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Use of biologically active substances in hops

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ABSTRACT

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In four-year experiments, hop was treated with 7 biologically active substances in two terms during vegetation: Lignohumate max (a mixture of humic acids and fulvic acids), Lexin (a mixture of humic acids and fulvic acids enriched with auxins), Lexenzym (a mixture of humic acids and fulvic acids enriched with auxins, phytohormones and enzymes precursors), *Ascophyllum nodosum* seaweed extract, synthetic auxin, humic acids and fulvic acids alone. The chlorophyll content was monitored after the application both in the vine leaves and in the branch leaves. After harvesting of the hops from the individual treatments, the yield of dry hops was determined and the cones were analysed for the content of alpha bitter acids. The results show that the most effective hop treatment was the application of Lexin and Lexenzym. The Lexenzym treatment provided a yield of dry hops of 1.86 t/ha, i.e. 0.47 t/ha higher compared with untreated control. The Lexin treatment provided yield higher by 0.41 t/ha of dry hops compared with the untreated control, while the harvested cones contained the most alpha-bitter acids (4.57%).

Keywords: *Humulus lupulus* L.; hops production; humic substance; green pigments; quality

Growing hops has a long-standing tradition in the Czech Republic. The onset of systematic hop growing is linked to the reign of Charles IV. Since then, Czech hops have been world-renowned. Cultivation of hop was gradually concentrated in areas with the most favourable conditions (Žatecko, Úštěcko and Tršicko). Throughout the centuries, there were various waves of decline and expansion of hops growing in Bohemia. Nowadays, the area of hops is growing again. The Czech Republic is the third largest hops grower in the world. The largest area is taken by Saaz, which is the world's most priced cultivar. The most important hop area – Žatec hop fields – is in the shadow of the Ore Mountains and Doupov Mountains. Climatic fluctuations of the past decades have affected all crops not only in the Czech Republic. These abiotic stresses can be partly compensated by agrotechnical measures, irrigation or the use of biologically active substances (Nesvadba et al. 2003, Rybka et al. 2014).

The results of many experiments (Pavlovic et al. 2012, Nováková et al. 2014, Procházka et al. 2015b) clearly show that the use of biologically active substances in the cultivation, not only of hops but also of other crops, leads to increased production and yield stabilization. According to these authors, plants with biologically active substances can react to different stress conditions and weather fluctuations.

One of the ways to increase the production potential of hop plants, and thus their production, is the application of biologically active substances during vegetation (Procházka et al. 2017). Dřímalová (2005) defines biologically active substances as various growth regulators, enzymes, substances associated with plant bioenergy or

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even photosynthetic pigments forming protein complexes that participate in the conversion of energy of electromagnetic radiation into the energy of chemical bonds. According to some authors, biologically active preparations based on a mixture of synthetic auxins, humic acids and fulvic acids may be very beneficial (Procházka et al. 2015a). A similar activity was shown in many experiments using synthetic analogues of some brassinosteroids; they, among other things, positively interact with auxins. The biologically active substances with anti-stress effects that act primarily on the cellular level, includ, for example, gibberellins or carbohydrates (Kohout 2001, Chen et al. 2004, Anuradha and Rao 2007).

MATERIAL AND METHODS

The experiment was established to determine the effect of biologically active substances on hops production parameters. The following biologically active substances were used in the experiment:

Lignohumate Max. Lignohumate Max is a product based on humins and is formed in the process of organic transformation of wood processing waste. It contains only active parts of the humus spectrum, namely humic acids and fulvic acids in a ratio of 1:1 (Procházka et al. 2015).

Lexin. Lexin is a liquid concentrate of humic acids, fulvic acids and auxins. It stimulates, for example, the division of cells and their long-term growth. Its positive effect was also observed on the formation of vascular bundles, the formation and growth of roots, and other anatomical-morphological properties and plant features including the increase in their yield (Procházka et al. 2016).

Lexenzym. A concentrate of humic acids and fulvic acids, enriched with phytohormones, vitamins, and enzymes.

Ascophyllum nodosum. Free cytokinins, purine bases and their nucleosides, abscisic acids, such as indolyl-acetic acid, are found in the *Ascophyllum nodosum* extract. Seaweeds also contain all major plant nutrients, trace elements and a wide range of vitamins (e.g. B, C, D, E, K, niacin) that can be used by plants. They also contain alginic acid, amino acids and mannitol.

Pure auxin. In the experiment, indole-3-acetic acid (IAA) was used. Auxin regulates many growth and development processes. It stimulates cell division, long-term growth and cell differentiation. In all these processes it interacts with cytokinins.

Humic acids. Humic acids are natural organic high molecular compounds formed by biological and chemical decomposition of organic matter (plant, animal, etc.) and synthetic activity of microorganisms (Senesi and Loffredo 1999, Veselá et al. 2005). These are colloidal, highly sorptive, easy-to- bind water, and with metal ions they often form complexes known as humates. Humic acids are fractions of humic substances that are not soluble in acidic and neutral aqueous solutions but are soluble in these solutions at higher pH values. These substances contain a large amount of carbon (52–65%) (Veselá et al. 2005).

Fulvic acids. Fulvic acids are low-molecular substances (a mixture of weakly aliphatic and aromatic organic acids), which have lower carbon content (about 30%) and water-soluble weakness compared to humic acids. Due to their small size, fulvic acids can easily enter the plant via roots, stems and leaves (Domingos et al. 2009).

Untreated control was sprayed only with water.

The experiments were carried out in the growing season 2014–2017 with the cvs. Saaz and Osvald clone No. 72. The experiment was carried out on four experimental sites. In 2014, a trial was made at the Tufa Tuchořice located in the Žatec hop area in the Louny district. The 1.8-hectare hop field is situated on a moderate slope of medium-heavy modal chernozem on carbon loess with a humus content of 2.4%. Hop cv. Osvald clone 72 was planted in 2013 in plant spacing 320 × 110 cm, along the north-south row. In 2015, the experiment was carried out at the Chmelex Hořesedly, Žatec hop area, Rakovník district. The 1.9-hectare hop field is free of irrigation and is situated on a mild slope and moderate cambisol with a humus content of about 2%. It was planted with cv. Osvald clone 72 in 2010 in spacing 280 × 115 cm, in the east-west direction. In 2016, the experiment was carried out in the hop farm of the Podlesí Ročov agricultural cooperative, which is also in the Žatec hop area, in the district of Louny. The hop field is free of irrigation, medium-heavy fluvisol, with a humus content of 2.1%, an area of 2.89 ha was planted in 1990, with the spacing of 280 × 80 cm, cv. Osvald clone 72, north-south exposition. In 2017, a trial was made at the MK AGRO in Čínov. The 1.46-hectare hop-garden is located on a plain and medium-hard Chernozem with a humus content of about 2%. It was planted in 1995, spacing 280 × 110 cm, cv. Osvald clone 72, in the north-south

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exposition. Details of individual experimental sites are presented in Tables 1 and 2.

For each experimental treatment, the relative chlorophyll content in the bine and branch leaves

Table 1. Growing technology at experimental localities

Tuchořice		Hořesedly		Ročov		Čínov	
autumn 2013	harrowing loosening to 15 cm	autumn 2014	harrowing loosening to 15 cm	autumn 2015	harrowing ploughing	autumn 2016	harrowing ploughing
22.3.2014	fertilizer LAV (300 kg/ha)	19.3.2015	fertilizer Amofos (150 kg/ha) + fertilizer SA (250 kg/ha)	15.3.2016	fertilizer Kieserit (150 kg/ha) + fertilizer MgS (150 kg/ha)	5.4.2017	harrowing
6.4.2014	cutting	11.4.2015	cutting	3.4.2016	harrowing	6.4.2017	fertilizer NPK 15 (300 kg/ha)
2.5.2014	fungicide Aliette 80 WG (1.5 kg/ha)	5.5.2015	1st training bines	7.4.2016	cutting	15.4.2017	cutting
8.5.2014	1st training bines	13.5.2015	fertilizer LAD (200 kg/ha)	10.5.2016	insecticide Actara (0.1 kg/ha)	10.5.2017	1st training bines
22.5.2014	fungicide Curzate K (1.5 L/ha)	20.5.2015	2nd training bines	12.5.2016	fungicide Aliette 80 WG (1.0 kg/ha)	22.5.2017	2nd training bines
26.5.2014	ridging	21.5.2015	fungicide Aliette 80 WG (1.0 kg/ha) + insecticide Actara WG (0.1 kg/ha)	12.5.2016	1st training bines	24.5. + 18.6.2017	1st and 2nd ridging
6.6.2014	fungicide Ridomil (1.5 kg/ha) + insecticide Teppeki (0.09 kg/ha)	22.5.2015	1st ridging	22.5.2016	2nd training bines	25.5. + 3.7.2017	fertilizer LAD (200 kg/ha) + fertilizer DAM (520 kg/ha)
23.6.2014	fungicide Ortiva (1.0 L/ha) + Fungicide Curzate K (1.5 kg/ha)	26.5.2015	fertilizer urea (100 kg/ha)	26.5.2016	ridging	14.5.2017	fungicide Aliette 80 WG (1.0 kg/ha) + fertilizer Zinkosol forte (1.5 L/ha)
23.6.2014	1st application – experimental treatments	2.6.2015	2nd ridging	9.6.2016	fungicide Curzate K (1.5 kg/ha) + fungicide Alliette 80 WG (1.0 kg/ha) + insecticide Teppeki (0.09 kg/ha)	24.5.2017	fungicide Aliette 80 WG (1.0 kg/ha) + fungicide Cuprocaffaro Micro (1.25 kg/ha) + MgS (1.0 kg/ha) + Zinkosol forte (1.5 L/ha)
4.7.2014	insecticide Movento 150 OD (1.0 L/ha)	15.6.2015	fungicide Curzate K (1.5 kg/ha) + insecticide Teppeki (0.09 kg/ha)	16.6.2016	fertilizer LAD (200 kg/ha)	3.6.2017	fungicide Ortiva (1.6 L/ha) + Confidor 200OD (0.6 L/ha) + fertilizer Vegaflor (6.0 L/ha)
18.7.2014	fungicide Ortiva (1.0 L/ha) + fungicide Curzate K (1.5 kg/ha)	22.6.2015	fungicide Ortiva (1.0 L/ha) + insecticide Nissuron 10 WP (1.5 kg/ha)	25.6.2016	1st application – experimental treatments	23.6.2017	fungicide Ortiva (1.6 L/ha) + insecticide Movento 150 OD (1.0 L/ha) + fertilizer Vegaflor (6.0 L/ha)
18.7.2014	2nd application – experimental treatments	29.6.2015	fungicide Lynx (0.75 L/ha)	25.6.2016	fungicide Aliette 80 WG (1.0 kg/ha)	7.7.2017	1st application – experimental treatments
6.8.2014	harvest	29.6.2015	1st application – experimental treatments	30.6.2016	fertilizer LAD (200 kg/ha)	7.7.2017	fungicide Revus (1.6 L/ha) + fertilizer Vegaflor (6.0 L/ha)
		15.7.2015	fungicide Bellis (2.0 kg/ha) + insecticide Movento 150 OD (1.0 L/ha)	8.7.2016	fungicide Bellis (2.0 kg/ha) + fungicide Curzate K (4.0 kg/ha) + insecticide Movento (1.0 L/ha)	24.7.2017	fungicide Ortiva (1.6 L/ha) + fertilizer Vegaflor (6.0 L/ha)
		15.7.2015	2nd application – experimental treatments	18.7.2016	2nd application – experimental treatments	24.7.2017	2nd application – experimental treatments
		9.8.2015	fungicide Flowbrix (3.5 L/ha)	18.7.2016	fungicide Cuprozin progress (5.0 L/ha)	12.8.2017	fungicide Cuproxat SC (7.0 L/ha)
		23.8.2015	harvest	9.8.2016	fungicide Cuproxat SC (7.0 L/ha)	25.8.2017	harvest
				28.8.2016	harvest		

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Table 2. Experimental treatments

Variant	Name	Dose
1	untreated control	only water
2	Lignohumate Max	0.4 L/ha
3	fulvic acids (FA)	equivalent FA content as in Lignohumate Max
4	humic acids (HA)	equivalent HA content as in Lignohumate Max
5	auxins	equivalent auxins content as in Lexin
6	Lexin	0.25 L/ha
7	Lexenzym	0.25 L/ha
8	*Ascophyllum nodosum*	0.5 kg/ha

Total water volume per 1 ha was 2000 L

was determined with the Yara N-tester (Konica Minolta, Inc., Tokio, Japan) every 3 weeks after each application.

The harvest of the individual experimental treatments was carried out by the drawn straw and then after the transfer to the stationary combine line the individual treatments were grazed, dried and weighed. At the same time, a sample of the harvested cones was taken and analysed for the content of alpha and beta bitter acids. These analyses were performed by the UV-VIS spectrophotometric method in an accredited laboratory.

Statistical analysis. The results of the field trials were processed by a general linear model (GLM ANOVA) using the statistical program SAS, version 9.4 (Carry, USA). Differences between the mean values were evaluated by the Tukey's *HSD* (honestly significant difference) test at the level of significance $P = 0.05$.

RESULTS AND DISCUSSION

The highest chlorophyll content in both bine and branch leaves was recorded in plants treated with Lexin and Lexenzym three weeks after the application. For both of these preparations, the chlorophyll content was more than 10% higher compared to the untreated control. It can be seen from Figures 1a,b that all used biologically active substances had a positive effect on the chlorophyll content in both leaf types. A similar

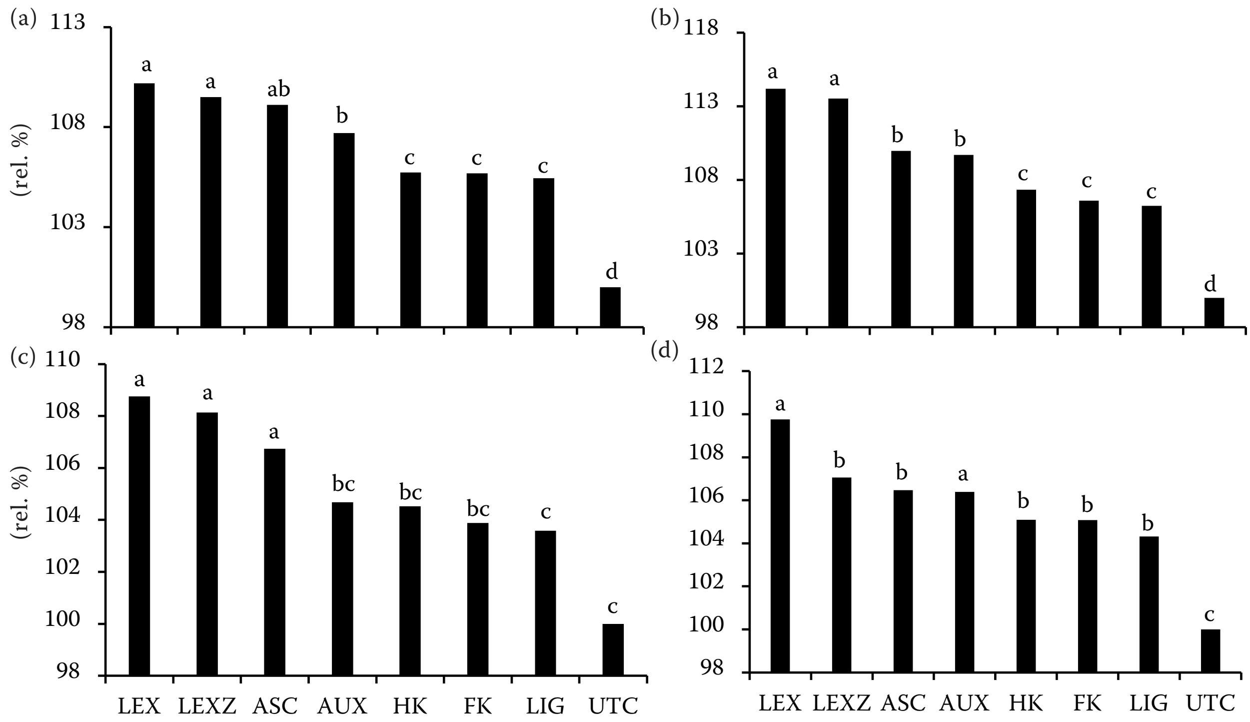

Figure 1. Average chlorophyll content in (a) bine and (b) branch leaves three weeks after the first application; (c) bine and (d) branch leaves six weeks after the first application. Average of years 2014–2017; in relative percentages of the control treatment. LEX – Lexin; LEXZ – Lexenzym; ASC – *Ascophyllus nodosum*; AUX – pure auxin; HK – humic acids only; FK – fulvic acids only; LIG – Lignohumate Max; UTC – untreated control; means with the same letters are not statistically significant

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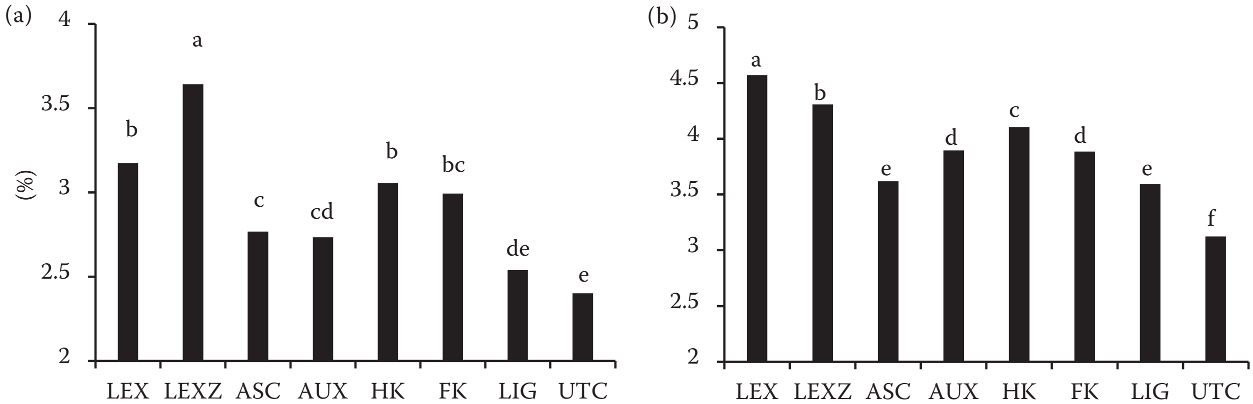

Figure 2. Average alpha bitter acids content in cones (a) two weeks before planned harvest, and (b) after harvest – average from 2014–2017. LEX – Lexin; LEXZ – Lexenzym; ASC – *Ascophyllus nodosum*; AUX – pure auxin; HK – humic acids only; FK – fulvic acids only; LIG – Lignohumate Max; UTC – untreated control; means with the same letters are not statistically significant

positive effect of the humic substances application on the chlorophyll content of the leaves was also observed by Lee and Barlet (1976) in corn. Figures 1c,d show that all used treatments had a positive effect on the chlorophyll content 6 weeks after the first application and 3 weeks after the second application of the investigated substances. The best results were achieved by preparations with a complex character, i.e., containing humic acids, fulvic acids and addition of auxins or microelements. The extract of *Ascophyllum nodosum* seaweed also had a positive impact. The positive effect of seaweed extracts on chlorophyll content was also observed by Řehoř et al. (2018), Khan et al. (2009) or Blunden et al. (1996) who observed this positive effect in tomato, wheat, barley, and corn. The chlorophyll content is to a large extent also a prerequisite for higher photosynthetic activity, as confirmed by the results of Pokorný et al. (2011). Khan et al. (2009) reported that seaweed extracts used to spraying leaves result in an increased ability to maintain chlorophyll content, which fully supports our results.

From the results of the analysis of the alpha-bitter acids content two weeks before the planned harvest, it is clear that all used biologically active substances had a positive effect on the production of alpha-bitter acids. It can be seen from Figure 2a that the highest average content two weeks before harvest had cones on plants treated with Lexenzym (3.64%) and Lexin (3.17%). Similar results were observed by Štranc et al. (2008) when the alpha bitter acid content in Lexin treated plants was by 48% higher than the untreated control. Analysis

Table 3. Results of statistic evaluation (average of the years 2014–2017)

			LEX	LEXZ	ASC	AUX	HK	FK	LIG	UTC	*HSD*
Chlorophyll content	bine leaves	3 weeks after application	110.19^{a}	109.51^{a}	109.12^{ab}	107.70^{b}	105.73^{c}	105.69^{c}	105.44^{c}	100.0^{d}	0.7813
	branch leaves		114.19^{a}	113.52^{a}	109.98^{b}	109.70^{b}	107.33^{c}	106.60^{c}	106.25^{c}	100.0^{d}	0.9627
	bine leaves	6 weeks after application	108.75^{a}	108.14^{a}	106.74^{ab}	104.68^{bc}	104.53^{bc}	103.88^{bc}	103.58^{c}	100.0^{c}	1.5304
	branch leaves		109.76^{a}	107.06^{b}	106.47^{b}	106.38^{ab}	105.10^{b}	105.08^{bc}	104.31^{b}	100.0^{c}	1.4178
Alpha bitter acids content	cones 14 days before harvest		3.17^{b}	3.64^{a}	2.77^{c}	2.73^{cd}	3.06^{b}	2.99^{bc}	2.54^{de}	2.40^{e}	0.1980
	harvested cones		4.57^{a}	4.31^{b}	3.62^{e}	3.89^{d}	4.10^{c}	3.88^{d}	3.60^{e}	3.12^{f}	0.1709
Yield of dry hops			1.80^{b}	1.86^{a}	1.62^{d}	1.68^{c}	1.62^{d}	1.57^{e}	1.69^{c}	1.39^{f}	0.0268

LEX – Lexin; LEXZ – Lexenzym; ASC – *Ascophyllus nodosum*; AUX – pure auxin; HK – humic acids only; FK – fulvic acids only; LIG – Lignohumate Max; UTC – untreated control; means with the same letters are not statistically significant; *HSD* – honestly significant difference

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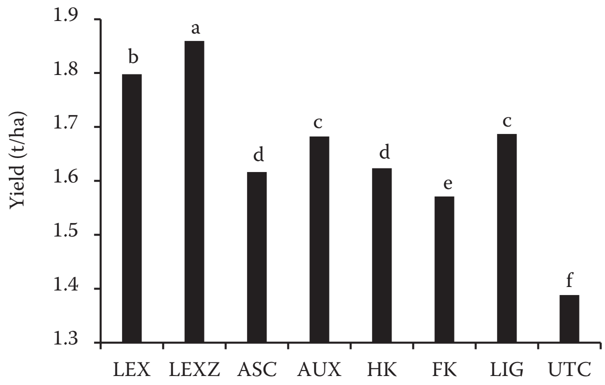

Figure 3. Average yield of dry hops – average from 2014–2017. LEX – Lexin; LEXZ – Lexenzym; ASC – *Ascophyllus nodosum*; AUX – pure auxin; HK – humic acids only; FK – fulvic acids only; LIG – Lignohumate Max; UTC – untreated control; means with the same letters are not statistically significant

of the alpha bitter acid content in hop cones at harvest showed a statistically significant increase over the control treatment (Table 3).

Figure 3 shows that all used biologically active substances had a positive effect on the yield of hops. The best results were achieved after the application of Lexenzym, where the yield of dry hops was 1.86 t/ha and after the application of Lexin, where the yield of dry hops reached 1.80 t/ha. Compared to the untreated control, the yield of dry hops increased by 0.47 t/ha in the case of Lexenzym and by 0.41 t/ha in the case of Lexin. It is therefore obvious that the application of these biologically active substances is highly efficient and beneficial for agricultural practice. Adamčík et al. (2016) reported that Lexin increased the yield of sorghum by 27.5% over a three-year trial compared to an untreated control. A positive influence of the use of biologically active substances in agricultural practice is summarized in Procházka et al. (2017) in the results of a four-year trial where soybean treated by Lexin in seeds had by 13% higher seed yield compared to the untreated control. Similarly, Pačuta (2013) after the Lignohumate Max leaf application increased the yield of sugar beet roots by 16% and sugar content by 2.3%, which represented 3.12 t/ha.

The above results suggest that the use of biologically active substances, in particular complex formulations consisting of several functional components, appears to be highly effective for the optimum hop production. Use of these substances helps plants to cope better with stress arising during vegetation due to changing climatic conditions in hop growing areas.

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