

THE EFFECT OF KELPAK SL BIOREGULATOR ON FUNGI ISOLATED FROM THE ROOTS OF HORSERADISH (*ARMORACIA RUSTICANA* GAERNT)

Summary

The Kelpak SL bioregulator concentrations of 0.4; 0.04; and 0.004 ml³/cm³ applied under laboratory conditions significantly modified the analysed features of tested fungi and the most their sporulation process. Intensified production of spores was observed in antagonistic fungi: *T.harzianum*, *T. kaningii* and enthomopathogenic *V. lecanii*, whereas inhibition in pathogenic *F. poae* and *V. dahliae*, respectively by 25.4% and 74.5%. Generally, at its lowest concentration in the medium (0.004), Kelpak SL contributed to reduction of the surface growth of the colony and increment of the analysed fungi biomass. On the other hand, the extract of algae (*Ecklonia maxima*) significantly inhibited the growth rate of *F. poae* and increment of *T. harzianum* biomass. Fungistatic effect of the applied concentrations of Kelpak SL was observed for *V. dahliae* - a dangerous horseradish pathogen and enthomopathogenic *V. lecanii* species on the media with 0.004 cm³.

Key words: *Ecklonia maxima*, phytopathogenic and antagonistic fungi, horseradish

ODDZIAŁYWANIE BIOREGULATORA KELPAK SL NA GRZYBY IZOLOWANE Z KORZENI CHRZANU (*ARMORACIA RUSTICANA* GAERNT.)

Streszczenie

W warunkach laboratoryjnych zastosowane stężenia (0.4; 0.04; 0.004 ml³/cm³) biostymulatora wzrostu Kelpak SL istotnie modyfikowały badane cechy testowanych grzybów, przy czym w największym stopniu ich proces sporulacji. Wzmoczone wytwarzanie zarodników stwierdzano u grzybów antagonistycznych: *T. harzianum*, *T. kaningii* oraz owadobójczego *V. lecanii*, a hamowanie u patogenicznych: *F. poae* i *V. dahliae* odpowiednio o 25,4% i 74,5%. Na ogół udział w podłożu hodowlanym najniższej koncentracji 0,004 Kelpak SL przyczyniał się do ograniczenia rozrostu powierzchniowego kolonii i przyrostu biomasy badanych grzybów. Z kolei niezależnie od zastosowanego stężenia, wyciąg z alg *Ecklonia maxima* istotnie hamował tempo wzrostu *F. poae* oraz przyrost biomasy *T. harzianum*. Fungistatyczne oddziaływanie zastosowanych stężeń Kelpak SL stwierdzono w odniesieniu do groźnego patogena chrzanu *V. dahliae* oraz owadobójczego gatunku *V. lecanii* na podłożach z udziałem 0,004 cm³.

Słowa kluczowe: *Ecklonia maxima*, grzyby fitopatogene i antagonistyczne, chrzan

1. Introduction

Application of bioregulators is an inseparable element of modern crop cultivation technology and in the first place protects plants against stressors, such as drought, frost, too high temperature and biological stress, whose main agent is presence of pathogens and other agrophages. According to Burchardt and Riederer [6], each stress greatly weakens the process of photosynthesis which leads undoubtedly, due to reducing the assimilation area, degradation of photosynthetic dyes and disturbance in stomata activity to disturbances in gaseous exchange in plant. Cavusoglu et al. [7] reported that stress factors disturb the hormonal balance in plants, in the first place limiting the activity of auxins, which are the most important for plants and participate in all their life processes. A decline in cytokinin content, responsible for cell divisions, growth and limiting the ageing process in plants, is observed at simultaneous intensified synthesis of abscisic acid and ethylene, which act in the opposite way. Foliar application of Kelpak SL biostimulant, which contains auxins and cytokinins obtained from algae (*Ecklonia maxima*) may effectively support plants under stress. High concentration and ratio of auxins (11.0 mg/l) to cytokinins (0.031 mg/l) cause that they are very fast absorbed by plants, and therefore ensure their high physiological activity. Many authors reported, that application of

the extract of brown sea algae (*Ecklonia maxima*) contributes to increase in crop yield and often also to its quality [15, 21, 22, 27, 28]. Cytokinins present in them influence the strengthening and growth of the plant root system [2, 29]. In the opinion of Khan et al. [14], extracts of brown algae favourably affect biological properties of soils, because they contribute to the development of beneficial microorganisms. Moreover they favour colonization of roots of citrus plants, papaya and passion flower by micorrhizal fungi, which play an important role in plant development [17]. Soil is the habitat for many phytopathogenic organisms, whose activity may change under the influence of plant growth biostimulants. There is relatively little information in the subject literature about these issues. Thompson's report [30] indicates that watering and sprinkling conifer tree seedlings in nurseries with extracts of sea algae hampers development of *Botrytis* pathogenic fungi, which infect the roots, shoots and needles. Extracts of sea algae may reduce the use of synthetic fungicides even by 80%. These extracts may be also useful in production and protection of plants, particularly in vegetative propagation, e.g. by seedlings, such as horseradish. *In vitro* research is an important stage in the assessment of biostimulant effect on plant health.

The paper aimed at evaluation of the effect of Kelpak SL bioregulator on linear growth, produced biomass and sporu-

lation of *Botrytis cinerea* Pers., *Sclerotinia sclerotiorum* (Lib) de Bary, *Fusarium oxysporum* Schlecht., *Fusarium poae* (Peck) Wollenw., *Verticillium dahliae* Kleb., *Verticillium lecanii* Zimm., *Trichoderma harzianum* Rifai and *Trichoderma koningii* Rifai fungi settling the horseradish roots.

2. Material and methods

The first stage of research consisted in isolation of fungi from the dry rotting skin of horseradish. The roots showing dry rot symptoms were cleaned superficially in running water. They were next cut in the places of lesion by means of sterile scalpel. Sections of 5mm were then collected from the border of healthy and diseased tissues, disinfected by immersing in 50% ethanol solution for 30 seconds, rinsed in sterile distilled water and dried on a filter paper. In the inoculation chamber the material was placed on Potato Dextrose Agar medium in 200 mm Petri dishes (10 pieces per dish). The culturing was conducted in a phytotron for 10 days at the temperature of 23°C. The appearing fungi colonies were successively split off. Subsequently, macro- and microscopic observations were conducted to classify the fungi to appropriate species using fungal identification keys and monographs [10, 18, 19, 20, 23, 24, 25]. Basing on the number of obtained isolates, the frequency of individual fungi species occurrence was established and expressed in percentage with reference to the total number of isolates (100%).

Biotic activity of Kelpak S1 biostimulant in inhibiting linear growth of the colonies, biomass increment and sporulation of dominant fungi species isolated from the roots was assessed at the second stage of investigations by means of the poisoned media method [16]. Three concentrations of the biostimulant were used: 0.40; 0.04 and 0.004 ml³/cm³. Petri dishes with PDA medium without Kelpak SL supplement were the control. The experiment was conducted in five replications. The linear growth rate of the tested fungi colonies was computed on the basis of everyday measurements of their increments, according to the following formula:

$$T = \frac{A}{D} + \frac{b_1}{d_1} + \dots + \frac{b_x}{d_x}$$

T – linear growth index;

A - mean from the measurements of fungal colony diameter in mm;

D – the duration of the experiment;

b₁,...b_x – increment of the colony diameter in mm;

d₁,...d_x – number of days from the last measurement.

After two weeks the number of spores produced by fungi was counted on each Petri dish by means of Thom's haemocytometer. The fungistatic activity of the biostimulant was assessed on the basis of linear growth inhibition, biomass increment and test fungi sporulation calculated using Abbott's formula [5].

$$I = \frac{K - A}{K} 100\%$$

Inhibition - stimulation coefficient according to Abbott's formula.

I – linear growth inhibition-stimulation coefficient (biomass and sporulation);

K – average fungi colony diameter on the control dish (mass of mycelium, number of spores);

A – average fungi colony diameter (mass of mycelium, number of spores) in the individual test objects.

The obtained results were subjected to statistical computations by means of analysis of variance, using Duncan test on the significance level $\alpha=0.05$.

3. Results and discussion

Altogether 214 fungi isolates were obtained in result of mycological analysis of horseradish roots showing dry rot symptoms, 169 colonies were classified to the species and the other 72 were counted to four genera (Tab. 1). Among the isolated phytopathogenic fungi, the *Fusarium* species, which accounted to 36.9% of the total isolated microorganism population were dominant. *F. oxysporum* (11.2%) and *F. poae* (9.5%) were the most frequently observed within this taxonomic genus. Among the phytopathogenic fungi, *Verticillium dahliae* (13.3) and *Sclerotinia sclerotiorum* (12%) were the most frequent. *Botrytis cinerea* (6.6%) was also registered in the dominant group. Dry rotting tissue of horseradish roots was also a habitat for antagonistic fungi: *Trichoderma harzianum*, *T. koningii* and phytopathogenic *Verticillium lecanii*, whose total share made up 17.4%. *Trichoderma* species mentioned above reveal a considerable pathogenicity towards nematodes but also towards many plant phytopathogenic fungi [9, 31]. The isolated fungi species are common in soils of various climatic zones, whereas a majority, such as *F.oxysporum*, *F. poae* or *B. cinerea* exist as saprophytes and plant pathogens. The common feature of all parasitic fungi species isolated from the horseradish roots is their polyphagia. *B. cinerea* infects over 200, whereas *S. sclerotiorum* 400 crop species, while developing inside their tissues cause severe losses in yields [4, 27, 3]. These species are typical necrotrophs, which first kill host plants and then settle the dead tissue [1]. On the other hand, *V. dahliae* is a pathogen settling vascular tissues causing brittle root (*Spiroplasma citri*) of horseradish roots [3, 11, 12, 13]. The habitat of the fungi isolated in the experiment is in soil whose physico-chemical changes undoubtedly affect the population of microorganisms of the rhizosphere and infecting plants.

Table 1. The dominant fungi species colonizing the roots of horseradish

Tab. 1. Dominujące gatunki grzybów zasiedlające korzenie chrzanu

Fungal species	Number of isolates	Per cent
<i>Botrytis cinerea</i> Pers.	16	6.6
<i>Fusarium oxysporum</i> Schlecht.	27	11.2
<i>Fusarium poae</i> (Peck) Wollenw.	23	9.5
<i>Sclerotinia sclerotiorum</i> (Lib.) de Bary	29	12.0
<i>Trichoderma harzianum</i> Rifai	15	6.2
<i>Trichoderma koningii</i> Oudem.	14	5.8
<i>Verticillium dahliae</i> Klebahn	32	13.3
<i>Verticillium lecanii</i> Zimm.	13	5.4
Total of other species belonging to the general	72	30.0

Source: own work / Źródło: opracowanie własne

Conducted laboratory experiments allowed for determining the immediate effect of *Ecklonia maxima* algae extract, applied as Kelpak SL preparation on development of pathogenic and antagonistic fungi isolated from horseradish

roots. At the same time, obtained results do not provide a basis for an explicit determination of the bioregulator effect on fungal organisms. Each analysed fungi species differently responded to Kelpak SL supplement in the culturing medium. The research demonstrated that the test fungi sporulation process was the most modified feature, as both very strong stimulation 292.97% and inhibition on the level of 78.63% were observed (Fig. 3). Similarly, the quantity of biomass obtained from the tested fungi ranged widely (-18.99 – 31.68) (Fig. 2). Relatively, Kelpak SL the least influenced surface growth of the fungi colonies – the registered growth inhibition coefficient assumed values from -6.87% to 18.54% (Fig. 1). Analysis of variance revealed a significant influence of the biostimulant on the surface growth rate of *Fusarium* fungi, *S. sclerotiorum*, entomopathogenic *V. lecanii* and antagonistic *T. koningii* (Tab. 2). However, the most significant inhibition of surface growth of the tested fungi was noted on the media with lowest concentrations of the biostimulant (Tab. 2, Fig. 1). Among the pathogenic fungi, *V. dahlia* revealed the weakest response to an addition of *Ecklonia maxima* extract to the medium, as evidenced by the values of the colony surface growth inhibition coefficients, which only at higher concentrations remained on the level between 1.66% and 2.08%. Similarly, inhibition of this species mass increment ranged between 2.43 and 7.28% (Fig. 2).

However, with increasing concentration of *E. maxima* in the medium, stronger inhibition of sporulation process was noted in *V. dahliae*, ranging from 70.23 to 78.63% (Fig. 3). In the pathogenesis fungi spores play an important role in spreading and infection of plants. In a pot experiment, other

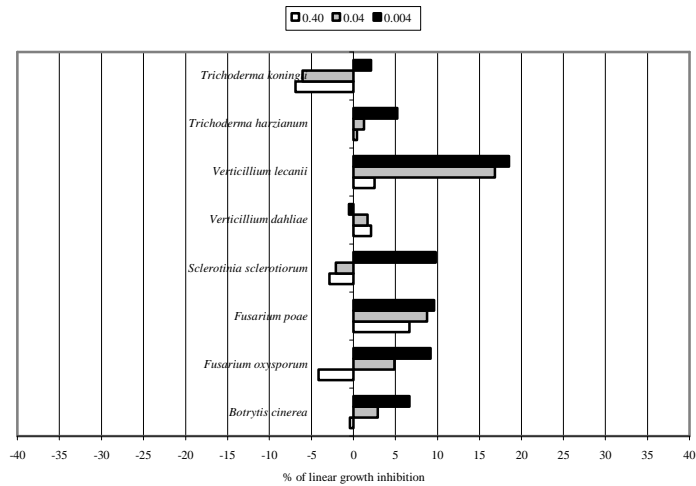
authors demonstrated a fungistatic effect of Kelpak SL on *V. dahliae* causing verticillium wilt of pepper [26]. Therefore, application of this biostimulant in horseradish cultivation may also contribute to reduction of brittle root (*Spiroplasma citri*) occurrence due to *V. dahliae*. In their own investigations the Authors registered significantly strong stimulation of sporulation in antagonistic species (Fig. 3). Especially intensified sporulation was noticed in *T. harzianum*, where almost thrice more spores were noted on the media containing 0.004 and 0.04 mm³·cm⁻³ than on the control. *Trichoderma* fungi play a crucial role in reducing the population of soil fungi, such as: *Rhizoctonia solani*, *Sclerotinia rolfisii*, *Phytophthora palmivora* and some genera of *Fusarium* and *Pythium* [31]. Cosoveanu et al. [8] reported a fungistatic effect of 2% *Ecklonia maxima* algae extract on *F. oxysporum* and *Botrytis cinerea*. In the Authors' own research only the lowest concentrations of Kelpak SL to some degree inhibited growth of these fungi colonies and biomass increment, but simultaneously increased sporulation intensity was observed (Fig. 1-3). In this situation it is difficult to conclude about a fungistatic effect of the tested bioregulator upon these fungi species. Summing up the results obtained for individual features, it may be stated that fungistatic effect of Kelpak SL was clearly visible only for *V. dahliae* and *F. poae* pathogenic fungi, whereas in its lowest concentration in entomopathogenic *V. lecanii*. It is necessary to conduct field experiments to assess the effect of Kelpak SL bioregulator on the healthiness of horseradish roots and soil fungi habitat, because of complicated relationships between many interacting factors existing in agricultural biocenosis.

Table 2. Kelpak SL bioregulator effect on tested features of tested fungi

Tab. 2. Wpływ bioregulatora Kelpak SL na badane cechy grzybów testowych

Concentration of Kelpak SL [mm ³ ·cm ⁻³]	<i>Botrytis cinerea</i>	<i>Fusarium oxysporum</i>	<i>Fusarium poae</i>	<i>Sclerotinia sclerotiorum</i>	<i>Verticillium dahliae</i>	<i>Verticillium lecanii</i>	<i>Trichoderma harzianum</i>	<i>Trichoderma koningii</i>
	Linear growth rate index [T]							
0.40	36.65	56.35	50.00	38.30	46.75	48.20	35.00	55.10
0.04	35.80	51.15	49.83	37.98	46.03	39.95	34.63	54.48
0.004	33.93	48.68	49.90	33.43	45.20	39.18	33.13	49.30
Control	36.20	52.95	54.00	37.18	46.52	48.68	35.13	50.40
NIR _{0,05}	r.n.	3.37	1.83	2.60	r.n.	6.52	r.n.	3.68
Concentration	Biomass [g]							
0.40	0.485	0.378	0.346	0.725	0.344	0.397	0.330	0.349
0.04	0.507	0.309	0.390	0.845	0.352	0.392	0.332	0.348
0.004	0.513	0.295	0.445	0.958	0.362	0.377	0.387	0.357
Control	0.500	0.413	0.374	0.871	0.371	0.401	0.483	0.372
NIR _{0,05}	r.n.	r.n.	r.n.	0.122	r.n.	r.n.	0.149	r.n.
Concentration	The number of spores in 1 cm ³ ·10 ⁷							
0.40	4.9	4.0	2.6	13,1	5.6	9.4	35.7	10.9
0.04	7.7	7.8	2.1	9,7	6.7	20.5	53.3	21.6
0.004	11.9	10.6	1.3	9,1	7.8	18.1	61.2	22.9
Control	4.5	3.9	2.7	9.0	26.2	12.7	15.8	13.5
NIR _{0,05}	0.9	1.1	0.3	1.2	3.2	0.6	3.4	2.7

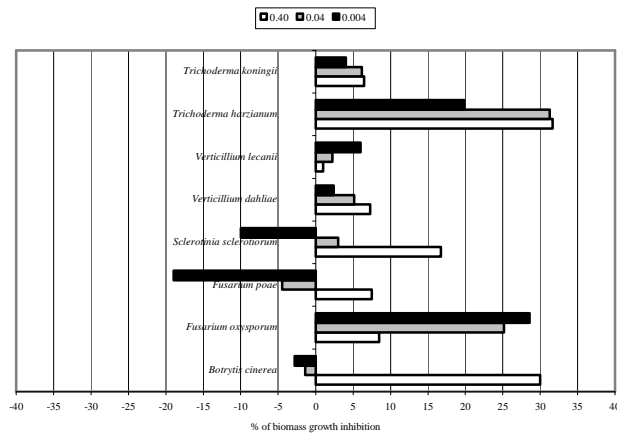
Source: own work / Źródło: opracowanie własne



Negative values denote stimulation of linear growth

Source: own work / Źródło: opracowanie własne

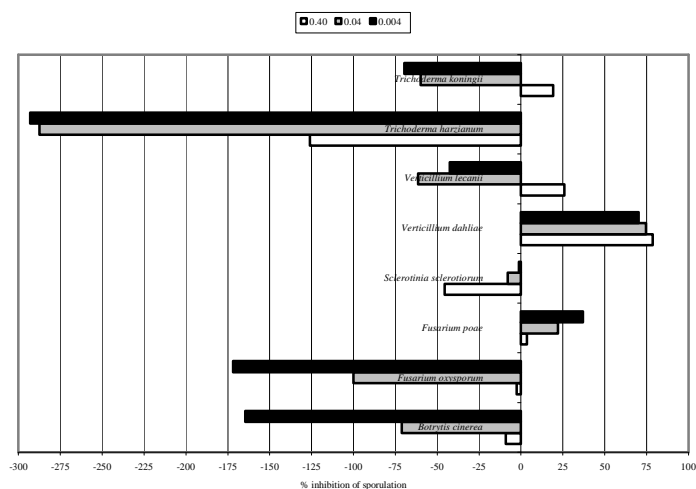
Fig. 1. Influence of Kelpak SL bioregulator on linear growth of tested fungi
Rys. 1. Wpływ bioregulatora Kelpak SL na wzrost liniowy badanych grzybów



Negative values denote stimulation of biomass

Source: own work / Źródło: opracowanie własne

Fig. 2. Influence of Kelpak SL bioregulator on biomass of tested fungi
Rys. 2. Wpływ bioregulatora Kelpak SL na biomasę badanych grzybów



Negative values denote stimulation of sporulation

Source: own work / Źródło: opracowanie własne

Fig. 3. Influence of Kelpak SL bioregulator on sporulation of tested fungi
Rys. 3. Wpływ bioregulatora Kelpak SL na zarodnikowanie badanych grzybów

4. Conclusions

1. The effect of Klepak S1 bioregulator on the colony linear growth, biomass and sporulation of the tested fungi under *in vitro conditions* depended on the fungus species and the concentration of the biostimulant applied to the culturing medium.
2. The most significant inhibition of the colony surface growth, increment of the tested fungi biomass and stimulation of sporulation in antagonistic fungi: *T. harzianum* (293%), *T. koningii* (70%) and in the pathogenic species: *F. oxysporum* (172%) and *B. cinerea* (164%) was observed on the medium containing the lowest - 0.004 concentration of Klepak S1.
3. Irrespectively of the applied concentration, the algae *Ecklonia maxima* extract revealed the strongest fungistatic effect upon *V. dahliae*, demonstrated primarily as a significant inhibition of sporulation process (70.23-78.63%) and to a lesser degree as limited surface growth and biomass increment.

5. References

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