INFLUENCE OF AMINO ACID-BASED STIMULANTS ON SELECTED QUANTITATIVE AND QUALITATIVE PARAMETERS IN BASIL (OCIMUM BASILICUM)

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Abstract

The aim of the work was to verify the effectiveness of selected stimulant based on amino acids. Tecamin Max (dose 2.5 ml.l^{-1}) and Controlphyt ID (3 ml.l^{-1}) preparations were foliarly applied to basil 4 times during vegetation. Two harvests were carried out and the influence of harvest time on selected parameters was also monitored. The change in fresh matter, dry matter, drying ratio and essential oil content in 4 varieties of Ocimum basilicum L. (´Genovese´, ´Letuce Leaf´, ´Bavires´, ´Pokemon´) was monitored. After foliar application of stimulants, the dry matter content and drying ratio were significantly (P ≤ 0.05) affected. The variety significantly (P ≤ 0.05) affected the content of fresh and dry matter and the content of essential oils. The time of harvest did not have a significant (P ≤ 0.05) impact on any monitored parameter. The total fresh matter content ranged from 10.83 to 19.64 t.ha^{-1}, the total dry matter content ranged from 1.19 to 3.17 t.ha^{-1}. The drying ratio represents the ratio of fresh to dry matter and ranges from 4.33 to 14.36. The essential oils contained in the dry matter reached values from 0.38 to 1.08 ml per 100 g.

Keywords: *basil, essential oils, fresh matter, dry matter, leaf-stimulant*

1. INTRODUCTION

The word "herbs" comes from the Latin word “herba” and means “green crops”. Today, the word has several meanings as non-woody plant, plant with useful properties beyond garden ornaments, plant except vegetable, grains for food and forage. Another meaning can be plants used for flavoring foods and beverages, for medicines, cosmetics dyes, perfumes and for other household and economic use (DeMuth 1996). Basil is one of the important herbs due to its properties. This herb has its origins in India and Iran, later it spread into the Mediterranean countries (Hiltunen and Holm 1999). The herbaceous species of basil (*Ocimum* spp.) belong to the family *Lamiaceae*. These annual plants grow to a height of 80-100 cm and are characterized by an upright habitus. The leaves of the plant are dark green to yellow-green color. Flowering tops and leaves are rich in essential oils (Sharma et al. 1996). Basil as a medical and aromatic plant has a significant use in the pharmaceutical industry. Barátová (2007) states that its main active ingredient is silica, which is present in all parts of the plant except the woody lower parts of the stems. Stanojevic et al. (2017) in their study showed that basil silica is important for its anti-inflammatory, antioxidant, anti-stress and antimicrobial effects. Basil contains 0.5 - 1.1% essential oil with a predominance of linalol (40%) and methylchavicol (25%), euganol, cineole and geraniol. It also contains folic acid, magnesium, calcium, vitamin A, vitamin C, potassium, sodium, phosphorus, iron, manganese (Kóňa 2013). Another important aspect of using basil is the use in the kitchen. Basil leaves are used to flavor soups, meat and fish dishes, cheeses, tomato salads, as well as an additive in vinegars and oils (Pushpangadan and George 2012). It is well known that basil is part of various spice mixtures as well as Provencal spices.

Biostimulants play an important role in controlling the adverse effects of abiotic stress, such as water shortages. According to the European Industry Council, plant stimulants are defined as biostimulants that contain substances and microorganisms that stimulate natural processes to increase nutrient utilization, increase their efficiency, increase plant tolerance to abiotic stresses. The benefits of biostimulants include: increased photosynthesis, increased antioxidant enzyme activity, improved plant quality, increased growth and yield, and more (Abobatta 2020).

The aim of the work was to evaluate the influence of leaf stimulants Tecamin max and Controlphyt ID based on amino acids on quantitative and qualitative parameters of selected varieties *Ocimum basilicum.* The content of fresh mass, dry matter content, drying ratio was determined. The content of essential oils was determined from dry matter.

2. MATERIALS AND METHODS

2.1. Materials

The experiment was based on the application of the leaf stimulants Tecamin max and Controlphyt ID to selected varieties of common basil. For the research work 4 varieties of basil (*Ocimum basilicum* L.) were used:

´Genovese´ - this variety comes from Italy. Its strong and large leaves have a sweet, slightly spicy taste. Flowers bloom white (Larum 2018).

´Letuce Leaf´ - this variety comes from Japan. it is often referred to as lettuce basil. It has huge wavy bright green leaves that can be up to 13 cm long (Grant 2019).

´Pokemon´ - this variety is a compactly growing annual plant with a very strong aroma. It has green leaves and white flowers (AgroMix-X 2021).

´Bavires´ - this variety with an intense scent comes from Germany. It has distinctive green leaves and white flowers. It belongs to the large-leaved basil (Barátová 2007).

Tecamin Max is a leaf biostimulant with anti-stress and regenerative effect. It contains - amino acids (14.4%), free L - amino acids (12%), organic substances (60%) - (phytohormones, organic acids, oligosaccharides, enzymes…), nitrogen (7%). In general, a 0.25 - 1.0% solution is used for foliar application. The pH of this foliar stimulant is 6.6. Tecamin Max is a product for overcoming stressful conditions. Strongly encourages vegetative growth and development of the plant, regenerates damaged tissues. Amino acids enhance the effect of pesticides (especially herbicides) due to their strong and fast absorption properties (Organix, s. r. o. 2020).

The Controlphyt ID is a leaf biostimulant that increases the alkaloid content of plants. It contains free L - amino acids (1.5%), nitrogen (N - 0.8%), phosphorus (P_2O_5 - 0.7%), potassium (K_2O - 0.7%), chitosan (1%). The pH of this foliar stimulant is 4.2. The active substances stimulate the plant's defense mechanisms and help to produce protective metabolites and alkaloids that have a defense function against pathogens (Organix, s. r. o. 2020).

Acronymns: ´Genovese´ (GEN), ´Letuce Leaf´ (LL), ´Pokemon´ (PO), ´Bavires´ (BA), Control (C), Tecamin Max (TM), Controlphyt ID (ID).

2.2. Methods

The experiment was conducted in 2020 in the conditions of the Slovak Republic in the Bánovce nad Bebravou (Horné Ozorovce district). Sowing of the plants was carried out on 2nd April 2020. In the phase of two cotyledons and four true leaves, the plants were transplanted into pots on 26th -27th April 2020. Healthy and hardened plants with 8-10 true leaves were planted on a plot on 27th – 28th June 2020. The plants were planted in 0.35 x 0.40 m spacing. The experimental field consisted of 3 variants: without foliar stimulant application (control), with Tecamin Max foliar stimulant application and with Controphyt ID foliar stimulant application. Each variety had 3 variants of 15 plants. There were 12 experimental fields, so 45 plants were planted in each variety in 3 different variants. The experimental field was regularly irrigated. Along with planting on 27th – 28th June 2020, granular Cererit was applied at a rate of 0.5 kg/m^2. Leaf stimulants Tecamin Max (2.5 ml.l^{-1}) and Controlphyt ID (3 ml.l^{-1}) were applied foliarly 4 times. The 1st dose was applied on 19th July 2020, the 2nd dose – 30th July 2020, the 3rd dose – 29th August 2020 and the 4th dose – 09th September 2020.

The harvest was carried out 2 times during the growing season, in the morning and in dry weather. The 1st harvest was carried out from 12th to 13th August and the 2nd harvest was carried out from 23rd to 24th

September. After harvest, the fresh mass was weighed and then allowed to dry. The dried samples were weighed again. The drying ratio (DR) was calculated according to formula: fresh weight / dry weight = DR (x:1). The content of essential oils was determined at the Institute of Horticulture SPU in Nitra, from dried ground herbal drugs by steam distillation.

3. RESULTS

The yield was evaluated in the fresh matter (FM) and in the dried matter (DM). The FM and DM yield values were converted to t.ha^{-1}. The drying ratio (DR) was calculated from FM and DM values.

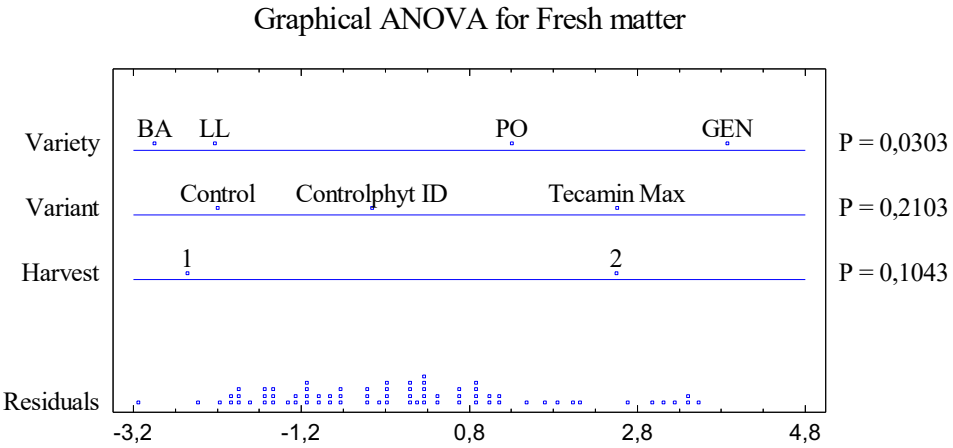
The total yield of FM ranged from 10.83 to 19.64 t.ha^{-1}, with the lowest yield in the variety BA C 1st harvest (5.30 t.ha^{-1}).The most optimal yields were achieved in the variant BA Controphyt ID in the 2nd harvest (11.38 t.ha^{-1}). A statistically significant effect of the biostimulants was recorded in total yield within variety between GEN ID - GEN C, GEN ID - GEN TM, BA ID-- BA C, BA TM - BA C varieties. Also the variety has a statistically significant effect on the harvest of fresh matter. It is in the case of the BA variety that a high increase in fresh mass is recorded after the application of stimulants.

	1st harvest	2nd harvest	Total
Control			
GEN	9.41^{de}	10.20^{cde}	19.61^{e}
LL	10.03^{e}	6.95^{ef}	16.98^{bcd}
BA	5.30^{a}	5.53^{a}	10.83^{a}
PO	9.49^{e}	7.45^{b}	16.94^{bcd}
Tecamin Max			
GEN	9.54^{e}	10.10^{de}	19.64^{e}
LL	6.40^{ab}	9.20^{ab}	15.60^{bc}
BA	8.47^{cde}	9.40^{cde}	17.87^{de}
PO	8.34^{cde}	9.59^{de}	17.96^{de}
Controlphyt ID			
GEN	7.66^{bcd}	8.67^{bc}	16.33^{bcd}
LL	6.69^{abc}	8.44^{ab}	15.13^{b}
BA	6.71^{abc}	11.38^{f}	18.09^{de}
PO	9.34^{de}	8.06^{bcd}	17.40^{cd}

Table 1. Yield of fresh matter [t.ha^{-1}]

Notes: ´Genovese´ (GEN), ´Letuce Leaf´ (LL), ´Bavires´ (BA), ´Pokemon´ (PO).

Same letters (a) within the same column means statistically non-significant difference (at 95.0% confidence level). Different letters (a; b) within the same column means statistically significant difference (at 95.0% confidence level).



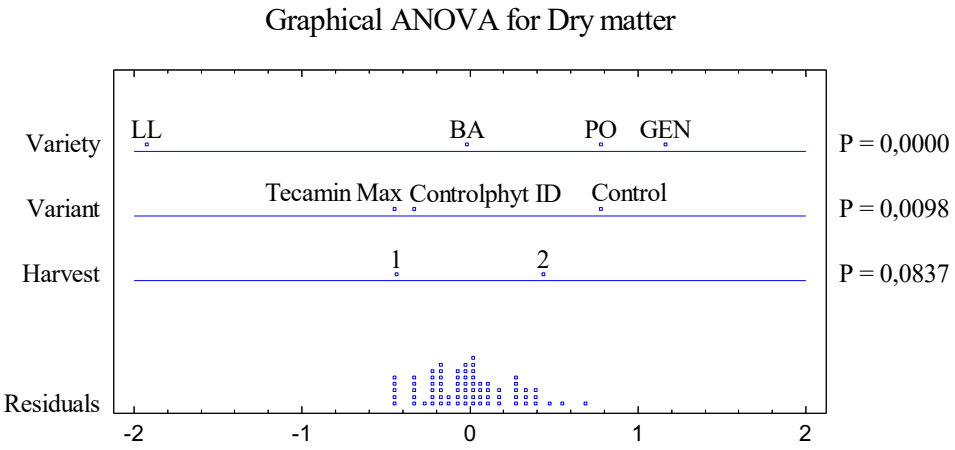
Dry matter total yield ranged from 1.19 to 3.17 t.ha^{-1}, with the lowest value in the variant LL C 2nd harvest (0.46 t.ha^{-1}) and the highest values was in the GEN C 2nd harvest (1.96 t.ha^{-1}). A statistically significant effect of the biostimulants was recorded in total yield of DM within variety between GEN ID – GEN C, GEN TM – GEN C, GEN ID - GEN TM, LL ID - LL C, LL TM – LL C, PO ID – PO C, PO TM – PO C, PO ID – PO TM. In general, varieties and biostimulant statistically significantly affected yields of DM. The use of both biostimulants has caused a significant decrease in dry matter in varieties GEN, LL and PO.

	1st harvest	2nd harvest	Total
Control			
GEN	1.21^{cde}	1.96^{g}	3.17^{f}
LL	1.17^{cde}	0.49^{a}	1.66^{b}
BA	1.00^{bc}	1.12^{de}	2.12^{cd}
PO	1.31^{de}	1.77^{g}	3.08^{f}
Tecamin Max			
GEN	1.35^{e}	1.34^{ef}	2.69^{e}
LL	0.62^{a}	0.72^{ab}	1.34^{a}
BA	1.21^{cde}	1.04^{cd}	2.25^{cd}
PO	1.11^{cd}	0.87^{bc}	1.98^{c}
Controlphyt ID			
GEN	0.98^{bc}	1.31^{ef}	2.29^{d}
LL	0.60^{a}	0.59^{a}	1.19^{a}
BA	0.83^{ab}	1.44^{f}	2.27^{d}
PO	1.29^{de}	1.37^{ef}	2.66^{e}

Table 2. Yield of dry matter [t.ha^{-1}]

Notes: ´Genovese´ (GEN), ´Letuce Leaf´ (LL), ´Bavires´ (BA), ´Pokemon´ (PO).

Same letters (a) within the same column means statistically non-significant difference (at 95.0% confidence level). Different letters (a; b) within the same column means statistically significant difference (at 95.0% confidence level).

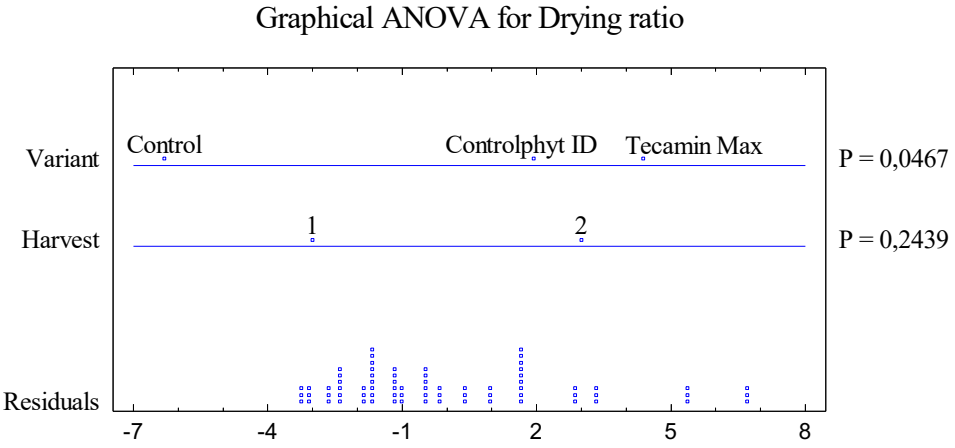


The drying ratio was determined after complete drying of the herbal drug. The values of the drying ratio ranged on average from 5:1 to 13:1. Variety PO C 2nd harvest had the lowest drying ratio (4.33), variety LL ID 2nd harvest had the highest drying ratio (14.36). It can be seen in the Table 3 that the lowest values were reached within the variety in the control variants and the effect of the variant caused statistically significant differences.

	1st harvest	2nd harvest	Average
Control			
GEN	7.80	5.19	6.50
LL	8.61	11.00	9.80
BA	5.32	4.93	5.13
PO	7.24	4.33	5.78
Tecamin Max			
GEN	7.06	7.56	7.31
LL	10.38	12.74	11.56
BA	7.01	9.05	8.03
PO	7.52	7.21	7.36
Controlphyt ID			
GEN	7.78	6.60	7.19
LL	11.12	14.36	12.74
BA	8.09	7.90	7.99
PO	7.25	5.90	6.58

Table 3. Drying ratio

Notes: ´Genovese´ (GEN), ´Letuce Leaf´ (LL), ´Bavires´ (BA), ´Pokemon´ (PO)



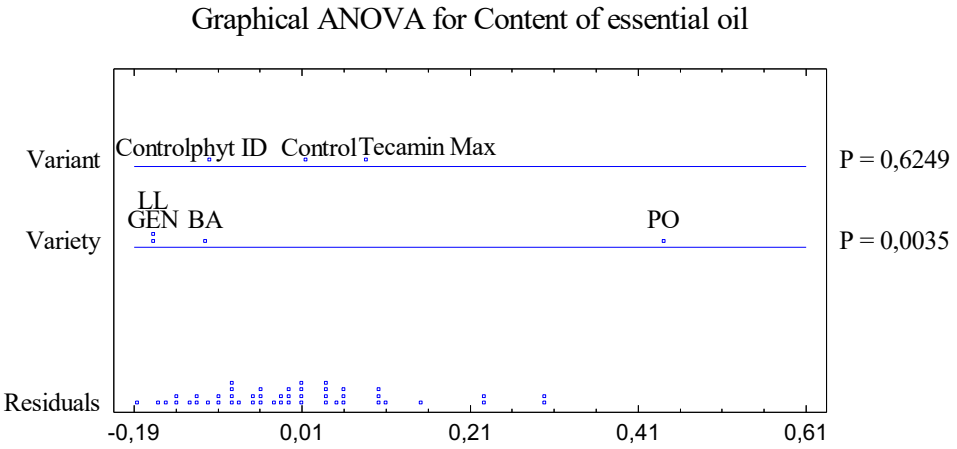
The total essential oil values ranged from 0.38 to 1.08 ml per 100 g. The essential oils had the lowest content BA ID 1st harvest (0.15 ml.100g^{-1}) and the highest content in PO TM 1st harvest (0.75 ml.100g^{-1}). The highest total content of essential oils was achieved by the variety BA in all variants. Individual varieties achieved a statistically significant differences in total content between the following variants: GEN ID – GEN C, GEN ID – GEN TM, LL TM – LL C, LL ID – LL C, BA ID – BA C, BA ID – BA TM, PO TM – PO C, PO ID – PO TM. The variety was shown to have a statistically significant effect on the essential oil content.

	1st harvest	**2nd harvest**	**Total**
Control			
GEN	0.23^{b}	0.20^{ab}	0.43^{ab}
LL	0.50^{d}	0.18^{a}	0.68^{cd}
BA	0.38^{c}	0.33^{c}	0.71^{cd}
PO	0.48^{d}	0.28^{abc}	0.76^{cd}
Tecamin Max			
GEN	0.23^{b}	0.30^{bc}	0.53^{b}
LL	0.18^{ab}	0.25^{abc}	0.43^{ab}
BA	0.33^{c}	0.33^{c}	0.66^{c}
PO	0.75^{e}	0.33^{c}	1.08^{e}
Controlphyt ID			
GEN	0.35^{c}	0.33^{c}	0.68^{cd}
LL	0.33^{c}	0.20^{ab}	0.53^{b}
BA	0.15^{a}	0.23^{abc}	0.38^{a}
PO	0.45^{d}	0.33^{c}	0.78^{d}

Table 4. Content of essential oil [ml.100g^{-1}]

Notes: ´Genovese´ (GEN), ´Letuce Leaf´ (LL), ´Bavires´ (BA), ´Pokemon´ (PO).

Same letters (a) within the same column means statistically non-significant difference (at 95.0% confidence level). Different letters (a; b) within the same column means statistically significant difference (at 95.0% confidence level).



4. DISCUSSION

It was found that biostimulants affected the growth of fresh matter both positively and negatively. The increase of FM can be explained by the statements which report those amino acids and small peptides have similar activity to the growth hormones auxins and gibberellins, which promote nutrient uptake and assimilation (Carrilo et al. 2019; Ceccarelli et al. 2021). Similarly, Rouphael et al. (2021) report that the use of plant protein hydrolysates caused an increase in photosynthesis and color status. An increase in ion content, yield and quality parameters of basil were also recorded. Protein hydrolysates derived from animal sources have shown worse effects than plant hydrolysates. In a study by Kim et al. (2019) also monitored the hormonal activity of protein plant biostimulants, they report that the biostimulants showed similar activity to auxin, thereby promoting the rooting of basil cuttings.

In our work, the application of biostimulants resulted in a decrease in dry matter, suggesting that protein hydrolysates could affect water uptake and its binding in plant tissue. The content of essential oils was fluctuating in our case. The content after the application of biostimulants significantly decreased or increased depending on the varieties.

The results of Noroozlo, Souri and Delshad (2020) suggest that application to leaves of medium to low concentrations (250 and 500 $mg.l^{-1}$) of glutamine or glycine improved basil growth. Fresh and dry weight of shoots, leaf area, and leaf chlorophyll content were improved by applying 500 $mg.l^{-1}$ glycine or glutamine per leaf compared to control plants. Saburi et al. (2014) states that amino acids significantly affect the increase of dry matter content in basil and the content of essential oils after the application of stimulants based on amino acids ranged from 0.25% to 0.35%, while the control variant contained 0.28%. Other studies report the effect of protein hydrolysates and amino acids on spice species of the *Lamiacea* family. Aktsoglou et al. (2021) claims that the application of protein hydrolysates did not affect the harvest of fresh and dry matter of *Mentha* spp. but caused an increase in the content of essential oils. Similarly, Mehrafarin et al. (2015) achieved an increase in yield parameters, dry matter, and the content of essential oils in *Melissa officinalis* L. after the application of stimulants containing free amino acids. Amino acid treatment also significantly increased the content of essential oils in *Satureja hortensis* L (Poorghadir 2020).

The harvesting time in our work did not significantly affect any monitored parameter. Some authors state that the intensity of sunlight affects the yield and content of essential oils. Saran et al. (2021) claim that shading the holy basil *Ocimum tenuiflorum* L. had a negative impact on fresh matter yield and essential oil content. Dou et al. (2018) in his study, present that basil achieved high yields in a 16-hour

photoperiod. Basil plants grown in the higher photoperiod had larger and wider leaves. Similarly, Frąszczak et al. (2011) states that a 16-hour photoperiod caused an increase in fresh mass yields compared to a 12-hour period.

5. CONCLUSIONS

The influence of amino acid-based leaf stimulants on selected quantitative and qualitative parameters of basils was variable. Significant changes were recorded in terms of quantitative parameters and drying ratio. Since the results were different depending on the variety, we can say that the response to the stimulant is closely related to the varietal assortment. We consider the experiment as a basis for future research tasks, and we recommend repeating in other soil - climate conditions or under stress conditions as it is known that stimulants support creation of secondary metabolites in response to stress.

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