper air circulation under plastic-house.
7. It is also strongly recommended to apply moringa leaf extract (% MLE) to lettuce growing in autumn as a foliar spray (15-20 mL plant^{-1}) or with irrigation. Because it improves not only some quality parameters but also reduces nitrate content in plant leaves.

8.0. SUMMARY

Lettuce (*Lactuca sativa* L.) is an *Asteraceae* family and one of the most prevalent leafy vegetables in fast food around the world. Lettuce is widely consumed fresh due to its high vitamin, polyphenol, flavonoids, carotenoids, and mineral element content. Even though lettuce (*Lactuca sativa* L.) is a popular and widely consumed vegetable, its nutritional value is underappreciated due to its high (95 %) water content. Lettuce is a cool-season crop grown on all continents, primarily in temperate and subtropical climates. Because lettuce is a seasonal crop, it necessitates a sufficient amount of fertilizer. However, it is clear that over-fertilization caused a number of environmental issues as well as harmful effects on human health, particularly due to nitrate accumulation in plant leaves.

To reduce the environmental and human health impact of chemical fertilizers, biostimulants can be used as a supplementary fertilizer to improve plant quality and reduce the amount of chemical fertilizer required in plant production while also improving the plant's ability to absorb and translocate nutrients. Plant biostimulants are considered to be a safe and environmentally friendly product that has been supported by scientists from a variety of natural sources to be used on plants as one of the most important agronomic ingredients.

From 2019 to 2021, studies were conducted at the University of Debrecen's Farm and Regional Research Institute in Hungary's Botanical and Exhibition Garden in early spring (under plastic house) and late autumn (under glasshouse). The goal of these studies was to investigate the impact of some natural plant biostimulants Bistep (B), Willow (W), and their combination Willow+ Bistep (W+B) as well as moringa leaf extract (MLE) on some quality parameters such as morphological parameters, bioactive compounds, and mineral content of three lettuce cultivars ("May King," "Kobak," and "Great Lakes").

The concentration of 0.5% Bistep and 6% moringa leaf extract (MLE) were applied to the lettuce varieties through spraying onto the plant leaves with the amount of 20 mL plant^{-1}, whereas 3% Willow bark extract was applied to plants through irrigation with the amount of 50-60 mL plant^{-1}.

The following vegetative characteristics were measured and evaluated: head weight (g head^{-1}), root weight (g head^{-1}), head diameter (cm), internal stem size (cm), head structure (scale 1....10), head closing (scale 1....10), and number of leaves (piece head^{-1}).

Furthermore, for the bioactive compounds, we measured vitamin C (mg 100 g^{-1} FW), total polyphenol (mg GAE 100g^{-1} FW) and nitrate content (mg kg^{-1}). Also, for the mineral content, we measured the most essential macronutrients as calcium, magnesium, sodium, potassium, phosphorus, sulphur and micronutrients as copper, manganese, zinc. To fellow the plant development over the growing seasons, we measured chlorophyll content using a portable chlorophyll meter SPAD-502 (Konica Minolta, Japan) and normalized difference vegetation (NDVI).

Our results display that plant biostimulants, lettuce genotypes, growth seasons, and the interaction between plant varieties and the application of various biostimulants had a significant influence on morphological traits in both seasons (spring and autumn). Over the period from 2019 to 2021, Willow bark extract (W) considerably increased head structure and head diameter compared to control plants cultivated in spring seasons, whereas Bistep (B) improved head and root weight. The application of Willow+ Bistep (W+B), on the other hand, could only enhance head structure, while moringa leaf extract (MLE) improved internal stem size. However, lettuce genotypes impacted leaf number more than biostimulants and environmental conditions. Among the lettuce cultivars, the "Kobak" variety had the best morphological criteria, including head structure, head closure, number of leaves, and internal stem size. The "Great Lakes" variety generated the only significantly greater head weight from 2019 to 2021.

Our findings show that genotypes, growth season, biostimulant treatments, and their interactions had an effect on the measured and evaluated parameters based on the growing season and climate conditions. In general, lettuce cultivars grown in the spring had much better physical parameters than those grown in the autumn. Among the treatments, the interaction of plant varieties and biostimulants had the greatest influence on the vegetative parameters of lettuce cultivars, especially in the spring. This could be because the climate in the spring season is much more conducive to physiological improvement, particularly in terms of light and temperature. In contrast, when lettuce cultivars were treated with the above treatments, the least impacted parameters were discovered for the autumn seasons.

The interaction of the "Great Lakes" variety with Willow bark extract (W) and Willow bark extract+ Bistep (W+ B) in the mean of years of spring growing seasons significantly improved the head weight, which is one of the most important commercial quality parameters. There was a slight improvement in the head weight of the autumn season for the interaction of 6% MLE with the lettuce cultivars but no significant difference was recorded.

The visualizing scaled characteristics, such as head structure (scale 1...10) and head closure (scale 1...10), were greatly enhanced in all lettuce cultivars grown in spring and autumn seasons. The treatment of 6% MLE being the most influential biostimulant on the "Kobak" variety, followed by "Great Lakes" and "May King". The interaction of plant biostimulants, growing season, and lettuce cultivars, on the other hand, had the least influence on head diameter (cm head^{-1}) and internal stem size (cm head^{-1}).

Plant genotypes, rather than treatments, had a large influence on the number of leaves. For example, the "Kobak" variety naturally produced the most leaves, ranging from 40 to 50 leaves, followed by "May King," which produced 35 to 40 leaves, and "Great Lakes," which produced the fewest leaves, ranging from 20 to 30 leaves. Although leaf number is largely determined by plant genotypes, the combination of Willow bark extract+ Bistep (W+B) could influence on the leaf production where the environment was much suitable. For example, in the 2020 season it could significantly boost this trait by 16 and 19 % in "May King" and "Kobak" cultivars, respectively, when compared to control plants. Furthermore, 6% MLE could increase number of leaves in all three lettuce cultivars.

Data from the spring seasons indicate that Willow bark extract+ Bistep (W+B) and Willow bark extract (W) were the most effective biostimulants in terms of macro- and micro-nutrient content improvements. The quantity of macronutrients calcium (Ca^{2+}), magnesium (Mg^{2+}), and phosphorous (P) in the plants treated with Willow bark extract+ Bistep (W+B) increased by 18%, 13%, and 9%, respectively, while potassium (K^{+}) decreased by 15%. Sodium content, on the other hand, was increased primarily by Willow (W) and Willow bark extract+ Bistep (W+B) treatments by 57%, and 72%, respectively. Among lettuce cultivars, "Great Lakes" lettuce has a higher level of macronutrient content (Ca^{2+}, Mg^{2+}, Na^{+}, and K^{+}) than "Kobak," despite "Kobak" having a significantly higher phosphorus (P) concentration at 301.41 (mg kg^{-1} DM).

The micronutrient content of lettuce varieties gave similar results. However, biostimulants Willow+ Bistep (W+B) had a positive influence on manganese, zinc and iron when the temperature was warmer in late April and May. Among the lettuce cultivars, the "Great Lakes" variety had much greater Cu, Mn and Fe content, while "Kobak" had the greatest Zn level.

The growing season had the greatest impact on total polyphenols, vitamin C, and nitrate levels, rather than plant biostimulants or lettuce genotypes. The combination of the factors, however, made some variations in the leaf content. The interaction of lettuce cultivars and the Willow+ Bistep (W+B) biostimulant significantly improved total polyphenol and vitamin C when compared to the control, primarily in the season when there was enough sunlight through the growing season and less temperature fluctuation. Similar results were seen for the 6% MLE in the autumn season.

This improvement was various based on the lettuce cultivars. Foliar spray of 6% MLE could significantly improve total polyphenol in the "Kobak" variety by 11%. Whereas vitamin C was improved in the same variety by 28% when it was treated with Willow+ Bistep (W+B). However, no significant improvement in vitamin C in the lettuce cultivars treated with 6 % MLE in autumn growing season. This might be because the light emission was much lower and this biostimulant could not influence as in spring season. A significant increase in total polyphenol was identified for the interaction of "May King" variety treated with Willow+ Bistep (W+B) by 9% in the spring 2020 season. Willow bark extract in combination with "Kobak" and "Great Lakes" could boost vitamin C levels by 16 % and 3%, respectively, in the 2021 season.

Total polyphenol was most impacted by the treatment of W+B and MLE in the mean of lettuce cultivars. Whereas, Willow, Willow + Bistep (W+B), and Bistep treatments had the greatest impact on vitamin C. In both growing seasons, the Willow bark extract (W) dramatically increased nitrate content. On the other hand, the growing season was the most important factor determining bioactive compounds (total polyphenols and vitamin C) and nitrate concentration in our three-year study. The spring season had the highest total polyphenol content (mg GAE $100g^{-1}$ FW), while the autumn season had higher vitamin C levels (mg 100 g^{-1} FW) and plants accumulated more nitrate in their leaves. The growing season and environmental factors, particularly light intensity and duration, influenced the nitrate content of lettuce cultivars. Similarly, genetic factors were among the most important factors in detecting nitrate levels. According to our three-year results, lettuce grown in the autumn season accumulates more nitrate than lettuce grown in the spring season. This is due to the longer daily light and better light quality in the spring season compared to the autumn season.

Our results also proof that the plant biostimulants is another factor influencing the nitrate level in lettuce cultivars. The Willow bark extract (W) being the sole treatment that significantly raised nitrate content in "Kobak" and "Great Lakes" varieties grown in spring season. Whereas Bistep, Willow+ Bistep (W+ B), and MLE could dramatically dropped nitrate levels especially in "Kobak" and "May King" cultivars. Moreover, the interaction of moringa leaf extract (MLE) with all three lettuce cultivars resulted in a significant reduction of nitrate in the autumn season, but this was less noticeable in the spring season.

Lettuce cultivars are different in terms of physical and nutrient content. The "Great Lakes" variety produces the largest head weight and size among lettuce cultivars, followed by "May King" and "Kobak." Furthermore, the "Great Lakes" variety contains more macro and micronutrients than the "Kobak" and "May King" varieties. The results show that the leaves of the "Kobak" variety contain more total polyphenols, whereas the "Great Lakes" variety contains more vitamin C. According to the findings, the "Great Lakes" variety was the best in terms of quality parameters, but it also accumulated the highest nitrate content when compared to the "May King" and "Kobak" varieties.

The mean results of the spring and autumn seasons show that the chlorophyll content or SPAD value was primarily affected by environmental and genetic factors, with the best results achieved in growing years with high light emission and in lettuce genotypes with greener colour, such as "Great Lakes." Spring experiments could have a higher SPAD value than autumn experiments. This might be because the climate condition in spring season was much suitable for the lettuce growth and improvement.
The growing season had the least influence on the normalized difference vegetation index (NDVI). The main distinction was the growing environment, where the best results were obtained in a fully controlled environment (autumn under glasshouse weather condition). Plant biostimulants could improve the SPAD and NDVI values slightly. The changes were most noticeable during the growing seasons (spring and autumn). Our correlation analyses indicate that total polyphenols had a negative correlation with nitrate content in both seasons, spring (-0.234**) and autumn (-0.541*). The correlation analyses for the other measured parameters differed depending on the season. The head weight has a negative correlation with nitrate in spring season only. While, vitamin C had a significant positive correlation with NDVI (0.231**) in spring season, but in the autumn season, it had a negative correlation at (-0.567*). The only high regression analysis found was for vitamin C improvement with temperature increase using plant biostimulants (Willow, Bistep, and Moringa leaf extract), with R^2 values of 0.588, 0.371, and 0.342, respectively.

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10.0. LIST OF PUBLICATIONS

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Registry number: DEENK/103/2022.PL
Subject: PhD Publication List

Candidate: Arshad Abdulkhalq Yaseen
Doctoral School: Kálmán Kerpely Doctoral School
MTMT ID: 10081851

List of publications related to the dissertation

Foreign language scientific articles in Hungarian journals (2)

1. **Yaseen, A. A.**, Takácsné Hájos, M.: The effect of Willow extract, Bistep and their combination on some quality parameters of lettuce (Lactuca sativa L.).
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2. **Yaseen, A. A.**, Takácsné Hájos, M.: Effect of Moringa leaf extract and set size on the bulb weight, diameter and yield of onions (Allium cepa L.).
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Foreign language scientific articles in international journals (5)

3. **Yaseen, A. A.**, Takácsné Hájos, M.: Evaluation of moringa (Moringa oleifera Lam.) leaf extract on bioactive compounds of lettuce (Lactuca sativa L.) grown under glasshouse environment.
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   Res. on Crops. 21 (2), 402-414, 2020. ISSN: 0972-3226.
   DOI: http://dx.doi.org/10.31830/2348-7542.2020.067

Foreign language abstracts (1)

8. **Yaseen, A. A.**: Effect of biostimulants on some mineral elements of lettuce (Lactuca sativa L.) grown under plastic tunnel.
   In: Scientific Conference of PhD. Studentsof FAFR,FBFS and FHLE SUA in Nitrawith international participation : Proceedings of abstracts, Slovak University of Agriculture, Nitra, 78, 2020. ISBN: 9788055222424

List of other publications

Foreign language scientific articles in international journals (5)

9. Ali, K. A., Noraldeen, S. S., **Yaseen, A. A.**: An Evaluation Study for Chlorophyll Estimation Techniques.
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13. **Yaseen, A. A.**, Ahmed, S.: Interaction effect of planting date and foliar application on some vegetative growth characters and yield of broccoli (Brassica olerasea var italica) grown under unheated plastic tunnel.
Journal of Garmian University. 4 (ICBS), 405-418, 2017. EISSN: 2522-3879.
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02 March, 2022

11.0. DECLARATION

This dissertation enables to obtain the doctoral (Ph.D.) degree of the University of Debrecen prepared by the Kálmán Kerpely doctoral school of the University of Debrecen.

Debrecen, 2022 …………………….

………………………… ..

the signature of the candidate

DECLARATION

I certify that Arshad Abdulkhalq Yaseen doctoral candidate 2018-2022. The above-mentioned Doctoral School carried out its work under my direction. The independent creative activity of the candidate is decisive for the results included in the dissertation; the dissertation is the independent work of the candidate. I suggest the acceptance of the dissertation.

Debrecen, 2022 ………

…………………………… ..

signature of the supervisor (s)

12. APPENDICES

Appendix 1.

Appendix 1. Effect of biostimulants and growing years on some measured vegetative parameters of lettuce in the mean of varieties in spring time, (Debrecen, 2019- 2021)

Treatments	Measured vegetative parameters											
	Head weight (g head^{-1})			Head diameter (cm)			Internal stem size (cm)			Root weight (g head^{-1})		
	2019	2020	2021	2019	2020	2021	2019	2020	2021	2019	2020	2021
Control	411.0^{b}	376.2^{d}	423.8^{ab}	19.9^{b}	24.1^{a}	19.2^{c}	5.5^{ab}	5.9^{c}	6.6^{a}	31.0^{a}	20.4^{c}	26.7^{b}
Willow	484.0^{a}	402.8^{c}	478.3^{a}	19.0^{b}	26.0^{a}	23.4^{ab}	6.3^{a}	5.0^{d}	6.2^{a}	35.3^{a}	22.8^{c}	18.8^{c}
Bistep	492.0^{a}	447.5^{b}	452.2^{ab}	20.0^{a}	24.0^{a}	25.5^{c}	5.5^{ab}	5.8^{d}	6.5^{a}	29.0^{b}	35.6^{a}	29.9^{a}
W + B	445.3^{a}	490.4^{a}	441.8^{ab}	20.2^{b}	27.2^{b}	25.2^{a}	5.0^{b}	6.8^{b}	6.6^{a}	22.3^{b}	28.8^{b}	19.5^{c}
MLE	-	402.4^{c}	405..2^{b}	-	26.7^{b}	24.1^{bc}	-	8.3^{a}	7.0^{a}	-	33.8^{a}	22.7^{bc}

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 Duncan
^{a,b} Multiple Range Test
FW: Fresh weight
W+B: Willow+Bistep

Appendix 2.

Appendix 2. Effect of biostimulants and growing years on some scaled vegetative parameters of lettuce in the mean of varieties in **spring** time, (Debrecen, 2019-2021)

Treatments	Scaled vegetative parameters								
	Head structure (1….10)*			**Head closing (1….10)****			**Number of leaves (Piece plant^{-1})**		
	2019	**2020**	**2021**	**2019**	**2020**	**2021**	**2019**	**2020**	**2021**
Control	7.93^{b}	7.79^{b}	8.33^{b}	8.00^{b}	7.91^{c}	8.33^{b}	41.26^{b}	34.83^{d}	38.77^{a}
Willow	9.00^{a}	9.16^{a}	9.11^{a}	8.93^{a}	8.94^{b}	9.11^{ab}	44.40^{a}	32.33^{d}	35.00^{b}
Bistep	8.13^{b}	9.16^{a}	9.27^{a}	8.53^{ab}	9.75^{a}	9.44^{a}	35.00^{c}	35.58^{c}	39.11^{a}
W + B	8.06^{b}	9.41^{a}	8.55^{ab}	9.06^{a}	9.83^{a}	8.77^{ab}	36.13^{c}	38.58^{b}	35.77^{b}
MLE	-	9.66^{a}	8.22^{b}	-	10.00^{a}	8.44^{ab}	-	46.66^{a}	34.22^{b}

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 Duncan ^{a,b} Multiple Range Test
(1…...10)*: 1 – loose ……10 – hard
(1….10)**: 1 – open ……10 – closed
FW: Fresh weight
W+B: Willow+Bistep

Appendix 3.

Appendix 3. Effect of plant variety on some vegetative parameters of lettuce in the mean of treatments in **spring** time (Debrecen, 2019-2021)

Varieties	**Measured vegetative parameters**											
	Head weight (g head^{-1})			**Head diameter (cm head^{-1})**			**Internal stem size (cm)**			**Root weight (g head^{-1})**		
	2019	**2020**	**2021**	**2019**	**2020**	**2021**	**2019**	**2020**	**2021**	**2019**	**2020**	**2021**
May King	433.90^{b}	313.90^{b}	388.66^{b}	16.25^{b}	31.25^{a}	22.80^{a}	6.02^{a}	6.12^{b}	6.55^{b}	27.60^{a}	36.65^{a}	28.56^{a}
Kobak	483.75^{b}	306.39^{b}	380.33^{b}	14.70^{c}	26.70^{b}	21.40^{a}	6.12^{a}	8.48^{a}	8.82^{a}	28.45^{a}	24.41^{b}	23.91^{a}
Great Lakes	666.90^{a}	383.16^{a}	560.33^{a}	18.60^{a}	28.95^{b}	21.53^{a}	4.62^{b}	4.62^{c}	4.56^{c}	32.27^{a}	23.80^{b}	18.22^{b}

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 Duncan ^{a,b} Multiple Range Test

Appendix 4.

Appendix 4. Effect of plant variety on some vegetative parameters of lettuce in the mean of treatments in **spring** time (Debrecen, 2019-2021)

Varieties	**Scaled vegetative parameters**								
	Head structure (1….10)*			**Head closing (1….10)****			**Number of leaves (piece head^{-1})**		
	2019	**2020**	**2021**	**2019**	**2020**	**2021**	**2019**	**2020**	**2021**
May King	8.80^{a}	8.73^{b}	7.76^{b}	9.40^{a}	8.76^{b}	7.66^{b}	45.45^{b}	33.20^{b}	38.53^{b}
Kobak	9.10^{a}	9.14^{ab}	9.13^{a}	9.55^{a}	9.55^{a}	9.53^{a}	49.65^{a}	48.40^{a}	46.46^{a}
Great Lakes	6.95^{b}	9.25^{a}	9.20^{a}	6.95	9.55^{a}	9.26^{a}	21.75^{c}	26.80^{c}	24.73^{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 Duncan ^{a,b} Multiple Range Test
(1…...10)*: 1 – loose ……10 – hard
(1….10)**: 1 – open ……10 – closed

Appendix 5.

Appendix 5. Interaction effect of plant biostimulants, growing years and lettuce varieties on some vegetative parameters of lettuce in **spring** time (Debrecen, 2019-2021)

Treatments	Varieties	Measured vegetative parameters											
		Head weight (g head^{-1})			Head diameter (cm)			Internal stem size (cm)			Root weight (g head^{-1})		
		2019	2020	2021	2019	2020	2021	2019	2020	2021	2019	2020	2021
Control	***May King***	459.40^{cd}	174.25^{i}	401.66^{de}	15.80^{de}	28.75^{bcd}	19.66^{def}	7.10^{a}	6.50^{bc}	7.26^{abcde}	31.20^{ab}	18.08^{fg}	31.70^{abc}
	Kobak	478.40c^{d}	234.00^{gh}	363.33^{e}	14.20^{e}	30.25^{b}	17.66^{f}	5.50^{ab}	7.88^{b}	9.23^{a}	30.60^{ab}	22.53^{defg}	27.10^{abcd}
	Great Lakes	595.20^{bc}	330.32^{d}	596.66^{ab}	17.80^{bc}	28.50^{bcd}	20.33^{bcdef}	4.10^{b}	3.50^{d}	3.43^{h}	31.40^{ab}	20.63^{efg}	21.53^{cde}
Willow	***May King***	492.60^{cd}	174.00^{i}	368.33^{e}	16.00^{cd}	30.00^{b}	22.66^{abcde}	6.80^{ab}	4.38^{d}	5.20^{efgh}	35.80^{ab}	29.03^{bcd}	23.56^{bcde}
	Kobak	523.20^{c}	204.42^{gh}	376.66^{de}	14.00^{e}	25.75^{de}	22.66^{bcde}	6.70^{a}	7.50^{bc}	9.06^{ab}	30.00^{ab}	16.05^{g}	13.30^{e}
	Great Lakes	736.20^{ab}	530.05^{a}	690.00^{a}	17.80^{bc}	34.50^{a}	24.66^{ab}	5.46^{ab}	3.38^{d}	4.56^{fgh}	40.20^{a}	23.30^{cdef}	19.73^{cde}
Bistep	***May King***	453.60^{cd}	456.20^{b}	428.33^{cde}	17.60^{bcd}	35.00^{a}	20.66^{bcdef}	5.50^{ab}	6.63^{bc}	7.00^{abcde}	24.60^{bc}	50.78^{a}	35.33^{ab}
	Kobak	509.40^{c}	361.50c^{d}	398.33^{de}	15.60^{e}	26.00^{cde}	20.00^{cdef}	6.80^{ab}	7.53^{bc}	8.83^{abc}	30.00^{ab}	30.10^{bc}	37.06^{a}
	Great Lakes	574.40^{c}	375.00^{c}	530.00^{bcd}	20.80^{a}	27.75^{bcde}	19.66^{def}	4.40^{b}	3.38^{d}	3.76^{gh}	31.00^{ab}	26.00^{cde}	17.30^{de}
W+B	***May King***	330.00^{d}	264.55^{fg}	380.00^{de}	15.60^{e}	29.25^{bc}	27.00^{a}	4.70^{b}	6.88^{bc}	6.76^{bcdef}	18.80^{d}	35.28^{b}	20.53^{cde}
	Kobak	424.00c^{d}	383.42^{c}	376.66^{de}	15.00^{e}	25.00^{e}	24.33^{abc}	5.50^{ab}	10.25^{a}	8.23^{abcd}	21.60^{cd}	27.78^{cde}	21.86^{cde}
	Great Lakes	761.80^{a}	430.25^{b}	569.00^{abc}	18.00^{b}	27.50^{bcde}	24.33^{abc}	4.80^{b}	3.38^{d}	5.06^{efgh}	26.50^{bc}	23.45^{cdef}	16.16^{de}
MLE	***May King***	-	500.50^{a}	365.00^{e}	-	33.25^{a}	23.66^{abcd}	-	6.25^{c}	6.53^{cdef}	-	50.12^{a}	31.70^{cde}
	Kobak	-	301.77^{ef}	386.66^{de}	-	26.50^{cde}	22.33^{bcde}	-	9.25^{a}	8.73^{abc}	-	25.62^{cde}	20.23^{cde}
	Great Lakes	-	297.02^{ef}	416.00^{de}	-	26.50^{cde}	18.66^{ef}	-	9.50^{a}	6.00d^{efg}	-	25.62^{cde}	16.36^{de}

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 Duncan [a,b] Multiple Range Test
W+B: Willow+Bistep

Appendix 6. Interaction effect of plant biostimulants and lettuce varieties on vegetative parameters of lettuce in **autumn** season (Debrecen, 2019-2021)

Treatments	Varieties	Head weight (g head^{-1}) 2019	Head weight (g head^{-1}) 2020	Head weight (g head^{-1}) 2021	Head diameter (cm) 2019	Head diameter (cm) 2020	Head diameter (cm) 2021	Internal stem size (cm) 2019	Internal stem size (cm) 2020	Internal stem size (cm) 2021	Root weight (g haed^{-1}) 2019	Root weight (g haed^{-1}) 2020	Root weight (g haed^{-1}) 2021
Interaction between MLE foliar application and plant variety													
May King	***Control***	73.70	93.46*	123.6	15.33	16.33	17.40	1.63	2.50	3.46	3.43	2.90	4.92
	MLE	75.90	124.66	146.4	12.66	15.33	17.60	1.83	2.83	3.74	6.60*	3.56	6.04*
Kobak	***Control***	76.10	53.33	142.2	13.00	12.00	18.40	2.30	2.16	3.86	2.63	2.76	6.00
	MLE	76.26	141.20*	158.0	12.00	15.66*	18.80	2.26	2.33	4.00	4.26*	3.06	5.78
Great Lakes	***Control***	99.93*	54.26	158.8	12.66	11.00	16.40	1.56	1.40	2.90	3.50	1.50	4.42
	MLE	75.90	121.20*	226.80*	13.00	12.66	19.60*	1.53	2.00	3.06	4.20	1.53	5.70*
Foliar applications of MLE													
Control	In the mean of	83.24	67.02	141.58	13.66	13.11	17.40	1.83	2.02	3.40	3.18	2.38	5.11
MLE		76.02	129.02*	177.09*	12.55	14.55	18.66*	1.87	2.38	3.60	5.02*	2.72	5.84*
Lettuce cultivars													
May King	In the mean of treatment	74.80^{b}	109.06^{a}	135.05^{b}	14.00^{a}	15.83^{a}	17.50^{a}	1.73^{b}	2.66^{a}	3.60^{b}	5.01^{a}	3.23^{a}	5.48^{a}
Kobak		76.18^{ab}	97.26^{a}	150.13^{b}	12.50^{a}	13.83^{ab}	18.60^{a}	2.28^{a}	2.25^{ab}	3.93^{a}	3.45^{a}	2.91^{a}	5.89^{a}
Great Lakes		87.91^{a}	87.73^{a}	192.82^{a}	12.83^{a}	11.83^{b}	18.00^{a}	1.55^{b}	1.70^{b}	2.98^{c}	3.85^{a}	1.51^{b}	5.06^{a}

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 Duncan ^{a,b} Multiple Range Test
Means within the same column followed by the same letter(s) are not significantly different at the probability level of ($P \leq 0.01$) according to independent sample *t-test* and Duncan a,b Multiple Range Test

Appendix 7.

Appendix 7. Interaction effect of plant biostimulants, growing years and lettuce varieties on some vegetative parameters of lettuce grown in **spring** season (Debrecen, 2019-2021)

Treatments	Varieties	Scaled vegetative parameters								
		Head structure (1….10)*			Head closing (1….10)**			Number of leaves (Piece head^{-1})		
		2019	2020	2021	2019	2020	2021	2019	2020	2021
Control	May	9.00^{ab}	6.42^{c}	8.00^{bc}	9.00^{abc}	6.50^{e}	7.00^{c}	51.00^{ab}	27.50^{f}	45.33^{abc}
	Kobak	9.40^{ab}	8.45^{b}	8.00^{bc}	9.40^{ab}	8.75^{bc}	9.00^{ab}	48.00^{b}	43.00^{cd}	47.33^{ab}
	Great	5.40^{d}	8.50^{b}	9.00^{ab}	5.60^{d}	8.50^{c}	9.00^{ab}	21.80^{de}	22.00^{g}	23.66^{e}
Willow	May	10.00^{a}	8.75^{ab}	8.00^{bc}	10.00^{a}	7.33^{d}	7.66^{bc}	50.80^{ab}	33.75^{e}	36.66^{d}
	Kobak	9.20^{ab}	9.25^{ab}	9.33^{ab}	9.00^{abc}	9.75^{a}	9.66^{a}	56.00^{a}	42.00^{cb}	43.00^{bc}
	Great	7.80^{bc}	9.50^{ab}	10.00^{a}	7.80^{c}	9.75^{a}	10.00^{a}	26.40^{d}	21.25^{g}	25.33^{e}
Bistep	May	8.00^{b}	9.50^{ab}	9.00^{ab}	9.40^{ab}	10.00^{a}	9.33^{a}	39.60^{c}	40.00^{d}	42.33^{c}
	Kobak	10.00^{a}	8.50^{b}	9.33^{ab}	10.00^{a}	9.25^{abc}	9.00^{ab}	44.40^{bc}	43.50^{cd}	48.33^{a}
	Great	6.40^{cd}	9.50^{ab}	9.66^{a}	6.20^{d}	10.00^{a}	10.00^{a}	21.00^{de}	21.75^{g}	26.66^{e}
W + B	May	8.20^{b}	9.50^{ab}	7.33^{cd}	9.20^{ab}	10.00^{a}	7.66b^{c}	40.40^{c}	32.75^{e}	35.33^{d}
	Kobak	7.80^{bc}	9.75^{a}	9.66^{a}	9.80^{a}	10.00^{a}	10.00^{a}	50.20^{ab}	53.00^{a}	47.00^{abc}
	Great	8.20^{b}	9.00^{ab}	8.66^{ab}	8.40^{bc}	9.50^{ab}	8.66^{ab}	19.80^{e}	20.00^{g}	25.00^{e}
MLE	May	-	9.50^{ab}	6.66^{d}	-	10.00^{a}	6.66^{c}	-	42.00^{cd}	33.00^{d}
	Kobak	-	9.75^{a}	9.33^{ab}	-	10.00^{a}	10.00^{a}	-	49.00^{b}	46.66^{abc}
	Great	-	9.75^{a}	8.66^{ab}	-	10.00^{a}	8.66^{ab}	-	49.00^{b}	23.00^{e}

Means within the same column followed by the same letter(s) are not significantly different at the probability level of (*P≤0.05*) according to Duncan ^{a,b} Multiple Range Test
(1…...10)*: 1 – loose ……10 – hard
(1….10)**: 1 – open ……10 – closed

Appendix 8.

Appendix 8. Interaction effect of plant biostimulants and lettuce varieties on vegetative parameters of lettuce in autumn time (Debrecen, 2019-2021)

Treatments	Varieties	Vegetative parameters								
		Head structure (1….10)*			**Head closing (1….10)****			**Number of leaves (Piece head^{-1})**		
		2019	**2020**	**2021**	**2019**	**2020**	**2021**	**2019**	**2020**	**2021**
Interaction between MLE foliar application and plant variety										
May King	**Control**	2.80	2.00	3.60	1.66	3.00	4.60	33.00	19.66	25.48
	MLE	5.50*	3.00	4.30*	2.13*	2.66	4.60	37.00*	25.33*	30.00*
Kobak	**Control**	2.26	1.33	4.10	1.43	1.66	3.60	29.66	20.66	26.80
	MLE	5.36*	3.33*	4.14	3.16*	2.66	4.30*	31.33	23.00*	29.60
Great Lakes	**Control**	1.26	1.00	3.80	5.00*	1.66	4.60	19.66	11.66	19.80
	MLE	2.33*	1.33	4.60*	2.60	1.33	4.80	17.33	14.33	23.40*
Foliar applications of MLE										
Control	In the mean of varieties	2.12	1.44	3.48	2.70	2.11	4.26	27.44	17.33	24.02
MLE		4.40*	2.55*	4.34*	2.63	2.22	4.56	28.55	20.88	27.66*
Lettuce cultivars										
May King	In the mean of treatments	4.16^{a}	2.50^{a}	3.95^{a}	1.90^{a}	2.83^{a}	4.60^{a}	35.00^{a}	22.50^{a}	27.74^{a}
Kobak		3.64^{a}	2.33^{a}	4.12^{a}	2.30^{a}	2.16^{ab}	3.95^{b}	30.50^{b}	21.83^{a}	28.20^{a}
Great Lakes		1.80^{b}	1.16^{b}	4.20^{a}	3.80^{a}	1.50^{b}	4.70^{a}	18.50^{c}	13.00^{b}	21.60^{b}

Means within the same column followed by the same letter(s) are not significantly different at the probability level of (*P≤0.05*) according to Duncan a,b Multiple Range Test
(1…...10)*: 1 – loose ……10 – hard
(1….10)**: 1 – open ……10 – closed

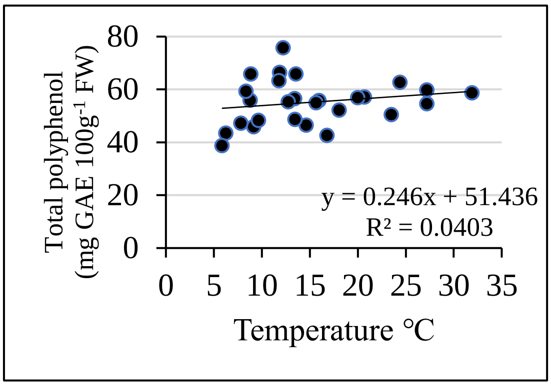
Appendix 9, I and II.

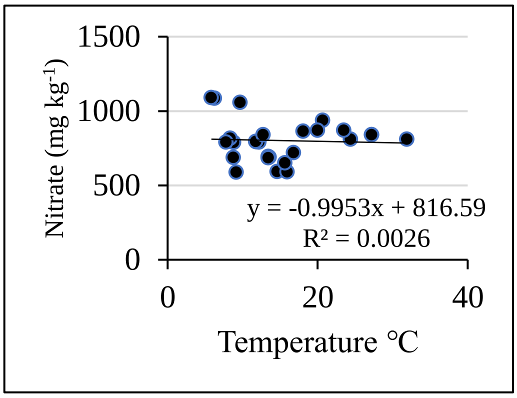
The calculated effect of biostimulants by regression analysis, compared with effect of measured environmental factors

I. Regression analysis for spring experiments

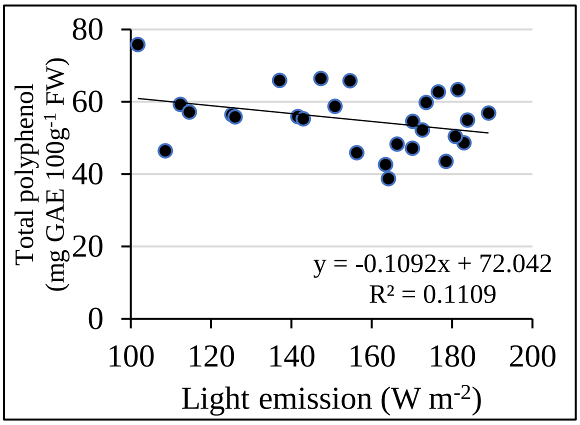
1. Willow bark extract

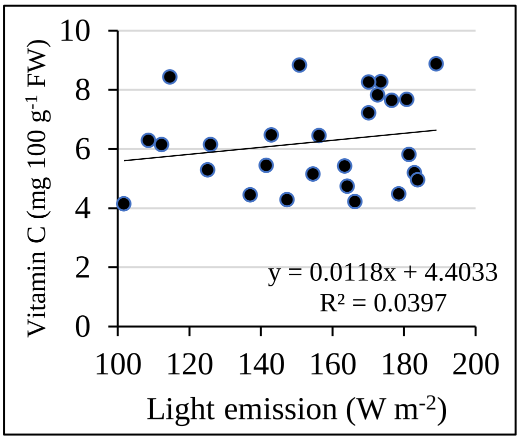
a. Temperature

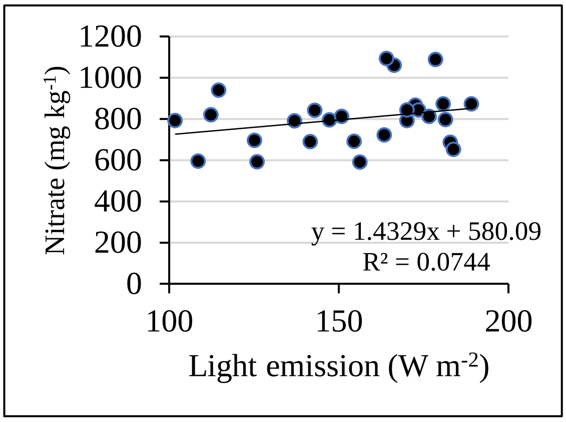




b. Light emission

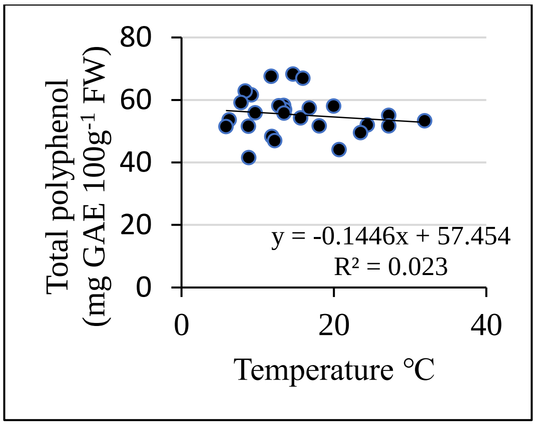


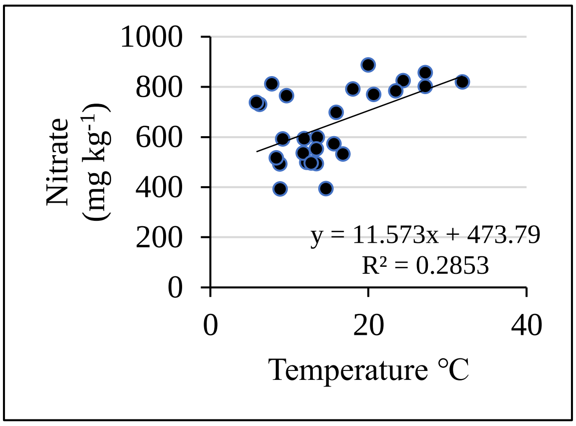




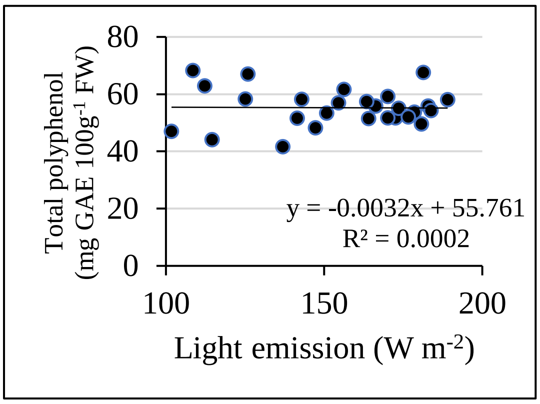
2. Bistep

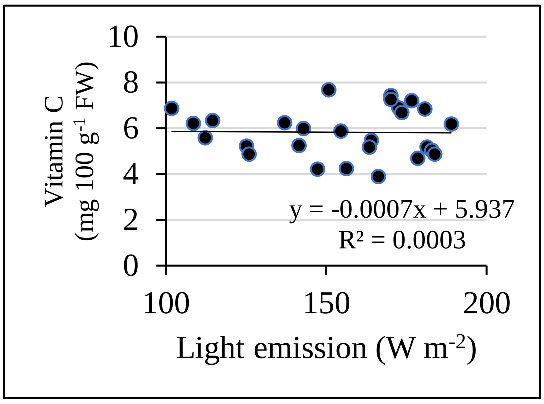
a. Temperature

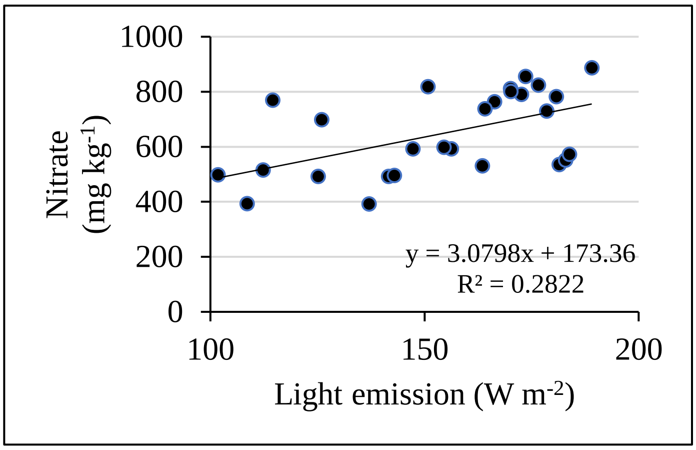




b. Light emission

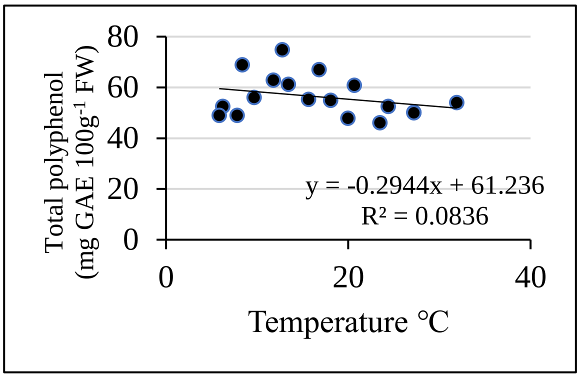


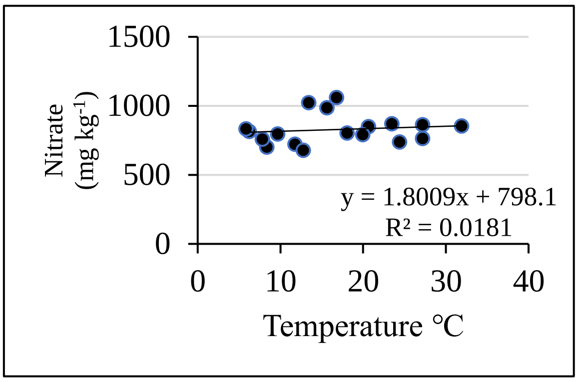




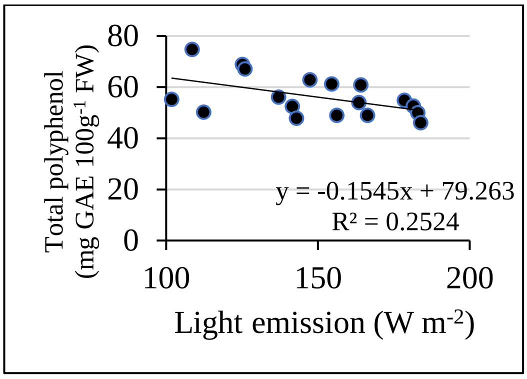
3. **Moringa leaf extract**

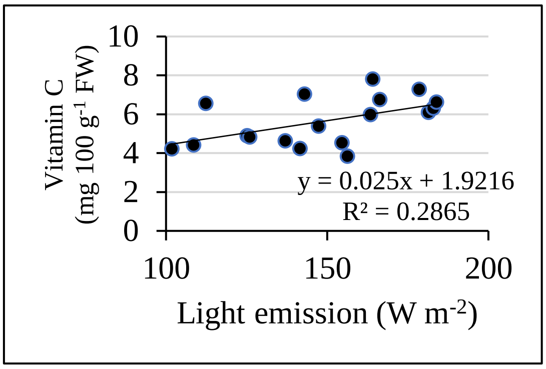
a. **Temperature**

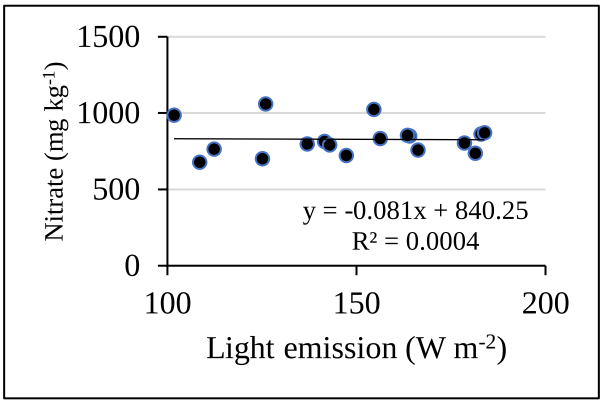




b. **Light emission**



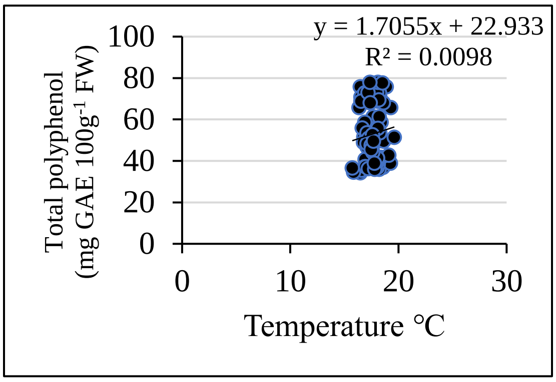


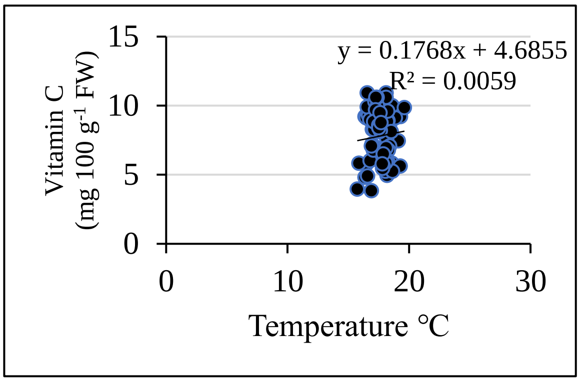


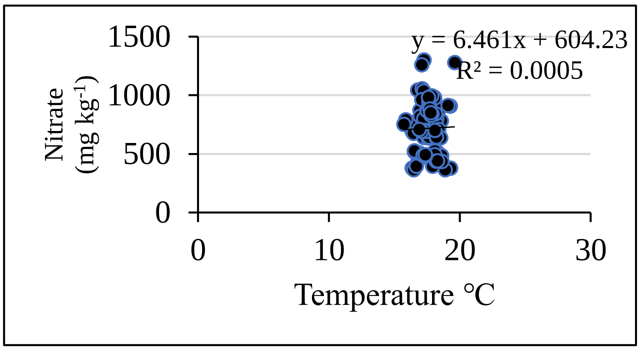
II. Regression analysis for autumn experiments

1. Moringa leaf extract

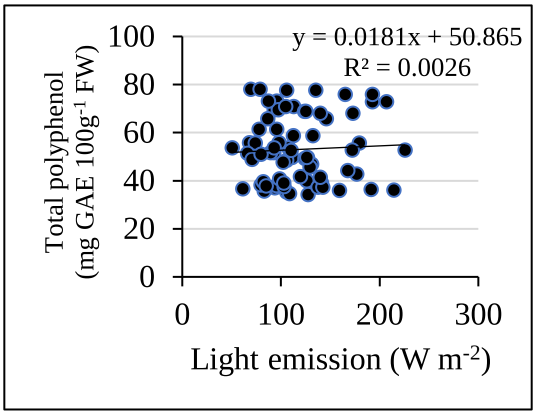
a. Temperature

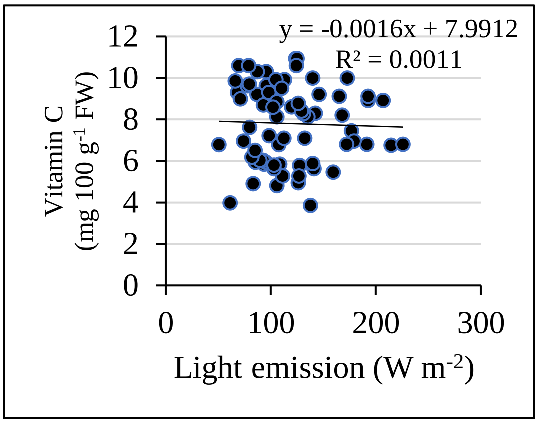


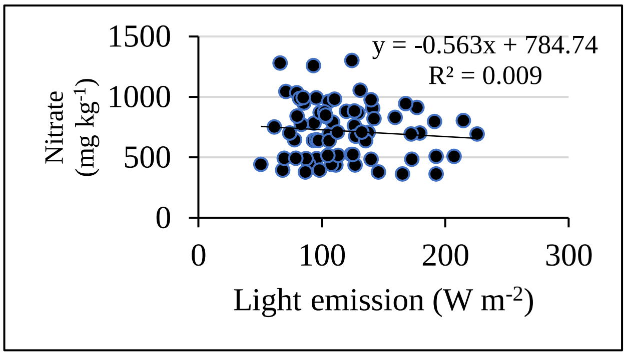




b. Light emission







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ABBREVIATIONS

Abbreviation	**Explanation**
BC	Before Christ
DW	Dry weight
EC	Electrical conductivity
ECe	Electrical Conductivity (EC) of a saturated soil paste extract
EU	European Union
EC	European Council
FW	Fresh weight
HPLC	High Performance Liquid Chromatography
KRG	Kurdistan regional government
MLE	Moringa leaf extract
NOP	National Organic program
NDVI	normalized difference vegetation index
pH	Potential of hydrogen
R^2	Coefficient of determination or goodness-of-fit of linear regression
SPAD	Single-photon avalanche diode
Sp.	Species
subs	Subspecies var. variety
W	Willow bark extract
W+B	Willow and Bistep

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