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Antioxidant status of Samarovski Chugas (Jugra) bryophytes and growth promoting activity of their water extracts

ABSTRACT

Relevance. **Despite valuable profile of biologically active compounds, high adaptability and beneficial effects on human health mosses have never been utilized in agriculture as growth stimulators.**

Materials and Methodology. **The present work aimed to evaluate the antioxidant status of 7 Jugra moss species and record the possibility of their water extract utilization for radish seedling growth.**

Results. **The total antioxidant activity (AOA) and polyphenol content (TP) determined via visual titration and Pholin-Chiocalteu methods accordingly were in the range of 14.6-30.4 and 8.7-24.4 mg GAE/g d.w. accordingly with the higher values typical to the Khanty-Mansiysk city (Misne) and lower – to the suburbs (Shapsha) and Uksovski spring compared to the high AOA, TP values for the *Pleurotium schreberi* of the southern Karelia and Vladimir. Direct correlations between AOA and TP for all species investigated (r=0.843) and between AOA and proline (0.936) for mosses gathered in Samarovski Chugas were indicated. Water extracts (0.02%) of five from 7 moss species recorded the ability to stimulate predominantly radish roots growth with the highest growth stimulation ability of *Sphagnum russowi* and *Callieregonella lindbegii extracts* and the lowest of *Pleurozium schreberi.***

KEYWORDS:

mosses, Khanty-Mansiysk, antioxidant status, radish seedlings, growth stimulation

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Антиоксидантный статус бриофитов Самаровского Чугаса (Югра) и ростостимулирующий эффект их водных экстрактов

РЕЗЮМЕ

Актуальность. **Несмотря на значительное содержание биологически активных соединений, высокую адаптационную способность и положительное действие на здоровье человека мхи до сих пор не использовали в растениеводстве в качестве стимулторов роста.**

Материал и методика. **Целью настоящей работы была оценка антиоксидантного статуса 7 видов мхов Югры и установление возможности использования их водных экстрактов в качестве стимуляторов получения проростков редиса.**

Результаты. **Общая антиоксидантная активность (АОА) и содержание полифенолов (ТР) установленные методами визуального титрования и Фолина-Чиокалтеу соответственно составили интервалы 14.6-30.4 и 8.7-24.4 мг-экв ГК/г с.м. с более высокими показателями, характерными для района Мисн
э Ханты-Мансийска и более низкими для пригорода (Шапша) и Уксовского родника, по сравнению с высокими уровнями АОА и ТР *Pleurotium schreberi* Северной Карелии и Владимира. Установлена прямая корреляция между АОА и ТР всех исследованных видов мхов Самаровского Чугаса (r=0,843), а также между уровнями АОА и содержанием пролина (0,936). Водные экстракты (0,02%) пяти видов мхов из 7 предпочтительно стимулировали рост корней редиса с наибольшим положительным эффектом, характерным для *Sphagnum russowi* и *Callieregonella lindbegii* и наименьшим для *Pleurozium schreberi.***

КЛЮЧЕВЫЕ СЛОВА:

мхи, Ханты-Мансийск, антиоксидантный статус, проростки редиса, стимулирование роста

1. Introduction

Non-vascular plants Bryophytes combine more than 24000 species of mosses (Bryopsida), liverworts (Hepaticopsida), and hornworts (Anthocerotopsida) and are characterized by high adaptability and stress resistance [1-3]. Being the major contributors to the forest green carpet they demonstrate high adaptability capable to survive in contrasting conditions from the Arctic to the Antarctic, including forests, lakes, and deserts. Though rarely utilized in agriculture [4] they provide the stability of ecosystems, decrease herbivore attack, provide sufficient water access protection and soil stabilization, and improve nutrient availability for the surrounding plants [5]. Lack of developed cuticles induces their utilization in eco-monitoring due to their high ability to absorb different pollutants including heavy metals and radionuclides [6, 7]. In agriculture bryophytes are valued for their high ability of water retention and nutrient absorption which benefits living plant transport and survival of potted plant species. The presence of terpenes, bibenzyls and bisbibenzyls, flavonoids, bibenzyl derivatives, and other antioxidants determines high adaptability of mosses to severe environmental condition [8] and increases surrounding plant resistance against mollusks [9]. Bryophyte phytohormones (predominantly auxin, cytokinines, and jasmonic acid) participate in defense responses [10] and may provide growth promoting properties to the surrounding plants [11, 12]. Antimicrobial, antifungal, anti-carcinogenic, antipyretic, diuretic, anti-neuroinflammatory, and hemostatic properties of bryophytes make them highly valued in traditional medicine [13, 14]. Thus, bryophytes are used by residents of Africa, America, Europe, Australia, New Zealand, Turkey, Japan, Taiwan, China, Pakistan, and India to treat skin, cancer, hepatic, and cardiovascular diseases, provide wound healing and improve antioxidant status [15, 16]. Their wide spectrum of biologically active compounds includes: terpenoids, polyphenols. flavonoids, lipids, polysaccharides, and alkaloids [14-17].

On the other hand, it should be noted, that growth promoting properties of bryophytes were investigated predominantly in natural ecosystems as a part of interaction with the surrounding plants either positive, neutral, or negative [5, 18-20]. Only one work discusses the utilization of *Bryum argenteum*, *Fissidens dubius*, and *Plagiochasma appendiculatum* extracts as growth stimulators of wheat seedling [21]. In this respect, the effect of moss extracts on growth stimulation of agricultural crops is still poorly investigated, though this question is widely discussed for different vascular plant extracts revealing high prospects of such an approach for improving plant yield, antioxidant status, resistance to abiotic and biotic stresses [22, 23].

Wide distribution of mosses in Arctic, lack of investigations devoted to their biochemical characteristics and investigations of their possible growth promoting effect provided the basis of the present investigation. Thus, the work aimed to evaluate the antioxidant status and growth stimulation effect of water extracts of 7 moss species obtained from the unpolluted area of Jugra on growth and development of radish seedlings.

Material and Methods

Investigation was achieved at the specially protected area Samarovski chugas situated in Khanty-Mansiysk Autonomic Okrug (Jugra; 60°59'21" N; 69°00'44" E) at the interfluve of Ob and Irtysh rivers (Fig. 1). The territory is characterized by prolonged cold winter and short hot summer. Mean annual temperature is between -1…-3°C. Mean annual precipitation level is 440-600 mm.

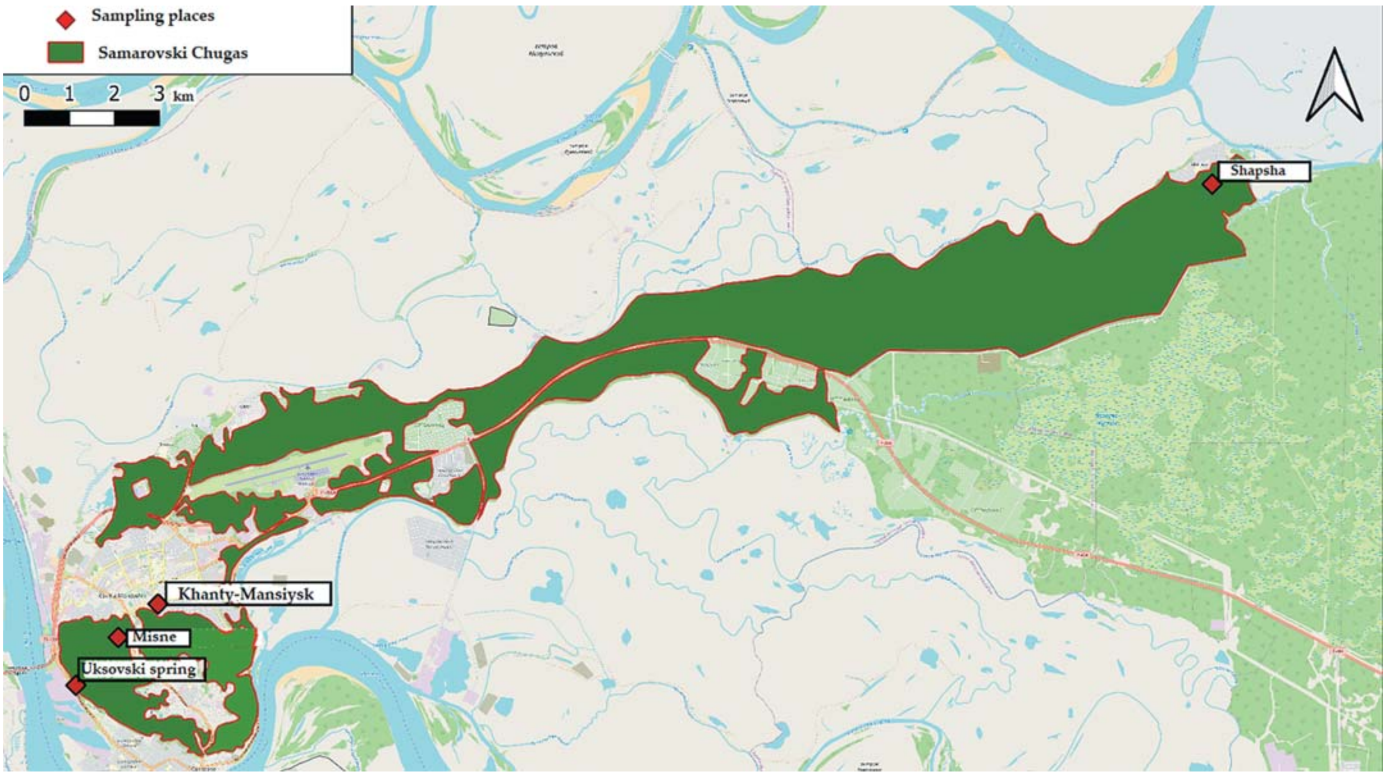

Fig. 1. Sampling places at the territory of Samarovski Chugas

Seven moss species (*Callieregonella lindbegii*; *Polytrichum commune*; *Pleurozium schreberi*; *Dicranum fuscescens*; *Sphagnum russowi*; *Plagiomnium cuspidatum*; Polytrichum juniperinum; and *Hylocomium splendens*) were gathered at the Special protected area of the Jugra (Samarovsky Chugas): Misne, Shapsha, and Uksovski spring (Fig. 1) during June 2025 – the period of optimal plant vitality. To evaluate the effect of habitat on moss properties several moss species (*Hylocomium splendens*; *Sphagnum russowi*; *Polytrichum commune*, and *Pleurozium schreberi*) were also gathered in the Vladimir region Lachuzhskie lakes Nature Reserve (55.946850 N; 38.955697 E) and the Southern part of Karelia, Kilpolna island Ladskoe lake (61.216488 N; 29.932401 E) After separation of soil particles samples were dried at room temperature and authenticated by Dr. Vladimir Fedosov, and cross-referenced with Check-list of mosses of the East Europe and North Asia [24-28]. After transferring moss samples to the laboratory they were homogenized and used for the determination of the total antioxidant activity (AOA), polyphenols (TP), and proline.

Evaluation of the efficiency of moss growth stimulation effect was tested on radish seed germination, cv. Mavr. 0.1 g of moss powder was mixed with 500 ml of distilled water and kept at room temperature with constant agitation during 24 hours. After filtration the extracts were used for radish seed germination. For germination, 30 seeds per replicate were placed on double-layered Whatman No. 1 filter paper in 90 mm petri plates. Each plate received 4 ml of extracts of 7 selected mosses or the same amount of distilled water. The determination of seed germination capacity (SGC) and seed germination energy (SGE) was achieved on the 6th and 3d days accordingly using the formula:

SGC (%) = (Total germinated seed after 6 days):(Total seed sown) × 100

SGE (%) = (Germinated seeds after 3 days):(Total seed sown) × 100

All determinations were performed in triplicate.

After 6 days, the following parameters were assessed:

Shoot Length (measured from the seed base to the shoot apex in mm using a meter rod, averaging three randomly selected seedlings per plate); *Root Length* (Measured from the seed base to the root tip, similarly averaged); *Fresh Weight* (Determined immediately after harvest using a Sartorius electronic balance (precision: 0.001 g), and Dry Weight (Recorded after oven-drying seedlings at 70°C for 48 hours until constant weight).

Antioxidant activity

The antioxidant activity of mosses investigated was assessed using a redox titration method [29] via titration of a 0.01 N $KMnO_4$ solution with the 70 % ethanolic extract of mosses. The reduction of $KMnO_4$ to colorless Mn^{+2} in this process reflects the quantity of antioxidants dissolvable in 70% ethanol. The values were expressed in mg gallic acid equivalents per g of d.w. (mg GAE g^{-1} d.w.).

Polyphenols

Total Polyphenols (TPs)

Total polyphenols were determined in 70% ethanolic extracts of dried moss powder using the Folin-Ciocâlteu colorimetric method as previously described [29]. Half a gram of dry homogenate was extracted with 20 mL of 70% ethanol/water at 80°C for 1 h. The mixture was cooled down and quantitatively transferred to a volumetric flask, and the volume was adjusted to 25 mL. The mixture was filtered through a filter paper, and 1 mL of the resulting solution was transferred to a 25 mL volumetric flask, to which 2.5 mL of saturated Na_2CO_3 solution and 0.25 mL of diluted (1:1) Folin-Ciocâlteu reagent were added. The volume was brought to 25 mL with distilled water. One hour later, the solutions were analyzed through a spectrophotometer (Unico 2804 UV, Suite E Dayton, NJ, USA) and the concentration of polyphenols was calculated according to the absorption of the reaction mixture at 730 nm. As an external standard, 0.02% gallic acid was used. The results were expressed as mg of gallic acid equivalent per g of dry weight (mg GAE g^{-1} d.w.).

Proline

Proline concentration was determined according to Ábrahám et al. [30], with slight modifications. About 50 mg of dry homogenized moss powder were grinded with 10 ml of 3% sulfur salicylic acid in a mortar. The mixture was filtered and 1 ml of the resulting filtrate was incubated with 2 mL of ninhydrin reagent, and 2 ml of acetic acid in tubes at 95°C for 1 hour. After tube cooling, samples were extracted with 3 mL of toluene and the proline concentration was assessed using the absorption value of the toluene extract at 520 nm and a calibration curve with 5 different proline (Merck) concentrations (0-40 μg mL^{-1}).

Statistics

All determinations were achieved in triplicate, and the data were processed by the analysis of variance and mean separations were performed through the Duncan's multiple range test, with reference to 0.05 probability level, using SPSS software version 27 (Armonk, NY, USA).

3. Results and Discussion

3.1. Antioxidant status of mosses

The total antioxidant activity of mosses investigated was in the range of 14.6-35.0 mg GAE g-1 d.w. with mean value of 20 mg GAE g-1 d.w. (Table 1). High interspecies and geographical variations of the parameter hamper the indication of moss antioxidant status peculiarities. The highest AOA was typical for *Polytrichum commune* and *Plurozium schreberi* while TP values predominated only for *Polytrichum commune* (Fig. 2). Contrary, among moss species tested *Plagiomnuim cupsidatum* recorded the lowest antioxidant activity which may be connected with optimal water and nutritional accessibility. On the other hand, taking into account that plant antioxidant status usually increases under environmental stress conditions [31] one may indicate relatively less favorable habitat conditions in Misne situated in the center of Khanty-Mansiysk city compared to the remoted Shapsha settlement and Uksovski spring. Indeed, compared to the *Plagiomnium cupsidatum* data characterized by the lowest AOA value, most of investigated moss species recorded significantly higher AOA values in Misne than in Shapsha (Fig. 2), except *Sphagnum russowi* known to control the hydrologic cycle and decrease plant nutrition due to the sequestration of nutrients from plants via cation exchange, thus leading to the predominance of atmospheric nutrition of the surrounding plants [32].

Table 1. Antioxidant status of Jugra mosses

Species	Sampling place	Proline	AOA	TP
		(mg g⁻¹ d.w.)	(Mg GAE g⁻¹ d.w.)	
Dicranum fuscescens	Shapsha Misne	0.314 ab 0.206 c 0.118 ef	14.2 e 18.9 cd 24.9 b	8.1 c 12.6 b 13.1 b
Callieregonella lindbegii	Misne	0.120 ef 0.099 g	19.8 c 19.0 c	10.5 c 10.4 c
Polytrichum commune	Shapsha Misne	0.105 g 0.140 de	18.7cd 30.4 a	13.6 b 24.4 a
Pleurozium schreberi	Misne Shapsha	0.150 d 0.124 e 0.317 ab	23.4 b 18.2 cd 21.3 b	10.6 c 11.7 bc 10.2 c
Sphagnum .russowi	Shapsha Misne	0.356 a 0.100 f	19.0 cd 14.6 e	9.8 c 9.3 c
Plagiomnium cuspidatum	Uksovski spring	0.122 e	15.1 e	10.3 c
Hylocomium splendens	Misne Shapsha	0.116 b 0.115 f	20.3 bc 17.9 d	8.7 d 13.8 b
Means M ±SD		**0.175±0.087**	**20.0±5.1**	**11.7±4.0**
CV (%)		**50.0**	**25.5**	**34.2**

AOA-total antioxidant activity; TP- total polyphenol content. Values in columns with similar letters do not differ statistically according to Duncan test at p<0.05

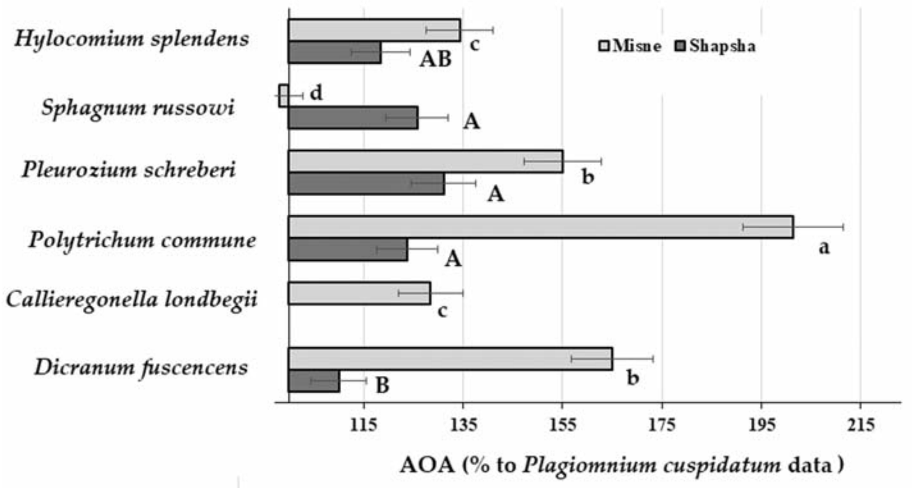

Fig. 2. Total antioxidant activity (AOA) of different moss species grown in Misne and Shapsha areas compared to the Plagiomnium cuspidatum data from the Uksovski spring. Values with similar letters do not differ statistically according to Duncan test at p<0.05

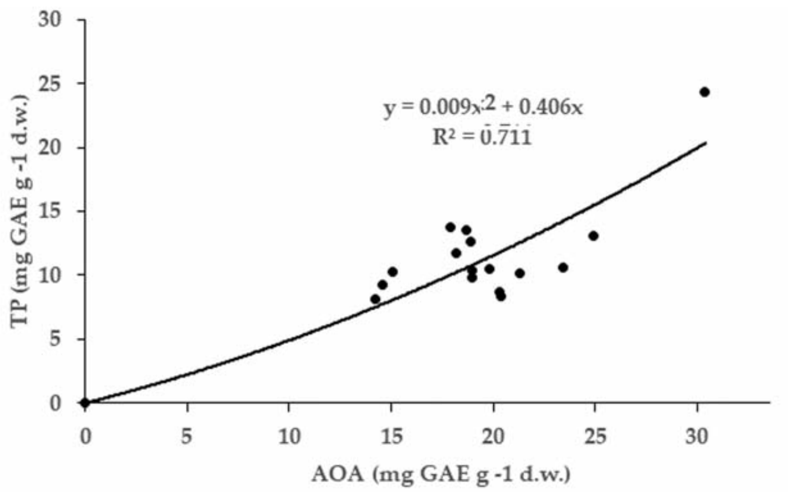

Fig. 3. Relationship between total antioxidant activity and polyphenol content in tested mosses (r=0.843; p<0.001)

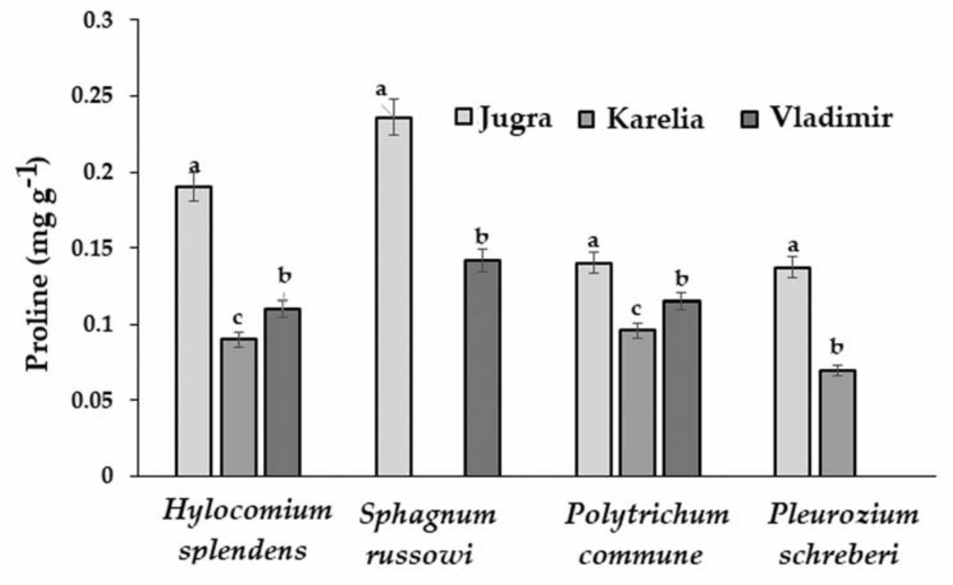

Fig. 4. Mean proline levels in Jugra, Karelia, and Vladimir mosses

Furthermore, despite significant interspecies and location differences in the total antioxidant activity and variations in the content of polyphenols a direct correlation between the latter parameters has been indicated (Fig. 3) which was in accordance with the investigation of Han et al. [33] who has demonstrated that polyphenols greatly contribute the bryophyte antioxidant capacity.

A direct correlation between TP and AOA in mosses confirms the unique antioxidant capacity of plants to withstand the unfavorable environmental conditions via phenolic biosynthesis. This phenomenon has been indicated in plants [34, 35] including tree bark [36], lichens [35], and mushrooms [37].

3.2. Proline accumulation

The proline level in plants under environmental stresses is another good indicator of stress severity [31]. This compound plays a crucial role in plant antioxidant defense, affecting cellular redox potential, and regulating osmolite balance protecting cellular structures and enhancing plant tolerance to various abiotic stresses [38, 39]. Indeed, mean individual values of proline accumulation by Jugra mosses were significantly higher that the appropriate values of mosses collected in milder climate of the Southern Karelia and Vladimir suburbs (Fig. 4).

On the other hand, one may indicate great differences in the proline accumulation not only by different species but similar species from different habitat. Taking into account proline participation in the antioxidant defense of plants one may expect the existence of a direct correlation between proline and AOA values in the investigated mosses. Indeed, such a correlation was confirmed for mosses grown in Misne stress conditions ($r=0.936$; $p<0.001$), but was absent for Shapsha mosses (Fig. 5).

Lack of significant environmental stress in the vicinity of Shapsha settlement decreases the detection possibility of pro-

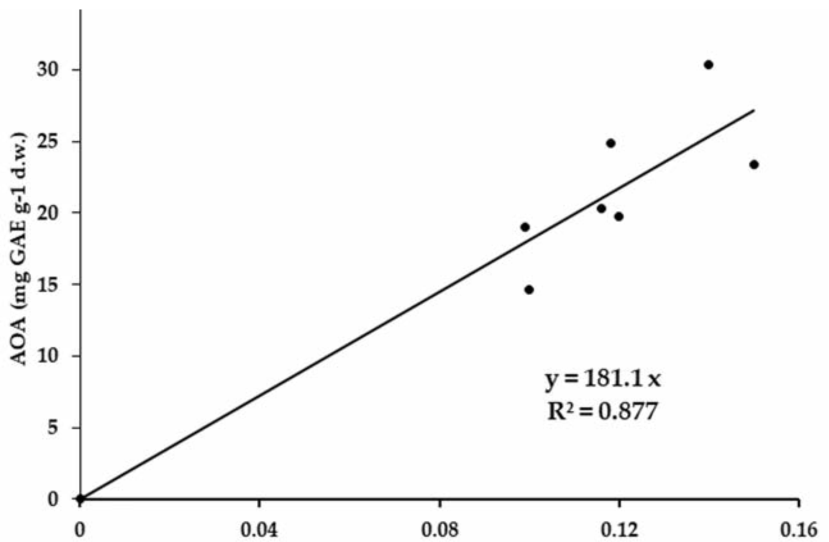

Fig. 5. Relationship between total antioxidant activity and proline content in mosses from Misne area ($r=+0.936$; $p<0.001$)

line-AOA interaction, while extremely high differences in the proline accumulation may be connected with the different stages of moss development, water regime, and differences in the growth rate [40].

3.3. Effect of Moss Water Extract on Radish Seed Growth and Development

In natural conditions the effect of mosses on plant seed growth and development is species specific and may either stimulate, inhibit seed germination and growth, or remain inactive [32]. Artificial conditions of the biologically active compound low concentrations in water extract, supposedly, may promote seed growth due to the presence of phytohormones with the predominance of jasmonic acid, auxins, and cytokinins [10]. Plant physiology data indicate root growth stimulation by auxins and shoot growth stimulation by cytokinins. Contrary, jasmonic acid may inhibit seed germination while at later stage of development it may induce increased growth [41].

The results indicate, that 5 moss species from 7 stimulate predominantly root growth with the highest beneficial effect typical for *Sphagnus russowi* and *Collieregonella lindbegii* (Table 2; Fig. 6). Interesting, while *sphagnum* stimulates exclusively root development, *C.lindbegii* promotes the growth of both roots and shoots which supposes the beneficial effect of auxin in the former case, and cytokins in the latter.

Table 2. Effect of moss extracts on radish seedling mass and length

Species	*Sampling place*	*Mass of 100 seedling (mg)*	*Root length (mm)*	*Shoot length* (mm)
Control (dist. water)		193±12 d	53.0±1.2 e	31.0±0.4 c
Dicranum fiscescens	Shapsha Misne	162±10 e 206±12 cd	50.0±1.2 f 53.5±1.2 e	36.0±0.5 a 34.0±0,5 b
Callieregonella lindbegii	Misne	221±11 bc	66.0±2.3 bc	35.0±0.5 a
Polytrichum commune	Shapsha Misne	179±11 e 259±13 a	51.0±1.2 ef 77.0±3.4 a	35.0±0.5 a 32±0.4 c
Pleuroziu schreberi	Shapsha	181±12 de	52.0±1.2 e	33.0±0.5 bc
Sphagnum russowi	Shapsha	231±12 bc	70.5±3.4 b	31.3±0.4 c
Plagiomnium cuspidatum	Uksovski spring	242±13 ab	63.0±2.3 cd	31.3±0.4 c
Hylocomium splendens	Shapsha	228±12 bc	61.0±2.3 d	32±0.4 c

Values in columns with similar letters do not differ significantly according to Duncan test at $p<0.05$

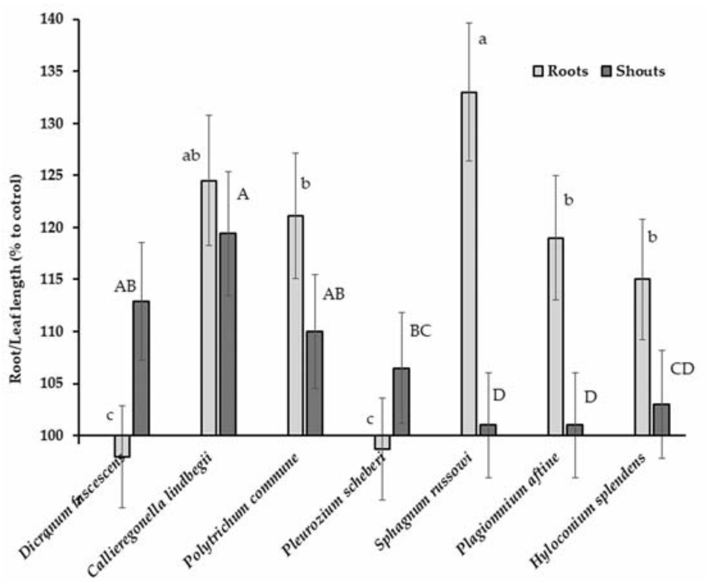

Fig. 6. Effect of moss water extracts on radish seedling length

It should be noted that the obtained results are not applicable to the natural environmental conditions, where significantly higher concentrations of moss soluble compounds in water may cause growth inhibition due to the development of the competition between the species [42].

Conclusions

Antioxidant activity of Jugra mosses varied from 14.2 to 30 mg GAE g^{-1} d.w. depending on genetic peculiarities and place of habitat with the highest values typical for *Pleurozium.schrebery* and *Polytrichum commune*. Antioxidant activity and proline content parameters confirm the existence of elevated stress conditions for moss grown in Misne contrary to Shapsha settlement. Most of moss species provided root growth stimulation effect with the exception of *Dicranum fiscescens* and *Pleurozium schreberi*. *Callieregonella lindbegii* was the only one capable to provide growth stimulation of both roots and shoots. The present results provide the first evaluation of Jugra moss antioxidant status and confirm prospects of diluted 0.02 % water extracts of several moss species as growth stimulators indicating species providing only radish root growth stimulation (*Sphagnum russowi*; *Plagiomnium aftine*, and *Hylocomium splendens*), exclusively shout growth (*Dicranum fiscescens*), and both root and shoot growth stimulation (*Callieregonella lindbegii* and *Polytrichum commune*) of radish seedlings along with negligible beneficial effect of *Pleurozium schreberi*.

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