Phytochemical composition of Humulus Lupulus L. in ontogeny under different treatments

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Abstract. Wild hop (Humulus lupulus L.) is a double-headed perennial climbing plant that belongs to the Cannabaceae family. Hops contain many biologically active compounds possessing powerful antimicrobial, antioxidant and antifungal activity, due to which its original use was for medicinal purposes and only later began to be used in beer production. The aim of the work was to identify the effects of growth regulators (hibbersib and epin-extra) and biofertilizer (powder) on the content of soluble phenolic compounds, flavonoids, tannins, carotenoids, vitamins C and B, protein and sugars in plants of common hop (Humulus lupulus L.) during ontogenesis. Root cuttings of hops were planted in the beginning of May 2022 in protected ground under laboratory conditions. In the second variant rootstock were potted in soil with 10 g/kg powders; in the third variant rootstocks were treated with hibbersib (666.6 µg/l) by spraying; in the fourth variant also, rootstocks were treated with epin-extra (500 µl/l) by spraying. After 4 weeks, all variants were transplanted to the open field. The samples for analysis (leaves) were taken 4, 8, 12 and 16 weeks after planting in the open ground, the average night temperatures were +16^{0}C, +24^{0}C, +14^{0}C and +7^{0}C, respectively. Phytochemical composition of leaves was determined by spectrophotometric method. In control plants the content of phenolic compounds and vitamin C reached the highest values in the twelfth week after planting. The synthetic growth regulators (epin-extra and hibbersib) and the biofertilizer (pudrete) were shown to change the phytochemical composition of the hop plants to different extents. Powdertet increased the vitamin and protein content to a greater extent whereas growth regulators increased the content of phenolic compounds and sugars, particularly under the influence of hibbersib. **Key words:** Humulus Lupulus L., epin-extra, hibbersib, pudrete, ontogeny, phytochemical composition.

1 Introduction

Humans have always needed the use of medicinal plants to treat certain illnesses. Although there has been a development in industry and the advent of synthetic drugs, herbal medicines have nevertheless continued to be an alternative form of treatment. The use of

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herbal preparations for therapeutic purposes has increased steadily in recent decades [1].

Common hop (Humulus lupulus L.) is a double-headed perennial climbing plant belonging to the Cannabaceae family that grows in temperate northern climates [2]. Almost all parts of the plant are rich in biologically active compounds with potent antimicrobial, antioxidant and antifungal activity [3, 4], so it was originally used as a medicinal plant, and only later became used for beer production.

These characteristics of this plant, combined with the growing interest in health-promoting biologically active substances, open up new and interesting perspectives for hops outside the beer industry [5]. For centuries and to date, the cultivation of hops has yielded several products, such as tea, fishing tackle (using the stems), foodstuffs (cooking young shoots) and even a preservative for bread [1, 4, 6].

Although the properties of hops have been extensively studied in the last 25 years [7], further research is required to clarify the chemical composition of the hop leaves to clarify whether they are as rich in bioactive compounds as cones and to identify whether the synthesis of useful compounds in hops can be improved using different approaches.

This work examines the effect of commercial growth regulators (hibbersib and epin-extra) and also of a biofertiliser (pudrete) on the content of different groups of phenolic compounds, carotenoids, vitamins C and B, proteins and sugars in common hop plants (Humulus lupulus L.) plants during ontogenesis.

2 Materials and Methods

Root cuttings of hops were planted in early May 2022 under controlled conditions in the laboratory. The experiment scheme consisted of 4 variants: the first variant - control, in the second variant rootstock cuttings were planted in pots with soil, in which pudrete was added (it is a bio-fertilizer from poultry manure dried in microwaves, consists of 88.4% organic matter, 4. 59% nitrogen, 1.80% potassium, 3.70% phosphorus) at a rate of 10 g/kg soil, in the third treatment the rootstocks were treated with hibbersib (666.6 µg/l) by spraying, and in the fourth treatment with epin-extra (500 µl/l), also by spraying.

After 4 weeks, all variants were transplanted to the open field (soil acidity was 6.9, organic matter (humus): 1.96%, nitrate nitrogen 35.5 mg/kg, ammonia nitrogen 11.3 mg/kg, mobile phosphorus 584 mg/kg, exchangeable calcium was 13.25 mmol/100g and exchangeable magnesium 1.5 mmol/100g).

Samples for analysis (leaves) were taken 4, 8, 12 and 16 weeks after planting in the open field. Average night temperatures were +16^{0}C, +24^{0}C, +14^{0}C and +70C, respectively.

Phytochemical composition of leaves was determined by spectrophotometric methods. The content of phenolic compounds, flavonoids, carotenoids, vitamin C, proteins and sugars was determined by the methods described in the study of Almugrabi et al. [8]. The tannin content was determined in terms of gallic acid, the optical density was measured at 277 nm [9]. The total riboflavin content was determined according to the method [10] at 445 nm.

The experiments were carried out under six biological conditions. Statistical processing of data was performed using GraphPad Prism version 5 software. Significance of the difference was determined by Mann-Whitney test with $P \leq 0.05$.

3 Results

Humulus lupulus L is an important source of primary and secondary plant metabolites [5]. The most notable primary metabolites produced by hops are ketones, alkanes, sugars, lipids, vitamins and amino acids, while the most common secondary metabolites of hops

are terpenoids present in essential oil and various groups of phenolic compounds [5].

Indeed, phenolic compounds show beneficial health effects, exhibiting sedative, anticancer, estrogenic, and antimicrobial properties. Other biological activities, including health effects against cardiovascular disease and type 2 diabetes, as well as neuroprotective effects, are mainly related to the hop-specific flavonoid (chalconoid) xanthohumol [11, 12].

Flavonoids represent the most diverse and widespread class of phenolic compounds with various metabolic functions in plants. Their biosynthesis often occurs in response to abiotic or biotic stress. Five groups of flavonoids are present in hops: chalcones, flavanones, flavonols, flavan-3-ols, and tannins, including some glycoside derivatives. The highest amount of flavonoids was found in the seeds and bracts of female inflorescences [5].

According to the results of our experiments, the content of soluble phenolic compounds and flavonoids in the control reached the highest values at the twelfth week, and then either did not change in the case of soluble phenolic compounds (Fig. 1), or even decreased in the case of flavonoids (Fig. 2).

Growth regulators and powders increased the content of soluble phenolic compounds and flavonoids. Powderet increased the phenolic content after 8 and 16 weeks after planting, but the flavonoid content only after 12 weeks. Epin-extra increased the phenolic content after 4 weeks only, while the flavonoid content increased at all growth stages. Hibbersib increased the content of phenolic compounds and flavonoids at 4, 12 and 16 weeks after planting (Fig. 1, 2).

The greatest effect on the content of soluble phenolic compounds had hibbersib, and on the content of flavonoids, hibbersib and epin-extra, the effect of these growth regulators increased at 12 weeks of growth at +14 ^{0}C.

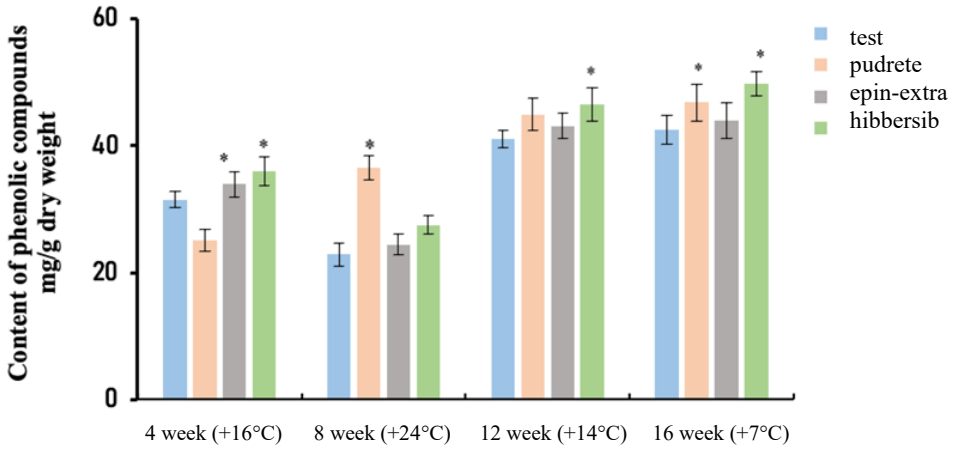

Fig. 1. Effect of different treatments on the content of soluble phenolic compounds in Humulus lupulus L. plants during ontogeny *- Statistically significant differences ($p<0.05$) compared with test (Source: authors).

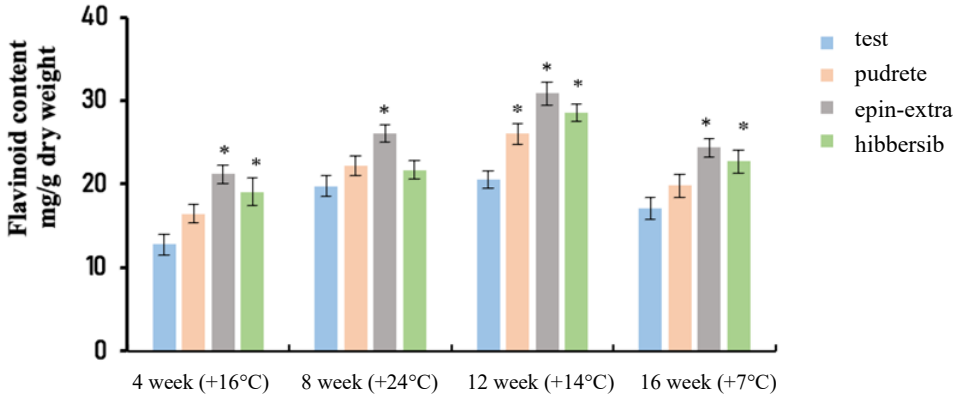

Fig. 2. Effect of different treatments on flavonoid content in Humulus lupulus L. plants in ontogeny
*- Statistically significant differences ($p<0.05$) compared to control (Source: authors).

One of the dominant groups of biologically active substances of Humulus lupulus L. raw material is tannins, which are a complex mixture of phenolic compounds with antioxidant activity [13].

In our experiments, it was found that in the control, the content of tannins, as well as other groups of phenolic compounds, reached the highest values at the twelfth week, and then decreased (Fig. 3).

As illustrated in Fig. 3, pudrete had no effect on tannin content. Synthetic biostimulants had a positive effect on this indicator, but to varying degrees. Epin-extra increased tannin content at 4 and 12 weeks, and hibbersib at 4.8 and 12 weeks after planting (Figure 3).

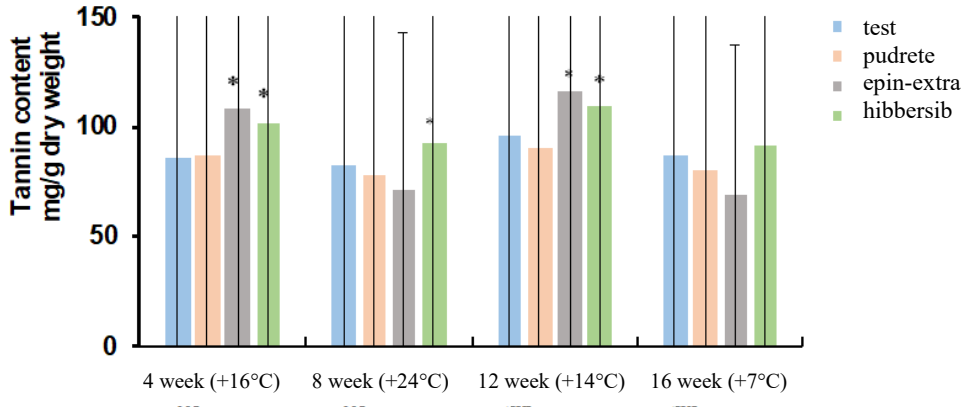

Fig. 3. Effect of different treatments on the tannin content of Humulus lupulus L. plants in ontogeny
*- Statistically significant differences ($p<0.05$) compared with test (Source: authors).

Protein content did not change during the development of the hop plants and was independent of the different climatic conditions (at +7^{0}C and +24^{0}C) (Figure 4). The epin treatment did not alter the protein content; however, the pudrete treatment increased it at 4, 12 and 16 weeks and the hibbersib treatment increased it at 8, 12 and 16 weeks after planting (Figure 4).

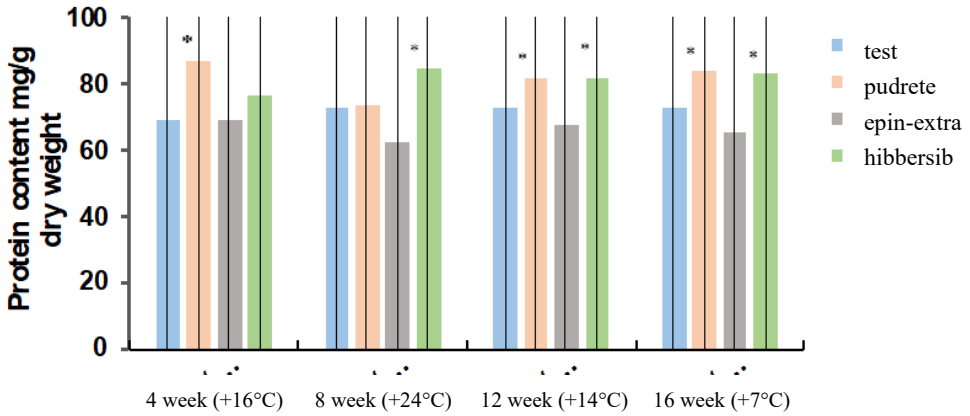

Fig. 4. Effect of different treatments on the protein content of Humulus lupulus L. plants in ontogeny *- Statistically significant differences ($p<0.05$) compared to control (Source: authors).

Sugar content decreased in ontogeny up to 12 weeks and then increased under conditions of low temperatures (+7^{0}C) (Figure 5). It should be noted that only hibbersib increased the content of sugars at all stages of plant development. Epin-extra had no effect on their amount, while pudrete reduced their content at all growth stages (Fig. 5).

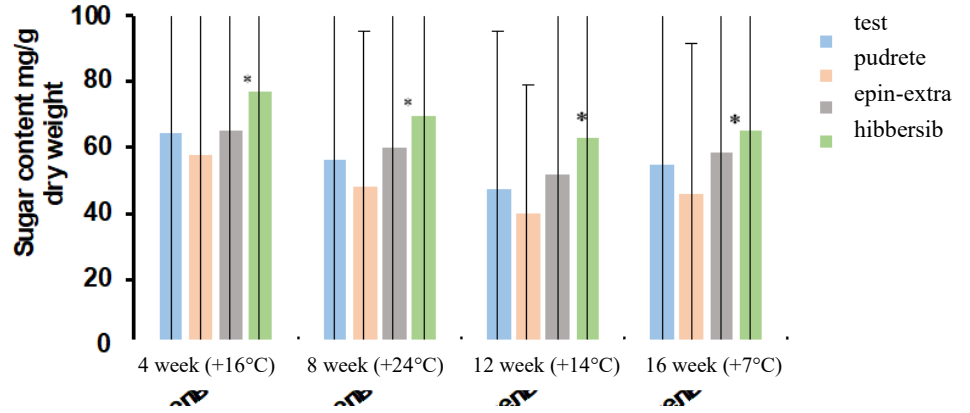

Fig. 5. Effect of different treatments on the sugar content of Humulus lupulus L. plants in ontogeny *- Statistically significant differences ($p<0.05$) compared with test (Source: authors).

Hops are an excellent source of vitamins such as provitamin A, vitamins B, C, E and PP [14]. Our results demonstrated that Humulus lupulus L., is also a rich source of vitamin C, the amount of which reached the highest values at the twelfth week and remained unchanged thereafter (Fig. 6). As for provitamin A and vitamin B2, their maximum content was observed at week sixteen (Fig. 7, 8).

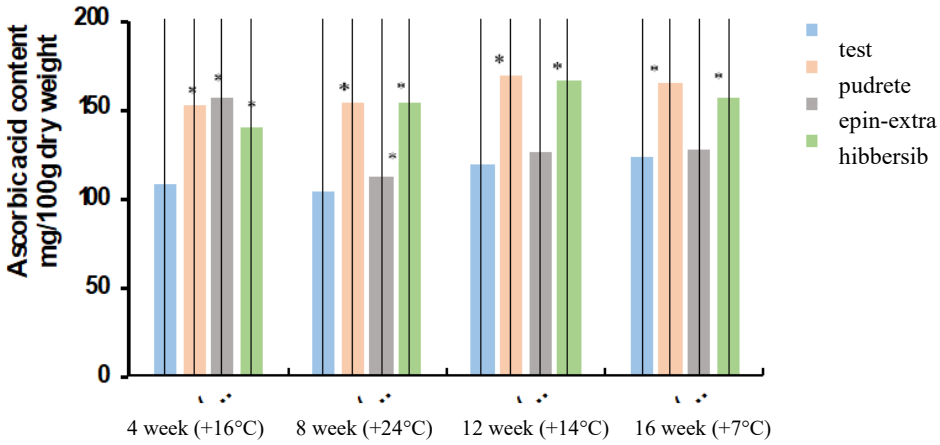

Fig. 6. Effect of different treatments on ascorbic acid content in Humulus lupulus L. plants during ontogeny *- Statistically significant differences ($p<0.05$) compared with test (Source: authors).

The growth regulators and powders tested altered the vitamin content. The most intensified formation of vitamins C, B_2 and provitamin A was caused by pudrete. It increased their content at almost all stages of growth and at different temperatures (Figures 6, 7, 8). Hibbersib had the same effect as pudrete on vitamin C content (the amount of vitamin C in the two variants was almost the same at 8, 12, 16 weeks) (Fig. 6). However, it had a lesser effect on provitamin A content, its effect only being evident at weeks 4 and 12 after planting (Fig. 7). Hibbersib practically did not change vitamin B_2 content at 4, 8, 12 weeks, but at 16 weeks reduced its content by 38.5 % compared with test (Fig. 8).

Epin extra increased vitamin C content only at the first stages of development (4 and 8 weeks) (Fig. 6), provitamin A content only at 12 weeks after planting (Fig. 7), and vitamin B2 content at all stages of growth, especially at 16 weeks (Fig. 8).

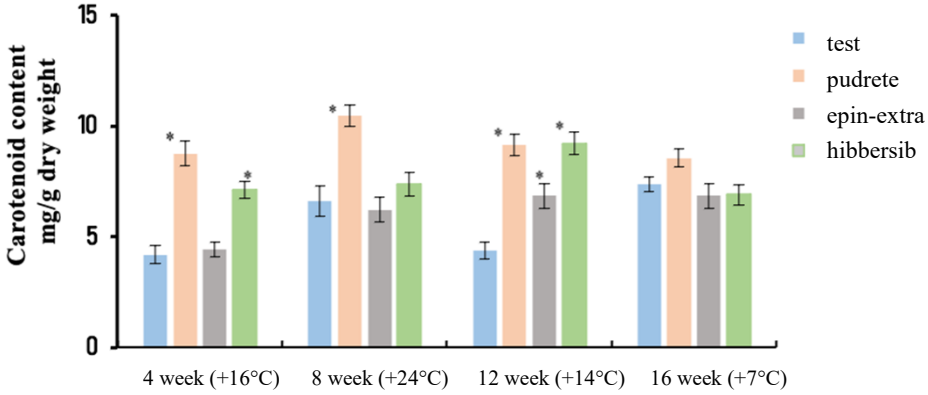

Fig. 7. Effect of different treatments on carotenoid content in Humulus lupulus L. plants during ontogeny *- Statistically significant differences ($p<0.05$) compared with test (Source: authors).

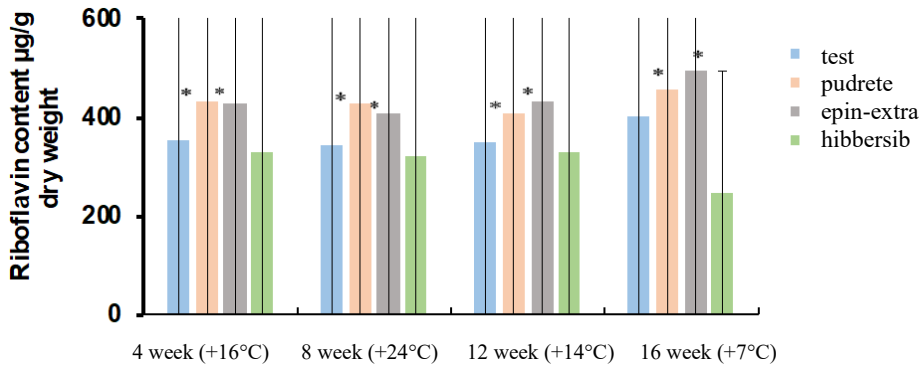

Fig. 8. Effect of different treatments on the vitamin B content of Humulus lupulus L. plants in ontogeny *- Statistically significant differences ($p<0.05$) compared with test (Source: authors).

4 Discussion

According to the results of our experiments, it was found that in the control the content of soluble phenolic compounds, flavonoids and tannins reached the highest values at the twelfth week of development, and then decreased or remained unchanged (Fig. 1, 2, 3). This increase in phenolic compounds by 12 weeks of growth (mid-maturity stage) is probably due to the active metabolism of the plants that accompanies rapid growth in the first few months.

Various studies have shown that the content of phenolic compounds increases initially in ontogeny and decreases in the later stages of maturity. Since the synthesis of primary and secondary metabolites comes from the same precursors, it is likely that in the early stages of ontogenesis active plant growth takes place and precursors are predominantly used for the synthesis of primary metabolites.

As plants develop, the content of phenolic compounds increases. This may be due to the fact that phenolic compounds are required for the biosynthesis of secondary cell walls and as protective substances. Our data on the increase in the content of phenolic compounds in hop ontogeny are in agreement with the results obtained in various studies [8, 15].

Our work has shown that synthetic growth regulators (epin-extra and hibbersib) and the biofertiliser powders can improve the phytochemical composition of hops, but to varying degrees.

Addition of powders has increased the content of soluble phenolic compounds and flavonoids at selected developmental stages but has done little to alter the tannin content. Growth regulators increased the levels of all the phenolic groups in almost all developmental stages of the hop plants. Hibbersib had the greatest effect on the content of these three parameters.

Some studies have shown that the treatment of plants with solutions and mixtures of mineral salts such as NPK increased the accumulation of polyphenolic compounds several times over in comparison with test plants [16, 17]. On the other hand, there is evidence of a significant decrease in the amount of soluble phenolic compounds and flavonoids in different plants under the influence of NPK [18,19].

According to our results, the powders we used (element content: 4.59 % N, 3.70 % P, 1.80 % K) increased the content of substances containing N, especially as proteins (Figure 4), and did not influence or reduced the content of substances containing large quantities of C, such as flavonoids (Figure 2), tannins (Figure 3) and sugars (Figure 5).

The ratio between N and C can regulate the interaction between primary and secondary metabolism (20). For each specific phytochemical and its respective biosynthesis pathway,

there is a different optimum ratio of these elements.

Phenylalanine is a precursor in the biosynthesis of phenols as well as an amino acid used in protein synthesis. Consequently, there may be competition for phenylalanine between protein synthesis and the synthesis of secondary metabolites, and therefore the biosynthesis of secondary metabolites may be inhibited due to the inclusion of phenylalanine in protein synthesis [21]. There is a positive correlation between the phenylalanine lyase activity (PHAL) and enzymes of the phenylpropanoid pathway and the accumulation of carbon-containing secondary metabolites in plants [22]. Plants grown on nitrogen-deficient soils increase ammonia input through increased PHAL activity, leading to increased accumulation of polyphenolic compounds [21]. Nitrogen is efficient for protein synthesis, and thus the phenolic content is reduced with the same amount of phenylalanine. In addition, phenylalanine gene expression increases with nitrogen depletion [23]. Thus, an increase or decrease in polyphenolic compounds may be associated with high or low activity of the enzyme phenylalanine lyase, indicating the vital role of this enzyme in the regulation of plant stress responses [24].

There is a negative correlation between protein and phenolic content because under conditions of excess nitrogen input, phenylalanine is used for protein synthesis rather than phenolics [25]. This may be the reason for the relatively low value of sugars (Fig. 5) and tannins (Fig. 3), and the high protein content observed in our study when using powders.

Epin-extra increased the content of soluble phenolic compounds only after 4 weeks, the flavonoid content at all growth stages and the tannin content after 4 and 12 weeks. Hibbersib significantly increased the content of phenolic compounds, flavonoids and tannins at 4, 12 and 16 weeks after planting (Fig. 1, 2, 3). It should be noted that the effect of these growth regulators increased at 12 weeks at temperature (+14^{0}C).

Brassinosteroids are known to promote resistance to stress, including high and low temperatures, drought and salinity by enhancing the expression of stress marker genes, including COR-gene and heat shock protein encoding [26]. It was shown that exogenous epibrassinolide can regulate the level of endogenous ABA which is an important anti-stress plant hormone [27].

It was found that as the level of foliar application of ABA increased, the amount of soluble sugars increased in Atractylodes macrocephala [28]. Carbohydrates are the main compounds required for the synthesis of phenolic compounds from shikimic acid, since the intermediate products of glycolysis, and the pentose phosphate pathway are involved in the synthesis of aromatic amino acids. It has been shown previously [20] that the amount of secondary metabolites largely depends on the balance between carbohydrate formation and consumption: the greater this ratio, the more secondary metabolites are formed.

In a study [29], it was shown that compounds of steroid nature (brasinolide and its analogue epin-extra) increase the content of phenolic compounds and flavonoids to a greater extent than compounds of terpenoid nature (hibberellic acid and their analogue hibbersib), which more effectively increase the content of proteins and sugars.

Hibberellins are able to induce the synthesis of specific transcription factors (GAMyb) that recognize sequences in the promoter sites of many genes, such as the barley α-amylase gene. It has been shown that hibberellin initiates the formation of not only α-amylase, but also other hydrolases, especially protease and ribonuclease [30]. This could be one reason for the increase in sugars under the influence of hibbersib in our study.

Our results demonstrated that Humulus lupulus L., is a rich source of vitamin C, provitamin A (carotenoids) and vitamin B2.

Vitamin C content reached its highest values at the twelfth week and then remained unchanged (Fig. 6), while provitamin A and vitamin B2 content was highest at the sixteenth week of cultivation (Fig. 7, 8).

The growth regulators and powders studied altered the vitamin content. The greatest

intensification of vitamin C, B2 and provitamin A formation was caused by pudrete, which increased their content at almost all stages of growth and at different temperatures (Fig. 6, 7, 8).

Data on the effect of nitrogen fertiliser on the vitamin content of plants are quite contradictory. Some studies have shown that nitrogen fertilizer application increases the vitamin C and carotenoid [16] and riboflavin [31] content in plants. There are also reports that nitrogen fertilizers may have no effect on vitamin C [32], carotenoids [33], and riboflavin [34] or even reduce ascorbic acid [35], riboflavin [34], and carotenoids [36] in plants when exposed to high concentrations.

Part of the disagreement may be related to different doses of nitrogen fertilizers in particular experiments [31-36].

Potassium is actively involved in cellular and physiological processes such as osmotic regulation, enzyme function, cation-anion balance, AFC detoxification and protein synthesis. The presence of potassium in the powder may have induced a variable increase in vitamin content in hops. A study [37] found that potassium could regulate the amount of chlorophyll by preventing its degradation.

Synthetic growth regulators increased vitamin synthesis in hops to a lesser extent than powders. Hibbersib had the same effect on vitamin C content as pudrete (Figure 6). However, it only increased pro-vitamin A content at 4 and 12 weeks after planting (Figure 7) and had practically no effect on vitamin B_2 content (Figure 8).

Epin-extra increased vitamin C content only at the first stages of development (4 and 8 weeks) (Fig. 6), provitamin A content only at 12 weeks after planting (Fig. 7), vitamin B_2 content - at all stages of growth, especially at 16 weeks (Fig. 8).

5 Conclusion

Our data show that analogues of natural hormones (epin-extra and hibbersib) and biofertilisers (pudrete) differentially enhance the synthesis of health beneficial primary and secondary metabolites in common hops (Humulus lupulus L.), thereby improving their quality and medicinal value.

Acknowledgements

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