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The effect of plant biostimulants on the macronutrient content and ion ratio of several lettuce (*Lactuca sativa* L.) cultivars grown in a plastic house

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A B S T R A C T

Plant biostimulants have been identified as one of the most important agronomic factors that scientists have been looking for in a range of natural sources over the last few decades. The purpose of these experiments was to see how plant biostimulants (Willow bark extract, Bistep, and the combination of Willow+Bistep (W+B)) affect macronutrient content and ion ratio in three lettuce cultivars (May King, Kobak, and Great Lakes) from 2019 to 2021. Willow bark extract was diluted to 3% and applied to the plants through irrigation with the amount of 50–60 mL plant^{-1}, whereas Bistep was sprayed every two weeks from transplanting in accordance with the manufacturer's recommendations at 0.05% with the amount of 20 mL plant^{-1}.

The data demonstrate that over the grown seasons, the treatments W+B and Willow bark extract were the most effective biostimulants impacting macronutrient content and ion ratio, whereas Bistep was the least effective. In comparison to untreated plants, the amount of macronutrients (Ca^{2+}, Mg^{2+}, and P) in lettuce leaves rose by 18%, 13%, and 9%, respectively, in plants treated with W+B over a period of years, while, potassium (K^{+}) significantly reduced by 15%. Sodium level, on the other hand, was significantly improved using plant biostimulants of Bistep, Willow and W+B by 33%, 57% and 72%, respectively. Ca^{2+} and Mg^{2+} levels in lettuce cultivars were substantially higher in the season of 2019 when the climate was cooler and the air humidity was higher, but Na^{+}, K^{+}, and P levels were higher in 2020 when the temperature was somewhat warmer and dryer. Among the lettuce cultivars, Great Lakes lettuce has a greater level of macronutrient content (Ca^{2+}, Mg^{2+}, Na^{+}, and K^{+}), however, Kobak contained much higher phosphorus (P) content at 301.41 (mg kg^{-1} DM).

Plant biostimulants enhanced the ion ratios of Na^{+}: Ca^{2+} and Na^{+}: Mg^{2+}, but the only substantial improvement in Ca^{2+}: P ratio was in plants treated with W+B from 2019 to 2021, particularly in the Kobak variety by 20%. Biostimulants, on the other hand, decreased the mass ratio of (K^{+}+Na^{+}): (Ca^{2+}+Mg^{2+}) in lettuce cultivars. Among plant biostimulants, Willow and the combination of W+B had the highest influence on nutritional absorption and ion ratio, although Bistep had the least. The macronutrient composition and ionic ratios of lettuce cultivars varied according to the cultivar differences and climate conditions. In our 3-year experiments, we discovered that the Great Lakes variety had a higher macronutrient content in the season of 2020, whereas the Kobak variety had a higher ion ratio.

1. Introduction

Lettuce (*Lactuca sativa* L.) belongs to the Asteraceae family and is one of the most popular leafy vegetables in fast food around the world. Lettuce is widely consumed raw because it is rich in vitamins, polyphenols, flavonoids, carotenoids, and minerals (Mou, 2012, 2009). Although lettuce (*Lactuca sativa* L.) is a popular and extensively consumed vegetable, it is underappreciated for its nutritional benefits, owing to its high (95%) water content (Kim et al., 2016).

In order to stay healthier, human body needs an adequate daily intake of essential vitamins, micro and macronutrients from different sources, animals and plants on a regular basis (Hart, 2016). Therefore, getting to know the nutrient fortification and nutritional value in plants is the current interest among researchers. Natural plant biostimulants are an interesting and ecologically friendly invention that improves flowering, plant growth, fruit development, crop yield, and

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nutrient utilization efficiency, as well as resistance to a wide range of abiotic stressors (Desoky et al., 2021a; Rouphael and Colla, 2020).

The European Council Regulation (EC) No 2019/1009 has defined certain substances, mixtures, and microorganism products as plant biostimulants, which shall be a European Union (EU) fertilizing product, its' function is to stimulate plant nutrition processes independently of the product's nutrient content with the sole purpose of improving one or more of the following plant or plant rhizosphere characteristics: (a) nutrient use efficiency, (b) tolerance to abiotic stress, (c) quality traits, or (d) availability of confined nutrients in the soil or rhizosphere" (EU, 2019). Depending on their content and composition, biostimulants can be applied to plants in low concentrations to the soil or plant leaves (Kunicki et al., 2010).

The weeping willow or Babylon willow tree (*Salix babylonica*) is long been considered as an important medicinal plant. In ancient times, willow extract was used as a pain reliever without knowing that the active ingredient in the extract is *salicin* (an alcoholic-glucoside of salicylic acid), from which a well-known drug, aspirin was derived (Alamgir, 2017; Fuster and Sweeny, 2011). Many other aspects of the beneficial effect of salicin in willow bark as an antitumor therapy have been demonstrated in recent studies (Bonaterra et al., 2010; Hostanska et al., 2007; Kong et al., 2014).

Aside from medicinal benefits, there are numerous research papers on the willow tree's agricultural benefits. Because the extract contains many essential mineral elements (N, P, K, Ca, B, Mn, Fe, Zn, and Mg) as well as 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). Thus, it has been applied for growth development in a variety of crops. Both willow leaf and bark extracts have an intriguing activity, with the bark extract being found to be more active than the willow leaf extract (Marchand et al., 2014). Though, no significant difference can be found between the use of willow leaf and bark extract in the experiment of (Mutlu-Durak and Kutman, 2021).

Some studies have shown that Willow bark extract improves seedling performance under saline stress (Mutlu-Durak and Kutman, 2021), improves rooting, and speeds up vegetative cutting propagation of lavender (softwood) and chrysanthemum (semi-hardwood) (Wise et al., 2020). The extract has also been revealed to have natural fungicidal properties against *Botrytis cinerea* and *Penicillium expansum* (Andreu et al., 2018; Hussain et al., 2011). The extract from the *Salix cortex* have been applied as a basic substance for plant protection such as *in vitro* tests on grapevine against downy mildew (*Plasmopara viticola*), decrease of leaf curl (*Taphrina deformans*) pathogen in peach trees, reduction of apple scab (*Venturia inaequalis*) diseases (Deniau et al., 2019).

In the last few years, the EU pesticide legislation, Regulation (EC) No 1107/2009, has approved the Willow bark extract of the common plant willow for agricultural purposes as a basic ingredient with fungicidal qualities (Deniau et al., 2019; Marchand, 2016). The active ingredient in the willow bark extract is Salicylic acid which exhibits an antifungal effect, according to several studies (da Rocha Neto et al., 2015; Tosun et al., 2003). However, there are few or no research papers on the effect of Willow bark extract on nutrient uptake and ion ratio in lettuce.

Bistep, on the other hand, is a kind of humic acid that is produced as an organic substance to increase plant growth, reduce the vegetative duration and help to increase nutrient absorption by plants through increasing nutrient availability and improving soil structure (Cimrin and Yilmaz, 2005). Humic acid encourages plant nutrient uptake as well as the availability and transportation of micronutrients in plants (Böhme and Thi Lua, 1996). Many studies have confirmed that humic substances or plant biostimulants improve lettuce growth and yield (Lucini et al., 2015; Russo and Berlyn, 1992; Smoleń et al., 2019; Yakhin et al., 2017). It can also improve crop tolerance to abiotic stress, particularly in saline soils, using irrigation water with high sodium chloride (NaCl) levels and reducing transplant stress (Lucini et al., 2015; Qin and Leskovar, 2020).

As far as we know, research on the impact of Willow extracts on plants has particularly focused on the root-enhancing and fungicidal properties. Willow bark extract has never been shown to have biostimulant properties as a basic substance in plant nutrient uptake. Furthermore, there have been very limited information on nutrient uptake and ion ratio in lettuce plants concerning Bistep humic acid or the combination of two plant biostimulants. These studies aimed to assess the effect of a manufactured biostimulant (Bistep) and a home-made basic substance (Willow), as well as the interaction of Willow+Bistep (W+B), on some macronutrient uptake and ion ratio in various lettuce varieties.

2. Materials and methods

2.1. Experimental setup and plant materials

These experiments were continuously carried out in three years of spring 2019–2021 at the Agrár Campus of the University of Debrecen, Hungary, in calcareous chernozem soil under plastic houses. The experiment soil properties are shown in Table 1. In 80-cell trays, lettuce seeds from three different lettuce varieties (May King, Kobak, and Great Lakes) were sown. The seedlings were transferred to direct soil 35 days after sowing, with a 30 cm spacing between two plants and 55 cm between rows. Plants were arranged based on a complete randomized block design with 3 repetitions (total of 20 plants) per variety and the following treatments.

- Control – sprayed with distilled water
- Irrigation with Willow bark extract 3% with the amount of 50–70 mL $plant^{-1}$
- Bistep 0.05% solution – sprayed onto the plant leaves with the amount of 15–20 mL $plant^{-1}$.
- Their combination Willow bark extract (3%) + Bistep (0.05%) – signed as (W+B)

For the treatment, we used 20 plants in every treatment and the plants were treated every two weeks starting two weeks after transplantation (Table 2).

To decrease the impact of chemical fertilizers on the environment and human health, biostimulants can be used as a supplemental fertilizer to improve quality and reduce the amount of chemical fertilizers used in plant production while also increasing the plant's ability to absorb and translocate nutrients (Pascale et al., 2017; Zodape et al., 2011).

Table 1
Soil chemical properties at the experiment site.

Soil chemical content	Years		
	2019	2020	2021
Soil pH	7.35	7.18	7.56
Organic carbon (humus content)	2.88	3.04	1.72
P_2O_5	3137	3140	1317
K_2O	459	381	291
Mg	584	192	240
Na	69.7	155	60.6
S	98.7	13.7	42.1
$CaCO_3$	4.20	1.62	0.72
NO_3^-	130	89.9	244
Zn	35.85	3.27	11.0
Cu	9.54	2.57	4.39
Mn	135.0	31.4	90.6
Total soluble salts	0.10	0.04	0.003
Soil plasticity (K_A)	42.00	38.00	33.00

Source: Agricultural Laboratory Center, University of Debrecen.

Table 2
Climate condition during the lettuce growing period under plastic tunnel.

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
	Transplanting		**Harvesting**
2019	25th	–	16th
2020	18th	–	12th
2021	20th	–	11th

2.2. Willow bark extract preparation

Young twigs (first-year) from a mature weeping willow tree (*Salix babylonica*) grown in Agrár Campus of the University of Debrecen, Hungary was collected in February 2019–2021 where the plant was in abscission period. The Willow bark extract was prepared based on the method described by Martin and Stephens (2008). To get 3% Willow extract, 100 g of the twigs were chopped to about 2–3 cm pieces and placed in a jar container. The container was filled with 400 mL of warm water (80 °C) and stored for 24 h. The mixture was constantly stirred (every 6 h) at 400 rpm. The mixture was filtered through a stainless-steel sieve, then diluted to 3% with deionised water, and the supernatants were stored in a fridge at -4 °C for spring use (Fig. 1). A sample extract was taken to the lab to measure some mineral contents (Table 3). The extract contains a high concentration of salicylate compounds and phenolics, as well as important minerals such as (N, P, K, Ca, B, Mn, Fe, Zn, and Mg) and plant growth regulators such as Indole 3-butyric acid (IBA) and Salicylic acid (SA).

2.3. Bistep preparation

Bistep is a humic acid manufactured in Hungary that is also known as Ferbanat L solution. It is a humic acid that is high in macro and micronutrients and is used to improve plant development and yield in a wide range of crops. This extract was foliar sprayed every two weeks from transplanting in accordance with the manufacturer's recommendations at 0.05% with the amount of 20 mL plant^{-1}. Some Bistep chemical compounds are shown in (Table 3).

2.4. Laboratory analysis

Macronutrient components in plant leaves were assessed at the Agricultural Laboratory Center of the University of Debrecen as follows:

Total dry matrix content was determined by drying the samples at 105 °C until mass constancy was reached (min. 4 h). Macroelements (Ca, K, Mg, Na, S, and P) were determined using the ICP-OES (iCAP 7400, Thermo Scientific) technique. From the properly prepared sample, 0.5000 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

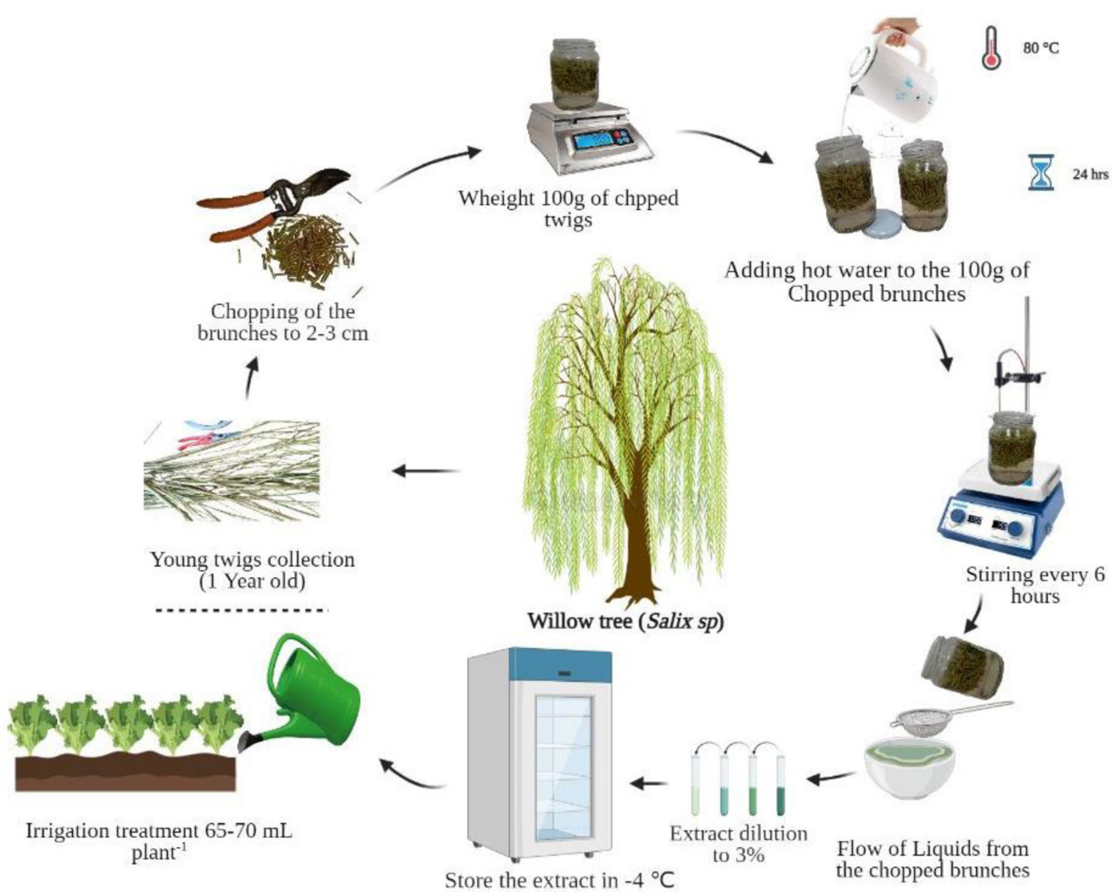

Fig. 1. Willow extract preparation practice
Created in (Biorender.com, 2021).

Table 3.
Some mineral composition in the used plant biostimulants of (Bistep and Willow bark extract).

Elements	Bistep	Willow extract
pH	7.4	7.22
Nitrate (NO^{3-}) (mg L^{-1})	0.02	< 0,200
Phosphorus (P_2O_5) (mg L^{-1})	0.03	78.8
Potassium oxide (K_2O) (mg L^{-1})	0.3	235
Magnesium (Mg) (mg L^{-1})	0.02	6.02
Manganese (Mn) (mg L^{-1})	0.007	0.386
Zinc (Zn) (mg L^{-1})	0.008	0.069
Molybdenum (Mo) (mg L^{-1})	0.09	–
Organic material (%)	25	–
Iron (Fe) (mg L^{-1})	0.01	–
Sodium (Na) (mg L^{-1})	–	14.7
Boron (B) (mg L^{-1})	0.0002	–
Sulphur (SO_2) (mg L^{-1})	–	5.21
Calcium (Ca) (mg L^{-1})	–	–
Copper (Cu) (mg L^{-1})	–	0.158
Total number of germs (number cm^{-3})	0.8 107	–
Micro fungus (number cm^{-3})	1.0 × 102	–

Source: Agricultural Laboratory Center, University of Debrecen.

(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.

2.5. Statistical analysis

The obtained data from three years of studies (2019–2021) were statistically analysed using one-way analysis of variance (ANOVA) in SPSS computer software, version 25 (IBM crop. Released, 2017). Duncan[a,b] multiple range tests were used to confirm the differences between the compared averages at the significance level of $p = 0.05$.

The ion ratio K^+: Ca^{2+}, K^+: Mg^{2+}, Na^+: Ca^{2+}, Na^+: Mg^{2+}, Ca^{2+}: Mg^+, (K^++Na^+): (Ca^{2+}+ Mg^+) and Ca^{2+}: P was calculated in excel software based on the method by Sahu (2013).

3. Results

3.1. Macronutrient content

In the mean of years, the treatment of W+B considerably increased calcium (Ca) content in May King and Kobak, but no significant variation was found in the Great Lakes variety (Table 4). On the other hand, magnesium (Mg), sodium (Na), and phosphorus (P) levels were significantly greater in plants treated with Willow bark extract. In contrast, no significant impacts were seen for the potassium content in lettuce types for Willow and the combination of W+B, although the Bistep biostimulant lowered the K accumulation in all three cultivars. Willow bark extract could increase the macronutrients Ca, K, and P in the colder season (2019) compared to warmer seasons (2020 and 2021). However, greater Na and Mg were found in Willow extracts throughout the seasons. The treatment of W+B substantially increased Ca, Mg, and P in the mean of years, whereas the Willow bark extract greatly improved Na in lettuce varieties.

The macronutrient composition of *Lactuca sativa* was determined by plant cultivars and climatic conditions during lettuce development (Table 5). Calcium and magnesium were higher in the 2019 growing season when the air temperatures were milder in the latter growing months of April and May, but sodium, potassium, and phosphorus were higher in the 2020 growing season, which was warm and fairly moist. Plants, on the other hand, reacted unfavorably in the warmer and drier season of 2021, when the temperature was high in March, April, and May and the environment was slightly dry. This is owing to the fact that when Na^+ level rises in drought and saline conditions, plants are subjected to severe abiotic stress (Desoky et al., 2021a).

Compared to untreated and Bistep treatment, Willow bark extract and the combination of W+B were the most influential biostimulant treatments in increasing nutritional content in lettuce cultivars. Plant biostimulants, on the other hand, dramatically reduced the potassium concentration of lettuce leaves. There was no clear discernible influence of soil chemical composition on the mineral content in the plants. Higher sodium, potassium and phosphorus levels in 2020, on the other hand, might be attributed to these ion availabilities and

Table 4
Macro nutrient content in lettuce in related to plant biostimulants.

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

According to Duncan's multiple-range test (*P = 0.05*), different letters within each column indicate significant differences.

Table 5
Macronutrient content in lettuce in related to growing season and cultivars.

Year and Cultivar	Ca	Mg	Na	K	P
	(mg kg^{-1} DM)				
Year					
2019	498.26a	290.52a	109.54c	1163.89c	242.33c
2020	476.87b	281.68ab	140.183a	1522.97a	301.41a
2021	405.46c	256.84c	91.96d	1042.06d	277.93b
Mean	460.19b	276.35b	113.89b	1242.98b	273.90b
Cultivar					
May King	440.96c	267.07c	107.151c	1202.43c	272.23ab
Kobak	455.10b	277.13b	115.28b	1250.93b	279.38a
Great Lakes	484.52a	284.84a	119.26a	1275.57a	270.06b

According to Duncan's multiple-range test (*P = 0.05*), different letters within each column indicate significant differences.

high phosphorus content in the soil (Table 1). In the mean of years, the Great Lakes cultivar contains higher levels of calcium, magnesium, sodium, and potassium, whereas Kobak and May King cultivars have higher levels of phosphorus (Table 5). Pérez-López et al. (2015) discovered similar results for the mineral concentrations of Ca, P, and Zn variations between two lettuce cultivars.

3.2. Macronutrient ion ratios

Changes in the ion ratios in lettuce was varied depending on the cultivar's response to the biostimulants and the grown seasons. Compared to the control, Bistep plant biostimulants significantly decreased the ion ratio of K^+: Ca^{2+}, K^+: Mg^{2+}, Ca^{2+}: Mg^{2+} and (K^++Na^+): (Ca^{2+}+ Mg^{2+}) in May King and Great Lakes varieties, while improving Na^+: Ca^{2+} ratio. There were no significant differences in Ca^{2+}: P when Bistep plant biostimulant was applied (Table 6). The use of biostimulant considerably reduced the mass ratio of (K^++Na^+): (Ca^{2+}+ Mg^{2+}), however, it didn't affect the ion ratio of Ca^{2+}: P. Willow bark extract could significantly increase the ratio of Na^+: $Ca^{2+}$and Na^+: Mg^{2+}, but reduced K^+: $Mg^{2+}$and no significantly influenced K^+: Ca^{2+}, ratio. The impact of the combination of W+B, on the other hand, was similar to that of the Bistep biostimulants.

The major difference for the ion ratios of K^+: Ca^{2+} between treated and untreated plants was discovered in the season of 2019 when the weather was colder, the air temperature was wet and the soil contained higher K_2O, Mg and $CaCO_3$. A similar result was found by Dziugieł and Wadas (2020) in potato tubers where they found greater differences among the growing season using plant biostimulants. Willow bark extract and the interaction of W+B were shown to be more influential during the colder season, nevertheless no significant changes in Bistep were identified under different climate conditions. Willow biostimulant substantially increased the mass ion ratios of Na^+: Ca^{2+} and Na^+: Mg^{2+} over time, while decreasing K^+: Ca^{2+}, Ca^{2+}: Mg^{2+}, and (K^++Na^+):(Ca^{2+}+ Mg^{2+}). In the mean of years, W+B was the only treatment that could significantly enhance the ion mass ratio of Ca^{2+}:P (Table 6).

The macronutrient ion ratios in lettuce cultivars were affected by environmental and genetic factors (Table 7). Except for the Ca^{2+}: P, all the mass ion ratios K^+: Ca^{2+}, K^+: Mg^{2+}, Na^+: Ca^{2+}, Na^+: Mg^{2+},

Ca^{2+}: Mg^{2+} and (K^++Na^+):(Ca^{2+}+ Mg^{2+}) were significantly higher in the season of 2020, where the weather was moderate and a bit wet. Among the lettuce cultivars, May King variety had higher K^+: Ca^{2+}

Table 6
Macronutrient ion ratios in lettuce cultivars in related to plant biostimulants.

Plant biostimulants	Cultivars			Years			Mean
	May King	Kobak	Great Lakes	2019	2020	2021	
K^+: Ca^{2+}							
Control	3.369a	3.460a	3.027a	2.240b	3.603b	4.121a	3.178a
Bistep	2.749b	2.522bc	2.401b	2.113b	2.990c	2.593b	2.532b
Willow	2.864ab	3.014ab	2.874a	2.503a	3.950a	2.337bc	2.879b
Willow+Bistep	2.266b	2.272c	2.320b	2.430a	2.480d	1.940c	2.295d
K^+: Mg^{2+}							
Control	6.013a	6.306a	5.996a	4.000a	7.189a	7.276a	5.946a
Bistep	4.409b	3.904b	4.215b	3.715b	4.830b	3.989b	4.170b
Willow	4.096b	4.440b	4.179b	4.224a	4.902b	3.562c	4.266b
Willow+Bistep	3.957b	3.921b	3.934b	4.009a	5.021b	2.844a	3.887c
Na^+: Ca^{2+}							
Control	0.177b	0.186c	0.183c	0.212b	0.163c	0.171c	0.181c
Bistep	0.255a	0.263b	0.246b	0.162c	0.318b	0.289a	0.250b
Willow	0.3.05a	0.335a	0.312a	0.251a	0.443a	0.263a	0.312a
Willow+Bistep	0.249a	0.231c	0.250b	0.240a	0.284b	0.204b	0.245b
Na^+: Mg^{2+}							
Control	0.316b	0.341b	0.356b	0.396c	0.326c	0.304c	0.341c
Bistep	0.406a	0.404b	0.431a	0.286d	0.511b	0.445a	0.412b
Willow	0.430a	0.493a	0.455a	0.425a	0.548ab	0.401b	0.463a
Willow+Bistep	0.442a	0.399b	0.427a	0.396b	0.578a	0.300c	0.416b
Ca^{2+}: Mg^{2+}							
Control	1.808a	1.841a	1.983a	1.788a	3.603b	1.844a	1.877a
Bistep	1.608bc	1.558b	1.766b	1.744a	2.990c	1.566b	1.644b
Willow	1.466c	1.491b	1.508c	1.688a	3.950a	1.533b	1.488c
Willow+Bistep	1.733ab	1.725a	1.675bc	1.665a	2.480d	1.844a	1.677b
(K^++Na^+): (Ca^{2+}+ Mg^{2+})							
Control	2.268a	2.349a	2.131a	1.573b	2.509a	2.727a	2.188a
Bistep	1.846b	1.689bc	1.684b	1.449c	2.044b	1.742b	1.729c
Willow	1.858b	1.989b	1.876ab	1.729a	2.429a	1.568b	1.906b
Willow+Bistep	1.595b	1.583c	1.605b	1.661a	1.849c	1.272c	1.597d
Ca^{2+}: P							
Control	1.589a	1.525b	1.889a	2.094a	1.697a	1.233c	1.648b
Bistep	1.599a	1.569ab	1.792a	2.063a	1.472b	1.448b	1.630b
Willow	1.603a	1.657ab	1.802a	2.029a	1.459b	1.589ab	1.671b
Willow+Bistep	1.776a	1.827a	1.776a	2.713a	1.701a	1.627a	1.773a

According to Duncan's multiple-range test (*P = 0.05*), different letters within each column indicate significant differences.

Table 7
Macronutrient ionic ratio in lettuce cultivars in related to growing season and cultivars.

Year and Cultivar	K^+: Ca^{2+}	K^+: Mg^{2+}	Na^+: Ca^{2+}	Na^+: Mg^{2+}	Ca^{2+}: Mg^{2+}	(K^++Na^+):(Ca^{2+}+ Mg^{2+})	Ca^{2+}: P
Year							
2019	2.322c	3.987d	0.216d	0.372c	1.725a	1.603c	2.06a
2020	3.255a	5.485a	0.302a	0.491a	1.722a	2.207a	1.582b
2021	2.748b	4.418c	0.232c	0.363c	1.602c	1.827b	1.474d
Mean	2.721b	4.567c	0.247b	0.408b	1.627b	1.855b	1.680b
Cultivar							
May King	2.812a	4.619a	0.247b	0.399b	1.654b	1.892a	1.642b
Kobak	2.817a	4.643a	0.254a	0.409a	1.654b	1.903a	1.644b
Great Lakes	2.655b	4.581a	0.248ab	0.417a	1.733a	1.824b	1.815a

According to Duncan's multiple-range test (*P = 0.05*), different letters within each column indicate significant differences.

and (K^++Na^+):(Ca^{2+}+ Mg^{2+}), whereas Great Lakes cultivar contained greater Na^+: Mg^{2+}, Ca^{2+}: Mg^{2+}, and Ca^{2+}: P. However, there were no significant differences in the mass ratio of K^+: Mg^{2+} amongst cultivars. Among the evaluated lettuce cultivars, Kobak has the highest ion ratios of any lettuce cultivar.

4. Discussion

The macronutrient content in lettuce, similar to any other characteristics, is influenced by genetic differences, environmental factors, agronomical practices, and genetic and environmental interactions (Mou, 2009; Rouchaud et al., 1984). Macronutrients in plants are responsible for various plant disorders such as blossom end rot due to Ca deficiency, tipburn incidence owing to a lack of K contents, marketable yield and quality as a result of Mg deficit, and so on (San Bautista et al., 2009). 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).

Several studies have demonstrated that plant biostimulants humic substances, seaweed extracts, and other plant-derived pH can improve nutrient uptake in plants (De Pascale et al., 2017). Biostimulants improve Ca, Mg and K uptake, this leads to the reduction of soil pH, EC and Na availability in the soil (Kumar et al., 2017). Ali et al. (2018) have demonstrated that foliar application of moringa leaf extract (MLE) could significantly improve N, P, K and Mg in geranium (*Pelargonium graveolens* L.) leaves. Some research studies also propose the use of a mixture of biostimulants as an alternative or partial replacement for chemical fertilizer in terms of plant growth performance and quality improvement. Abou-Sreea et al. (2021) have proposed cattle manure (CMn) and bio-nutritious substances such as royal jelly (RJ), as well as a combination of CMn and RJ (2 g L^{-1} as a foliar feeding) along with CMn (30 m^3 ha^{-1}) as a partial alternative to chemical fertilizer in the production of organic *Hibiscus sabdariffa* L. Also, the results by Desoky et al. (2021b) show that the combination of clove fruit extract (CFE) and/or salicylic acid (SA), results in reduction or limitation of the drought stress in potato production.

Our three-year study shows that plants treated with Willow bark extract and W+B had the highest macronutrient contents. This might be due to the high content of plant growth regulators (Indole 3-butyric acid) (IBA) in Willow extract (Deniau et al., 2019; Mutlu-Durak and Kutman, 2021). Bistep, on the other hand, is a kind of humic acid that improves mineral absorption by plants and boosts nutrient availability in the soil (Cimrin and Yilmaz, 2005). Indole 3-butyric acid improves the root system (De Pascale et al., 2017), helping the plant to absorb more nutrients to the upper parts (Dziugieł and Wadas, 2020; Halpern et al., 2015; Khan et al., 2009). This extract was positively working during the colder months of April and May in the season of 2019 which was colder and air humidity was higher when the plants begin to develop their heads. However, the combination of W+B may have a greater impact on nutrient absorption and mass ion ratio, particularly when it comes to Na uptake because Willow bark extract contains a greater quantity of Na (14.7 mg L^{-1}), but no sodium was discovered in the Bistep extract (Table 3). The reason might be because the willow extract was administered as a root treatment and Bistep was foliar sprayed.

In our research studies, it is clear that the environmental conditions could acts a substantial impact on the influence of plant biostimulants on plant response. Pecha et al. (2012) have proposed that foliar sprays of plant biostimulants can be applied when the air humidity is near saturation point to obtain the most impact on nutrient absorption by the plants. This is because among the forces that drive the evaporation of the biostimulant solution is relative air humidity.

It is worth noting that W+B dramatically enhanced Ca content in lettuce leaves, which may be useful for reducing salt tolerance in dry and semiarid environments (Ilyas et al., 2021; Naeem et al., 2017). A Bistep humic material could slightly enhance Na content in lettuce leaves, whereas the same treatment lowered K absorption in the plants. Chinnusamy et al. (2006) have described that K uptake can be inhibited by high Na concentrations. According to research investigations on lettuce produced in the Czech Republic by Kopta et al. (2018), the combination of biostimulants has a greater beneficial impact on lettuce quality under salt stress circumstances for spring and summer growing season. The combination of root and foliar biostimulant treatment has also been demonstrated to boost physiological and bioactive compounds in lettuce by Cristofano et al. (2021).

Aside from soil ion availability, environmental factors, particularly humidity, could impact nutrient availability via the plant root system. Low air humidity and high temperatures may be a cause for declining macronutrient levels in the 2021 season. Because in this causes the soil dry out faster, the soil's water content becomes limited for nutrient delivery to the root surface, resulting in decreased nutrient absorption due to poor root development in dry soil (Marschner and Rengel, 2012).

In the mean of years, plants with low concentrations of potassium resulted in higher calcium and magnesium contents. Similarly, some research studies have shown that higher potassium levels cause potential depolarization and a decrease in the driving force for the absorption of other cations such as Ca and Mg via plasma membrane transporters (El-Nakhel et al., 2020; Rietra et al., 2017).

Moreover, the concentrations of Ca, Mg, Na, and K in lettuce leaves were substantially greater in the Great Lakes, followed by Kobak and May King cultivars. Recent research by Corrado et al. (2021) has discovered that two lettuce cultivars had distinct metabolic strategies in terms of the abundance of biochemical classes to adapt to environmental change. El-Nakhel et al. (2020) found a substantial variation in bioactive and mineral content between two butterhead lettuce cultivars (Green and Red Salanova) based on color differences.

It is also essential to pay attention to the interaction between ion cations in plant cells, as antagonistic or synergistic actions which can produce nutritional imbalances in plant tissues, compromising quality (Zhang et al., 2017). Scientists place a high value on the ion ratio in our daily food since it has a direct influence on human health. As a result, the daily intake of the ion ratio of certain minerals has been determined. For example, the optimal Ca: Mg ratio in the human diet varies between 1.70 and 2.60 (weight to weight), and excessive consumption or the imbalance consumption increases the risk of different types of cancer and metabolic syndrome (Costello et al., 2021; Moore-Schiltz et al., 2015). However, there is a very limited information on the influence of plant biostimulants on the mass ratio in lettuce varieties.

Apart from the health benefits of our discovery, lettuce grown in saline conditions may get benefit from this, since lettuce is recognized for moderately sensitive to salinity (De Pascale and Barbieri, 1995; Miceli et al., 2003) primarily the application of W+B. Biostimulants used in our experiments could improve the mass ratio of Na^{+}: Ca^{2+} and Na^{+}: Mg^{2+}, but did not significantly influence Ca^{2+}: P in lettuce cultivars. On the contrary, biostimulants (Bistep and interaction of W+B reduced the mass ratio of K^{+}: Ca^{2+}, K^{+}: Mg^{2+}, and (K^{+}+Na^{+}): (Ca^{2+}+ Mg^{2+}), however, no significant difference was recorded for the K^{+}: Ca^{2+} through applying Willow bark extract, this might be because this extract is rich for potassium content.

5. Conclusion

Plant biostimulants can be an environmentally friendly product for improving various macronutrients in lettuce vegetables; nevertheless, a mixture of biostimulants is recommended to minimize some ion ratios. Apart from its antifungal and hormonal effects, Willow bark extract enhanced certain micromineral content, however, it also improved sodium levels in plant leaves. Thus, the W+B combination was the best for reducing the ion ratio of (K^{+}+Na^{+}): (Ca^{2+}+ Mg^{2+}) and improving the nutrient quality in different lettuce cultivars. Our findings reveal that plant biostimulants function better in colder and wet seasons; nonetheless, the results show that biostimulants can improve macronutrients in all three seasons, suggesting that they can reduce environmental stress caused by air temperature and soil conditions. As a result, improving the nutritional value of vegetables is commercially relevant since it increases nutrient intake without increasing consumption and reduces fertilizer requirements by plants, resulting in fewer production costs. More research on such plants is needed to back up the current report.

Declaration of Competing Interest

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

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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