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ASSESSMENT OF MINERAL NUTRIENT IMPACT ON METABOLITES ACCUMULATION IN KALE (*BRASSICA OLERACEA VAR. SABELLICA*)

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Green leafy kale (Brassica oleracea var. sabellica) has huge scientific attention because of its health-promoting functionality. In the present study the impact of NPK, energen, biostim and humate on flavonoid, phenolic compounds, vitamin C, carotenoids, malondialdehyde (MDA), protein, proline and soluble sugar in kale was investigated. The mineral nutrients mostly increased but in some cases maintained the studied metabolites. The stimulatory effect of applied mineral nutrients on the phytochemicals analyzed varied with the different combinations of macro and microelements. Lipid peroxidation was minimized in leaves treated with mineral nutrients hence a reduction in MDA levels. Contrary to the correlation between nitrogen deficiency and increase in polyphenol and vitamin C content in plants, NPK and biostim did not reduce phenolic compound levels. The results of this study showed that NPK maximized the synthesis of vitamin C and proline; energen - phenolic compounds, carotenoids and sugar; biostim – phenolic compounds, proteins and sugar; humate – flavonoids and sugar in curly kale. Therefore, the type of macronutrient and micronutrients combination increases phytochemicals in differently. To enhance the synthesis of phenolic compounds and vitamins, the most promising additives are those containing humic acids (humate and energen), and biostim proved to be more effective for the synthesis of proteins.

Background. *The understanding of how diet affects the incidence or treatment of disease has led to a rise in consumer's demand for functional foods as well as created the market for natural sources of health benefitting compounds rather than the synthetic sources. Curly kale has gained scientific attention as a functional food because it contains higher levels of phytochemicals than most vegetables. These phytochemicals have shown antioxidant, antimutagenic, cytotoxic, antifungal, and antiviral activities. However, the content levels of these metabolites are influenced by not only genetic but environmental factors. It was of interest to evaluate how various mineral nutrients can elicit the accumulation of these compounds that minimize the risk of chronic diseases or aid in their treatment.*

Purpose. *Evaluate how the mineral nutrients, NPK, energen, biostim and humate affect the content of metabolites (proteins, sugars, flavonoids, phenolic compounds, vitamin C, carotenoids, MDA and proline) in curly kale (Brassica oleracea var. sabellica).*

Materials and methods. *Sprouts from kale seed kept wet in a Petri dish for 7 days were transferred to the field. At 6 weeks old four mineral nutrients (NPK, energen, humate and biostim) were added to the soil. Control variants were treated with water. A week later, the leaves were harvested after which, the phenolic compound, flavonoid, protein, sugar, vitamin C, carotenoid, MDA and proline contents were determined using spectrophotometric methods.*

Results. *It was shown that humate fertilizer elicited the highest accumulation of flavonoids. Kale plants fertilized with energen were observed to have the highest phenolic compound content. NPK, energen and humate caused a similarly positive effect on vitamin C content in leaves, unlike biostim whose effect did not significantly differ from control plants. Energen treated kale had the highest increment of carotenoids. A varied reduction of MDA levels in plants treated with all four mineral nutrients was observed in kale leaves. Plants fertilized with biostim accrued the highest protein content in leaves. Proline content increased under the influence of all fertilizers studied. Sugar levels for all kale plants treated with the studied mineral nutrients were enhanced equally*

Conclusion. *Macro and microelements supplied by mineral nutrients differentially boost the biosynthesis of health-promoting metabolites in curly kale, thereby enhancing its quality.*

Keywords: *Brassica; kale; mineral nutrients; proteins; sugar; proline; MDA; flavonoids; phenolic compounds; vitamin C; carotenoids*

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ОЦЕНКА ВЛИЯНИЯ МИНЕРАЛЬНЫХ ВЕЩЕСТВ НА НАКОПЛЕНИЕ МЕТАБОЛИТОВ В КАПУСТЕ КЕЙЛ (*BRASSICA OLERACEA VAR. SABELLICA*)

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Зеленая листовая капуста кейл (Brassica oleracea var. sabellica) привлекает огромное научное внимание благодаря своей полезной для здоровья функциональности. В настоящем исследовании изучалось, как NPK, энерген, биостим *и гумат влияют на содержание флавоноидов, фенольных соединении, витамина С, каротиноидов, малонового диальдегида (МДА), белков, пролина и растворимых сахаров в капусте кейл. Минеральные питательные вещества в основном увеличились, но в некоторых случаях сохраняли содержание изученных метаболитов. Стимулирующее действие применяемых минеральных питательных веществ на анализируемые фитохимические вещества различное в зависимости от различных комбинаций макро- и микроэлементов. Перекисное окисление липидов было сведено к минимуму в листьях, обработанных минеральными питательными веществами, что привело к снижению уровня МДА. Вопреки корреляции между дефицитом азота и увеличением содержания полифенолов и витамина С в растениях, NPK и биостим не снижали уровень фенольных соединений. Результаты этого исследования показали, что NPK увеличивает синтез витамина С и пролина; энерген – фенольных соединений, каротиноидов и сахара; биостим – фенольных соединений, белков и сахара; гумат – флавоноидов и сахара в кудрявой капусте кейл. Таким образом, тип макронутриентов и комбинация микронутриентов по-разному увеличивает фитохимические вещества. Для усиления синтеза фенольных соединений и витаминов наиболее перспективны добавки, содержащие гуминовые кислоты (гумат и энерген), биостим оказался более эффективным для синтеза белков.*

Состояние вопроса. *Широко известно, как диета влияет на заболеваемость или лечение заболеваний. Это понимание привело к росту потребительского спроса на функциональные продукты питания, а также создало рынок для натуральных источников полезных для здоровья соединений, а не синтетических источников. Кудрявая капуста кейл привлекла внимание ученых как функциональная пища, потому что она содержит более высокий уровень фитохимических веществ, чем большинство овощей. Эти фитохимические вещества проявляют антиоксидантную, антимутагенную, цитотоксическую, противогрибковую и противовирусную активность. Однако на уровень содержания этих метаболитов влияют не только генетические, но и экологические факторы. Было интересно оценить, как различные минеральные питательные вещества могут вызывать накопление этих соединений, которые уменьшают риск хронических заболеваний или помогают в их лечении.*

Цель работы *– оценить, как минеральные питательные вещества, NPK, энерген, биостим и гумат влияют на содержание метаболитов (белков, сахаров, флавоноидов, фенольных соединений, витамина С, каротиноидов, МДА и пролина) в кудрявой капусте кейл (Brassica oleracea var. sabellica).*

Материал и методы. *Проростки из семян капусты, выдержанные влажными в чашке Петри в течение 7 дней, были перенесены в поле. На 6-ой недели,* *в почву были добавлены четыре минеральных питательных вещества (NPK, energen, гумат и biostim). Контрольные варианты обрабатывали водой. Через неделю листья собирали и определяли содержание фенольных соединений, флавоноидов, белка, сахара, витамина С, каротиноидов, МДА и пролина спектрофотометрическими методами.*

Результаты. *Было показано, что гумат вызывал наибольшее накопление флавоноидов. Было отмечено, что растения капусты кейл, обработанные энергеном, имели самое высокое содержание фенольных соединений. NPK, энерген и гумат аналогично оказывали положительное влияние на содержание витамина С в листьях, в отличие от биостима, эффект которого существенно не отличался от контрольных растений. У капусты кейл, обработанной энергеном, был самый высокий прирост содержания каротиноидов. В листьях капусты кейл наблюдалось различное снижение уровня МДА у растений, обработанных всеми четырьмя минеральными питательными веществами. Растения, удобренные биостимом, имели самое высокое содержание белка в листьях. Содержание пролина повышалось под влиянием всех изученных удобрений. Уровень сахара для всех растений капусты, обработанных изученными минеральными питательными веществами, был одинаково повышен.*

Заключение. *Макро- и микроэлементы, поставляемые минеральными питательными веществами, дифференцированно повышают биосинтез полезных для здоровья метаболитов в кудрявой капусте, тем самым повышая ее качество.*

Ключевые слова: *Brassica; капуста кейл; минеральные питательные вещества; белки; сахар; пролин; МДА; флавоноиды; фенольные соединения; витамин С; каротиноиды*

Для цитирования. *Антех Д.Д., Тимофеева О.А., Мостякова А.А. Оценка влияния минеральных питательных веществ на накопление метаболитов в капусте кейл (Brassica oleracea var. sabellica) // Siberian Journal of Life Sciences and Agriculture. 2021. Т. 13, № 3. С. 208-224. DOI: 10.12731/2658-6649-2021-13-3-208-224*

Introduction

The rise in consumer’s demand for quantity and quality of natural sources of health benefitting compounds due to concerns about the side effects of synthetic drugs, fuels effort in increasing the quantity as well as the nutritional value of curly kale (*Brassica oleracea var. sabellica)*. Improving crop cultivation practices has gained unprecedented importance as one of the breakthroughs to address this issue.

Nutrients are primarily considered as food components that either cannot be synthesized in the body or whose formation require some specific factors that may in some situations be insufficient or absent. The different health benefitting organic compounds are products of both primary and secondary metabolism in plants.

Primary metabolites are found across all species within broad phylogenetic groups and are produced using the same or similar biochemical pathways. Secondary metabolites determine the colour of vegetables, protect plants against herbivores and microorganisms, attract pollinators and seed-dispersing animals, and act as signal molecules under biotic and abiotic stress conditions [5].

It is widely recognized that other biochemical compounds in plants, such as flavonoids, phenolic acids, and glucosinolates are associated with minimizing the incidence of diseases. A large number of phytochemicals capable of antioxidant, antimutagenic, cytotoxic, antifungal, and antiviral activities have been identified in kale and other Brassica plants [14]. Curly kale *(Brassica oleracea var. sabellica)*, compared to other leafy vegetables, such as mustard greens and collard greens, rocket salads, lettuce, etc. is more nutritionally and functionally important for human health as it contains higher levels of metabolites that prevent or minimize the risk of some chronic diseases [28].

Since the level of biochemical components of kale can be influenced by biotic and abiotic factors, it was important to evaluate how the mineral nutrients, NPK, energen, biostim and humate affect the content of metabolites (proteins, sugars, flavonoids, phenolic compounds, vitamin C, carotenoids, MDA and proline) in curly kale.

NPK as a mineral nutrient supplies nitrogen to promote leaf growth, phosphate for root, flower and fruit enhancement, and potassium for stem & root growth in plants [25]. Humate supplies potassium humate and micronutrients essential for plant growth and soil fertility enhancement [29]. Energen is made up of potassium humate salts and trace elements which enhances plant growth as well as other ranges of physiological functions [18, 21]. Biostim increases plant's resistance to drought and increases crop yield by supplying a balanced composition of nitrogen, potassium, sulphate and amino acids [19].

Materials and Methods

Kale sprouts from seed which were moistened with water and kept in a Petri dish for 7 days, were planted in the fields at the Kazan Federal University botanical garden in June 2020. A week after treating 6-weeks-old kale plants with NPK 15-15-20 applied at 20kg/ha, energen (K salts of humic acids and trace elements, 80g/l), biostim (a mixture of macro - and microelements, 1l/ha), and humate (K- salts of humic acids and microelements, 80g/l), the leaves were collected to determine the content level of vitamin C, MDA, flavonoids, carotenoids, phenolic compound, proteins, proline and sugar. Control plants were treated with water.

Using the aluminium chloride colourimetric method [23] with little modifications, flavonoids (quercetin) were determined. Phenolic compounds were determined by the modified Folina of Ciocalteu method [20]. Measurement was done at 725 nm and results were expressed in gallic acid equivalent. Determination of Vitamin C was done by the summation of ascorbic acid and dehydroascorbic acid using potassium hexacyanoferrate solution at the absorbance value of 680 nm [12]. The content of carotenoids was calculated using the modified spectrophotometric method of Costache et. al. [6]. The content of MDA was evaluated by the accumulation level of the product formed from the reaction of malondialdehyde and thiobarbituric acid [26]. By the modified Lowry method, protein content was calculated [16]. Determination of sugar was done by the modified anthrone method [10]. Proline content level was determined by methods in the publication of [26].

Experiments were carried out in three biological repeats. The statistical analysis was performed using the GraphPad prism program, v. 8.4. The significance of the mean was determined using the one-way analysis of variance method with subsequent unpaired t-test for pairwise comparison (significance difference was estimated at $P < 0.05$).

Results and discussion

Curly kale is perceived to be a very promising leafy green food product, which promotes health due to its high phytochemical composition. In this study, the possibility of increasing the content of health-enhancing biochemical compounds in curly kale was observed.

Quercetin one of the most ubiquitous flavonoids in kale that is a potent free radical scavenger, and is thus considered to protect humans against several types of cancer and cardiovascular diseases [7]. NPK, energen and humate increased the content of flavonoids. Humate had the greatest effect on the content of flavonoids (Fig. 1). Flavonoid levels in biostim treated plants did not significantly differ from the controls.

It was important to assess the effect of the studied mineral nutrients on phenolic compound content as it is one of the major antioxidants in *Brassica* vegetables which accounts for more than 70% of their total antioxidant capacity[17].

According to our results the mineral fertilizers, especially energen increased the phenolic compound levels (Fig. 2).

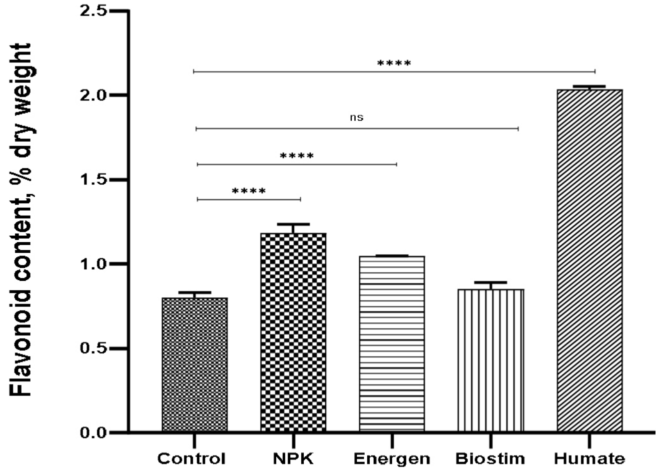

Fig. 1. The influence of various mineral nutrients on flavonoids in kale *Brassica oleracea var. sabellica* (L.). Error bars signify 95% confidence intervals. Pairwise comparison was conducted using student's t-test. ns, ****symbolize P > 0.05 and P ≤ 0.0001, respectively. *Source*: «Compiled by the authors».

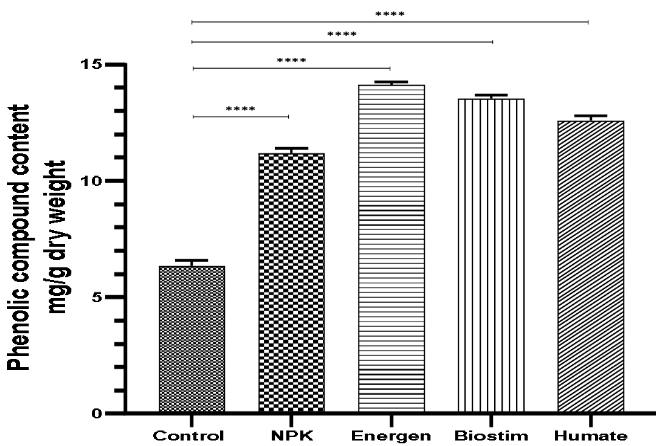

Fig. 2. The influence of various mineral nutrients on phenolic compounds in kale *Brassica oleracea var. sabellica* (L.). Error bars signify 95% confidence intervals. Pairwise comparison was conducted using student's t-test. ****, symbolizes P ≤ 0.0001, respectively. *Source*: «Compiled by the authors».

Ascorbic acid is a major nutrient and antioxidant in vegetables. Cruciferous vegetables, especially kale, are a good source of ascorbic acid for the diet [4]. NPK, energen and humate approximately had an equally positive effect on vitamin C content, while biostim's effect did not cause a rise (Fig. 3).

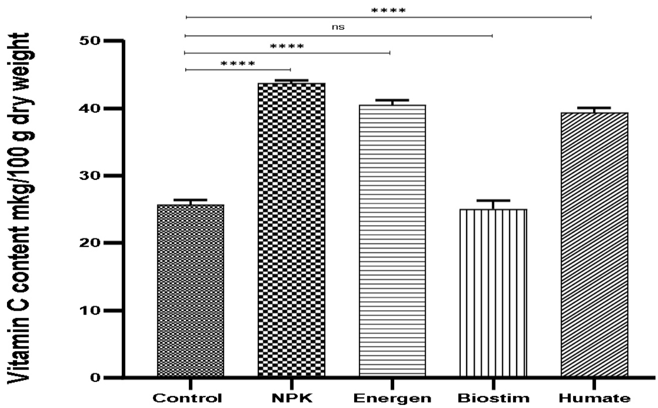

Fig. 3. The influence of various mineral nutrients on vitamin C in kale *Brassica oleracea var. sabellica* (L.). Error bars signify 95% confidence intervals. Pairwise comparison was conducted using student's t-test. ns, and **** symbolize P ≤ 0.0001, respectively. *Source*: «Compiled by the authors».

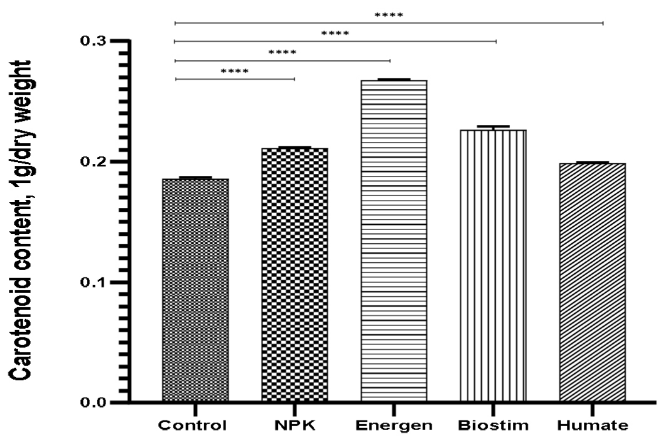

Fig. 4. The influence of various mineral nutrients on carotenoids in kale *Brassica oleracea var. sabellica* (L.). Error bars signify 95% confidence intervals. Pairwise comparison was conducted using student's t-test. ****, symbolizes P ≤ 0.0001, respectively. *Source*: «Compiled by the authors».

Carotenoids, as accessory pigments in the light-harvesting steps of photosynthesis, play an important role in the human diet by virtue of their metabolism to vitamin A. It exhibits high antioxidant properties and is one of the major classes of phytochemicals found in kale [2]. As observed in Fig. 4, carotenoids increased variably for all fertilized plants with energen treated kale having the highest level of pro-vitamin A (Fig. 4).

Malondialdehyde (MDA) is a reliable biomarker of lipid peroxidation and membrane damage [8]. NPK, energen, biostim and humate effectively reduced this product of biomembrane deterioration in curly kale (Fig. 5).

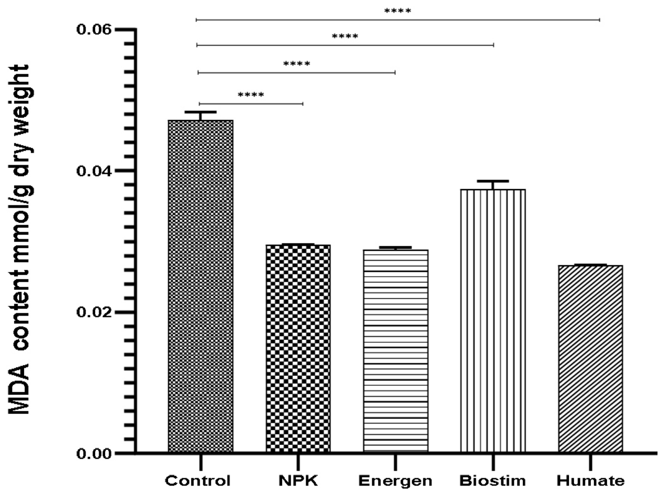

Fig. 5. The influence of various mineral nutrients on malondialdehyde in kale *Brassica oleracea var. sabellica* (L.). Error bars signify 95% confidence intervals. Pairwise comparison was conducted using student's t-test. ****, symbolizes $P \leq 0.0001$, respectively. *Source*: «Compiled by the authors».

Characterized as a protein-rich green matrix, kale has a beneficial nutritional composition and potential health benefits [3]. With the exception of energen treated plants whose protein content did not differ from the controls, NPK, biostim and humate had a rising effect on kale's protein level (Fig. 6).

Proline enhances plants' adaptability to environmental stress via osmotic adjustment. An increase in proline content is beneficial to plants' resistance to stress [9]. According to our data, proline content increased under the impact of all fertilizers studied (Fig. 7).

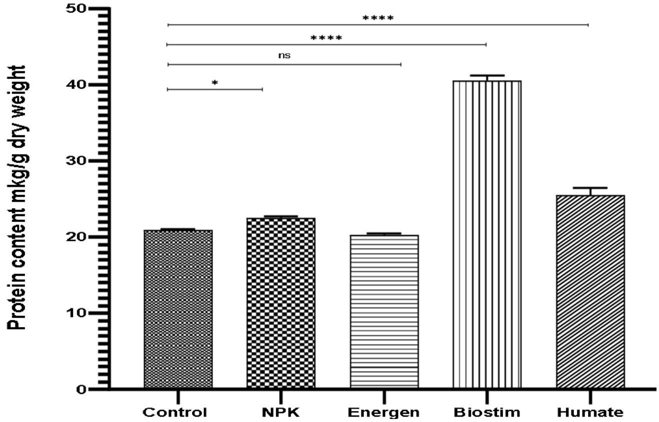

Fig. 6. The influence of various mineral nutrients on proteins in kale *Brassica oleracea var. sabellica* (L.). Error bars signify 95% confidence intervals. Pairwise comparison was conducted using student's t-test. ns, * and ****, symbolize $P > 0.05$, $P \leq 0.05$ and $P \leq 0.0001$, respectively. *Source*: «Compiled by the authors».

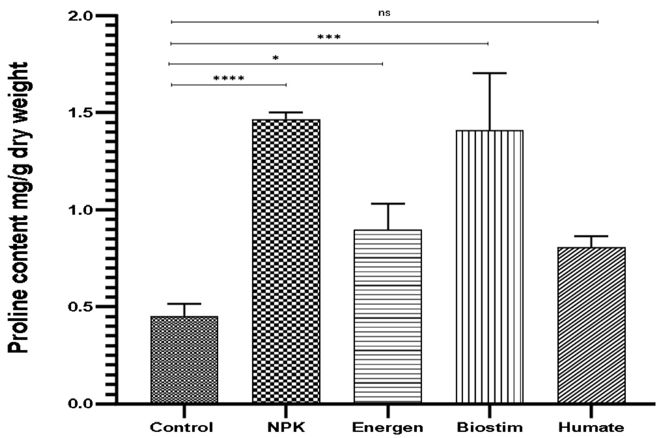

Fig. 7. The influence of various mineral nutrients on proline content in kale Brassica oleracea var. sabellica (L.). Error bars signify 95% confidence intervals. Pairwise comparison was conducted using student's t-test. ns, *, ***, ****, symbolize $P > 0.05$, $P \leq 0.05$, $P \leq 0.001$, and $P \leq 0.0001$, respectively. *Source*: «Compiled by the authors».

Sugar does not only enhance the taste of kale but helps reduce the cell's osmotic potential, thereby minimizing water loss [11]. Sugar levels for all kale plants treated with the studied mineral nutrients were enhanced equally.

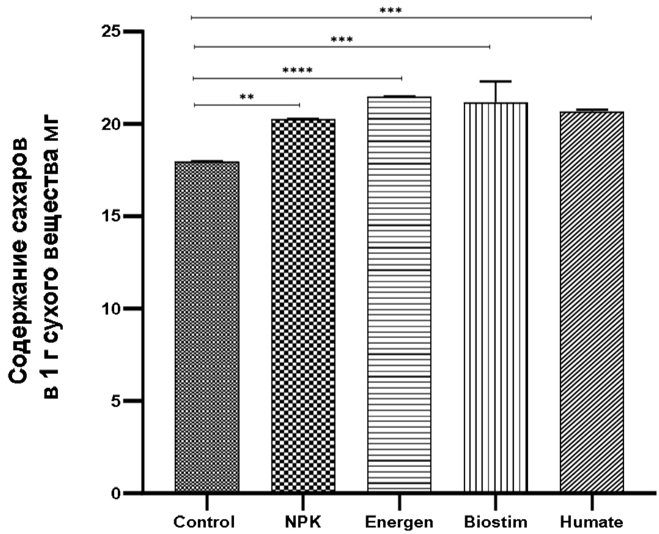

Fig. 8. The influence of various mineral nutrients on sugar content in kale *Brassica oleracea var. sabellica* (L.). Error bars signify 95% confidence intervals. Pairwise comparison was conducted using student's t-test. **, *** and ****, symbolize $P \leq 0.01$, $P \leq 0.001$, and $P \leq 0.0001$, respectively. *Source*: «Compiled by the authors».

The biosynthesis of polyphenols (phenolic compounds and flavonoids) in plants from phenylalanine/tyrosine in a shikimate pathway is greatly influenced by the availability of macronutrients and micronutrient supply. Expression of chalcone synthase (CHS) the primary enzyme that produces an intermediate used in flavonoid synthesis is triggered by the macro and microelements in the fertilizers applied to our sample of the study. Some scientific researches show that polyphenols' content increases in response to phosphorus and nitrogen deficiency in plants[15]. However, the positive effect of nitrogen deficiency on phenol levels in plants accompanied by an increase in phenylalanine ammonia-lyase activity happens only in the case of prolonged nitrogen depletion due to inhibition of primary metabolism. In the fields, lack or excess of nitrogen may be assuaged or accentuated by climate conditions. For these reasons, different authors will observe varied effect of nitrogen on the accrual of phenolic compounds content [22]. This could account for the reason why in our research, we observed an increase in the content of flavonoids and soluble phenolic compounds when kale was treated with the mineral nutrient NPK as well as Biostim increasing phenolic compound content.

Some works reveal that nitrogen fertilizers decrease vitamin C levels in leafy vegetables. At the same time, other experiments on Brassica plants have shown that depending on the plant type, a decrease, an increase, or no effect in vitamin C levels occurs with nitrogen increase. In the case of kale, the optimal dose for the highest amounts of vitamin C was from 0.6 to 1.2 g N per dm^{-3} [13]. The nitrogen content of our fertilizer corresponds to 0.6 g / dm^{-3}, which is consistent with the literature data. In light of this connection, it seems that a stimulating effect of this supplement on the content of vitamin C was observed. Potassium actively participates in cellular and physiological processes such as osmotic adjustment, enzyme functioning, cation-anion balance, detoxification of ROS and protein synthesis. The presence of potassium in NPK, humate energen and biostim may have synergetically induced the variable rise in carotenoid, protein and proline content in curly kale. Wang et. al [27] in their research explain that potassium helps in regulating the amounts of chlorophyll levels by preventing its decomposition. Protein synthesis is triggered by potassium treatment. A similar increase in proline levels in Brassica juncea by potassium treatment was observed by Yousuf et al [30].

During our study, all mineral nutrient treatments lowered MDA content, signifying their involvement in the accumulation of ROS scavenging molecules that limit membrane damage associated with lipid peroxidation. Our results agree with the findings of Ahmad et. al [1] who observed reduced levels of MDA in broad bean under the effect of potassium.

The positive effect of humate, energen, and biostim which contain several trace elements is probably associated with the activation of enzymes involved in the synthesis of the studied compounds.

In our experiment, soluble sugar content in kale fertilized with the studied mineral nutrients slightly increased. This is coherent with the observation by Sung et. al [24] in a research that soluble sugar in nitrogen, phosphorous, or potassium deficient plants was present at concentrations several times higher compared to NPK sufficiency.

The type of macronutrients and micronutrient combination differentially increases the phytochemicals. To enhance the synthesis of phenolic compounds and vitamins, additives containing humic acids (humate and energen) are most promising. At the same time, energen exerted a stronger influence, in comparison with humate, on the formation of these compounds, possibly due to silicon. Biostim proved to be more efficient for protein synthesis. Therefore, it is assumed that the type of mineral processing used in growing kale will determine its nutritional value.

Conclusions

Our data establish that mineral nutrients supply macro and microelements which differentially enhance the synthesis of health-promoting metabolites in curly kale *(Brassica oleracea var. sabellica)* thus improving its quality and medicinal value.

Conflict of interest information. The authors assert that there is no conflict of interest.

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