Cultivation of energy crops by ecological methods under the conditions of global climate and environmental changes with the use of diatom extract as a natural source of chemical compounds

K. Piotrowski¹ · Z. Romanowska-Duda¹ · B. Messyas²

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Abstract

Modern agriculture must be subject to some adaptation processes due to unpredictable climate changes. One of the activities that enables the production of plant biomass in adverse climatic conditions is the development of ecological and innovative crop technologies using natural plant extracts. The elimination of synthetic fertilizers and their replacement with products based on organic matter will increase the plant's resistance to negative conditions of environmental stress, will have a positive effect on the development and yielding of plants and will reduce cultivation costs. The plant material: willow (*Salix viminalis*), Jerusalem artichoke (*Helianthus tuberosus*) and Virginia mallow (*Sida hermaphrodita*). The natural extract was obtained from *Navicula* sp. (Bacillariophyceae) monocultures, which was applied to plants in three variants: watering, spraying (foliar application) and watering and spraying. Every 2 weeks: plant height and chlorophyll content index were determined and at the end of the growing season, an analysis was made of: fresh and dry biomass, gas exchange activity in plants (net photosynthesis, transpiration, stomatal conductivity and intercellular CO₂ concentration). The enzymatic activity of acid (pH = 6.0) and alkaline (pH = 7.5) phosphatase, RNase, dehydrogenases, as well as the integrity of cytoplasmic membranes was determined. The obtained results confirmed the positive effect of diatom monoculture extract on the growth and development of the plants. The analyzed physicochemical parameters were characterized by 15–20% higher values in comparison with the control. The use of natural extracts from *Navicula* sp. can be an alternative to chemical fertilizers and is considered one of the promising strategies in organic agriculture.

Keywords *Salix viminalis* · *Helianthus tuberosus* · *Sida hermaphrodita* · Bacillariophyceae · Biostimulators · Biofertilizer

Introduction

Limiting the use of chemical fertilizers in agriculture due to their negative impact on the environment and human health is one of the priority assumptions of modern, sustainable

European policy. The latest regulation of the European Parliament from June 2019 radically changes the approach in the use and production of artificial fertilizers in the European Union. Thanks to the new regulations, only fertilizers that meet stringent EU-wide quality and safety requirements will be allowed to enter the EU market. Current contaminants in EU phosphate fertilizer products (heavy metals) may pose a threat to human and animal health, as well as all levels of the ecosystem. A modern and sustainable approach in organic farming must be based on natural organic substances (biostimulators) (Grzesik et al. 2015). The conducted research and numerous literature data indicate that bio-fertilization may become an alternative to dangerous synthetic fertilizers and pesticides. Their main advantage is primarily ecological and environmentally friendly origin and a positive impact on the growth, development and yielding of cultivated plants. Silicon is one

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✉ Z. Romanowska-Duda
zdzielawa.romanowska@biol.uni.lodz.pl

¹ Department of Plant Ecophysiology, Faculty of Biology and Environmental Protection, University of Lodz, Banacha Str. 12/16, 92-237 Lodz, Poland

² Department of Hydrobiology, Faculty of Biology, Institute of Environmental Biology, Adam Mickiewicz University in Poznan, Str. Umultowska 89, 61-614 Poznań, Poland

of the most common elements in the earth's crust. However, due to its specific chemical properties, it occurs in a form most often inaccessible to plants, often as silica in the form of quartz — a substance insoluble in water. The form available for plants is silicic acid which is very unstable and easily turns back into forms inaccessible to plants (Abro et al. 2009; Sommer et al. 2006). Research to date has shown that silicon fertilization increases plant resistance to diseases and pests, water shortage, light and salt stress. The use of silicon also mitigates the effects of phytotoxic excess of some ions in the root environment, such as sodium, manganese, aluminum and heavy metals. Despite the undoubted benefits of using silicon in plant development, the use of this element in crops is still underestimated, and one of the reasons is the lack of a sufficiently stable fertilizer product (Meena et al. 2014; Farooq and Dietz 2015). Technological advancement, producer awareness and increasingly restrictive international regulations have given the chance to use natural organisms including diatoms as biostimulants and a natural source of silicon. The use of specific and unique properties of diatoms in modern and sustainable economy is highly prospective and creates many opportunities, including as a base for the production of new generation fertilizers. These organisms are diverse and cover over 100,000 species. These organisms live as single cells or form colonies that resemble several-centimeter threads, starfish or bushes (Witkowski et al. 2006; Prygiel et al. 1993; Round et al. 1992; Simental and Sanchez-Saavedra 2003). Their cultivation is relatively simple, does not generate high costs and they are a source of many bioactive chemical compounds widely used in the pharmaceutical industry to produce antiviral and antibacterial drugs (Fimbres-Olivarria et al. 2018). It is an interesting and positive fact that the biochemical composition of diatoms is variable and depends on the properties of the environment in which they live. The presence of red light during microalgae growth stimulated the synthesis of carbohydrates, and the exposure of blue light significantly increased the protein content (Jungandreas et al. 2014). High content of antioxidant compounds in diatoms is successfully used in the cosmetics and nutraceutical industry (Karthikeyan et al. 2013; Abd El Baky et al. 2013). High content of silicon, nitrogen, phosphorus and biologically active compounds allows the use of these organisms as a base for innovative preparations for growing plants. Diatoms are unicellular organisms with the ability to form colonies, they occur in freshwater and marine environments, as well as on rocks, in soil and bark of trees — wherever there is moisture and light (Grzesik et al. 2015; Descy et al. 1991; Kwadrans et al. 1998). They inhabit not only clean waters, but they can also be found in highly polluted places, such as sewers, where no other organisms can survive (Broda et al. 2015). Being highly sensitivity to the content of nutrients and availability of light, diatoms are used as an indicator of the eutrophication process (Noga et al. 2013). High content of heavy metals in water makes diatom cells deformed and much

smaller than under conditions favorable to their development (Peszek et al. 2014). Characteristic structure and composition of a cell wall distinguish these algae from other organisms. Hydrated silica ($\text{SiO}_2 \times n\text{H}_2\text{O}$) and diatopetines — an acid polysaccharide are — their main components. (Round et al. 1992). The specific properties of diatom structure, such as the ability to form silica armor and to produce mucus rich in mannose, fucose and galactose can be used in the process of applying fertilizers from diatoms, and thus can have a positive effect on biomass and crop yielding (Round 1990). Due to the high content of fats and proteins, they form the base of the food chain in oceans, seas and inland waters. They are thought to be responsible for a quarter of global oxygen production. They are capable of phytoremediation — absorption of heavy metal ions (lead, zinc, nickel, cadmium, titanium) (Rakowska 2003). Despite the lower content of nitrogen and phosphorus in diatoms compared to synthetic fertilizers, these elements are better absorbed by plants. In addition, diatomaceous bacteria are present on the surface of diatom cells, which can be directly absorbed by plants or stored in soil through denitrification processes (Allen et al. 2011). The use of diatoms as new generation fertilizers can stimulate growth and development of cultivated plants as well as their resistance to pathogens and stressors, such as sudden climate changes. Research to date and literature data have confirmed the positive effect of other microorganisms (*Cyanobacteria*) on crop growth and the ecosystem (Piotrowski et al. 2016; Grzesik and Romanowska-Duda 2014). Diatoms, including *Navicula* sp., can play a key role in improving soil quality and biological life and in stimulating plant and microorganism growth. The aim of the study was (1) analysis of the growth and metabolic activity of energy plants (*Salix viminalis*, *Helianthus tuberosus*, *Sida hermaphrodita*) grown under the conditions simulating climate change and treated with an extract obtained from diatoms (2) assessment of the usefulness of diatoms as a source of natural silicon in agro. The positive effect of diatoms on the growth, development and metabolic activity of energy plants has been demonstrated. The possibility of using diatoms in the production of new generation fertilizers which are based on natural and ecological ingredients of organic origin was pointed out. The development and application of diatom preparations can significantly reduce or completely eliminate synthetic fertilizers and pesticides that are dangerous to humans and ecosystems.

Materials and methods

Selection of plant material

The research was carried out using three popular plant species: willow (*Salix viminalis*), Jerusalem artichoke (*Helianthus tuberosus*) and Virginia mallow (*Sida hermaphrodita*). The choice of plant material was dictated by their specific

properties: rapid growth, low cultivation costs, occurrence in various climatic zones, high calorific value. The course of the experiment strictly depended on the growing season of the selected plant species. The experiment was carried out in three variants: Variant I—vegetation room (controlled conditions: soil moisture, temperature, lighting); Variant II—greenhouse (controlled soil humidity, temperature dependent on weather), soil drought alternating every 3 weeks (20% soil moisture) and excessive soil moisture (60%), the soil moisture level was monitored using a soil moisture measurement ThetaProbe ML3, Delta-T; Variant III—field (weather-dependent conditions), soil moisture, temperature, weather dependent on sun exposure, time of day, season of the year. In variant I, the experimental plants were subjected to variable temperatures that may occur in the conditions of a changing climate. The simulation of temperature changes is presented in Table 1. 20/0/20 °C—plants grown at 20 °C were lowered three times every 14 days to 0 °C for 24 h; 20/40/20 °C—plants grown at 20 °C were raised three times to 40 °C for 24 h every 14 days; 20/0/40/20 °C—plants grown at 20 °C were lowered to 0 °C twice for 24 h after 14 and 42 days of cultivation and one temperature increase to 40 °C for 24 h on the 28th day of the experiment; 10/−5/20/40/20 °C—the plants for the first 14 days of the experiment were grown at 10 °C, then the temperature was lowered to −5 °C for 24 h, for the next 42 days, the plants were grown at 20 °C, meanwhile, the 28th day the temperature was raised to 40 °C for 24 h.

Plants in all variants (I–III) were grown in 3-L pots filled with a mixture of sand and peat (1:1 = v:v). The first applications of diatomaceous fertilizer were made at a plant height of 15 cm. The experiment was carried out in

five replicates for each treatment. Each lot consisted of 30 plants of each species, arranged by random blocks. The obtained means from each parameter were grouped using the Duncan test at significance level $\alpha = 0.05$.

Diatom cultivation and preparation for plant treatment

During the growing season, all plants were treated with *Navicula* sp. monoculture extract. The biopreparation was applied every 3 weeks in three ways: by direct watering, spraying (fertilizer in the form of an aerosol), and watering and spraying. Monoculture *Navicula* sp. came from the KER collection, Department BiOŚ, UŁ, cultured in a phytotron room on BG11 medium (ATCC Medium 616) rich in micro- and macroelements at 27 °C with Farel lamps (18 W), according to the procedure developed by Romanowska-Duda et al. (2010). The number of *Navicula* sp. cells was determined using a Fuchs and Rosenthal hemocytometer. In the monocultures used for the experiments, the number of cells in 1 mL of water was 600,000. Then, the diatom cells were subjected to a 2-min centrifugation (4000 rpm) and suspended in water. Before starting the application of the monoculture, it was subjected to a sonication process of 15 min (ultrasound frequency 20 kHz, with an amplitude of 80% and impulses of 0.7 s at intervals of 0.3 s) using an ultrasonic homogenizer (Omni-Ruptor 4000, Omni International). Depending on the experimental variant, each plant was sprayed with 20 ml, 20 ml watered or 40 ml *Navicula* sp. monoculture mixture sprayed and watered.

Table 1 List of variants according to which the experiment was conducted

<i>Salix viminalis</i>	<i>Helianthus tuberosus</i>	<i>Sida hermaphrodita</i>
Variant I—vegetation room (controlled conditions: soil moisture, temperature, sunlight—controlled)		
Variable temperature 20/0/20 °C; 20/40/20 °C 20/0/40/20 °C; 10/−5/20/40/20 °C		
Diatom monoculture application in the form of watering	Diatom monoculture application in the form of spraying	Diatom monoculture application in the form of watering and spraying
Variant II—greenhouse (soil humidity controlled, temperature dependent on weather), applied every 3 weeks, alternately soil drought (20% soil moisture) and excessive soil moisture (60%)		
Temp. 20 °C, Variable soil moisture 20–60%, every 3 weeks		
Diatom monoculture application in the form of watering	Diatom monoculture application in the form of spraying	Diatom monoculture application in the form of watering and spraying
Variant III—field (weather-dependent conditions), soil moisture, temperature, weather-dependent sun exposure, time of day, time of year		
Temperature and humidity vary		
Diatom monoculture application in the form of watering	Diatom monoculture application in the form of spraying	Diatom monoculture application in the form of watering and spraying

Research methodology

The assessment of the usefulness and effectiveness of the *Navicula* sp. monoculture mixture as a bio fertilizer were determined on the basis of biometric measurements of plants. Every 4 weeks, plant growth and development were determined, and the chlorophyll content index of plant leaves was determined using a Minolta SPAD-502 apparatus (Konica Minolta) (Górnik et al. 2002). The measurement was made by closing the measuring head on a leaf. Since the leaf is not cut off or damaged in any other way, the same leaf was measured during the growth period of the plant. At the end of the growing season, fresh and dry biomass as well as gas exchange activity in plants (net photosynthesis, transpiration, stomatal conductivity and intercellular CO₂ concentration) using the TPS-2-PP Systems analyzer, USA were assessed. Measurement of gas exchange parameters was carried out in laboratory conditions at a constant temperature of 24 °C and constant lighting 2 × 18 W/840. Gas exchange parameters have been marked in accordance with the manufacturer instruction: “using infrared gas analysis techniques, where CO₂ concentration was determined with an accuracy of 1 ppm. The TPS-2 passes a measured flow of air over a leaf sealed into a chamber called the “leaf cuvette”. Using a “gas switching” technique, the TPS-2 first samples the CO₂ and H₂O of the air going to the cuvette (reference) and then the air leaving the cuvette (analysis). From the flow rate and the change in concentrations, the assimilation rate of CO₂ and transpiration rate of water can be determined. This technique is commonly referred to as the “open” system method of measurement and is the method employed by the TPS-2. Designed for use under the most demanding field conditions (high temperature and humidity, dust), the system is equipped with an internal, hydrophobic filtering system ensuring that all internal parts are fully protected. The TPS-2 is a high-precision instrument. It features a single, non-dispersive infrared gas analyzer for accurate measurement of CO₂. The gas analyzer includes an infrared source, a gold-plated and highly polished sample cell and detector. The analyzer acts as an absorptiometer measuring only in the 4.26 μm waveband ensuring accurate, rapid and stable CO₂ results. The optical bench is thermostatted and completely insulated to ensure accurate measurements under changing ambient temperatures. The enzymatic activity of acid (pH = 6.0) and alkaline (pH = 7.5) phosphatases (Knypl and Kabzińska 1977), RNase (Knypl and Kabzińska 1977) (using the UVmini-1240 Shimadzu spectrophotometer) were measured. Phosphatase 8 mM *p*-nitrophenylphosphate in glycine-maleate-Tris-citrate buffer was used as a substrate for phosphatase assay at pH 6.0 and 7.5 (as measured in a whole assay mixture at 30 °C) The assay mixture contained 0.1, 0.2 and 0.5 ml eluate in a final volume of 1.5 ml. Reaction was allowed to proceed for 20 min at 30 °C and stopped

by adding 1.0 ml of 0.3 N NaOH. Absorbance at 404 nm was read and corrected for blanks into which NaOH solution was added before the eluate. The phosphatase activity is expressed as $\Delta A_{404} \text{ nm (0.5 ml eluate)}^{-1}$ per an equivalent of fresh weight of tissue, or per such a volume of nutrient medium which was equivalent to 1 g of floating plants at the very time of analyses. Phosphatase activity in crude nutrient media is expressed as $\Delta A_{404} \text{ nm (0.2 ml eluate)}^{-1}$. RNase 0.1 ml of highly polymerized RNA was mixed with 0.1 ml of 0.1 M citrate buffer (pH 6.0), and 0.1 ml of eluate. Reaction was allowed to proceed for 60 min at 30 °C, then stopped by adding 3.0 ml of magnesium–lanthanum precipitating reagent (Ambellan and Hollander 1966). Absorbance at 260 nm was read and values of A 260 nm corrected for 11-fold dilution with the precipitating reagent and for to blanks into which enzyme was added after the precipitating reagent. RNase activity is expressed as $\Delta A_{260} \text{ nm (0.1 ml eluate)}^{-1}$ per equivalent of g fresh weight. The integrity of cytoplasmic membranes was determined using a conductivity meter (CC-551 Elmetron) (Górnik et al. 2002).

Results

The obtained test results confirmed the positive effect of the *Navicula* sp. monoculture mixture on the growth and development of energy plants of all three species. The substances used accelerated growth, which was expressed by greater height, they improved quality of plants and biomass yield as well as their metabolic activity.

The conducted experiment showed that treating plants with the mixture of diatom monocultures positively affected the growth of energy plants (Figs. 1, 2, 3). The use of the biostimulator increased the plant height compared to the control sample by 12–25%. In the variant carried out in the phytotron room, all three plants were characterized by better growth and higher resistance to adverse temperature conditions. It was shown that the most optimal form of application of the tested biostimulator in the form of diatom extract (15% higher) was simultaneous watering and spraying of plants.

A positive effect of the *Navicula* sp. monoculture extract was also observed in the greenhouse plant cultivation variants (with variable weather-dependent temperature and variable soil humidity 20–60% (simulation of drought and flooding) (Fig. 2) and in completely weather-dependent conditions—in field (3). Analyzing the dynamics of plant growth, it was indicated that simultaneous watering and foliar application (spraying) with the sonicated diatom extract accelerated the growth and development of the tested energy plant species compared to the control series (15% more favorable growth), a slightly weaker effect was obtained in the other variants, i.e., watering alone (increase

Fig. 1 Growth dynamics of plants (I) Virginia mallow (*Sida hermaphrodita*), (II) willow (*Salix viminalis*), (III) Jerusalem artichoke (*Helianthus tuberosus*) grown in a phytotron room with variable temperature and constant soil humidity. Diatom monoculture extract applied in the form of watering and spraying every 3 weeks. Variable temperature: 20/0/20 °C—plants grown at 20 °C were lowered three times every 14 days to 0 °C for 24 h; 20/40/20 °C—plants grown at 20 °C were raised three times to 40 °C for 24 h every

14 days; 20/0/40/20 °C—plants grown at 20 °C were lowered to 0 °C twice for 24 h after 14 and 42 days of cultivation and one temperature increase to 40 °C for 24 h on the 28th day of the experiment; 10/-5/20/40/20 °C—the plants for the first 14 days of the experiment were grown at 10 °C, then the temperature was lowered to -5 °C for 24 h, for the next 42 days, the plants were grown at 20 °C, meanwhile, the 28th day the temperature was raised to 40 °C for 24 h

Fig. 2 Growth dynamics of plants (I) Virginia mallow (*Sida hermaphrodita*), (II) willow (*Salix viminalis*) and (III) Jerusalem artichoke (*Helianthus tuberosus*) grown in a greenhouse with vari-

able temperature (depending on weather) and variable soil moisture 20–60% (drought and flooding). A mixture of diatom monocultures applied in the form of watering and spraying every 3 weeks

by 5%), foliar application (increase by 7%). It was also found that the plants treated with the diatom mixture were characterized by higher resistance to adverse environmental conditions, showed much less sensitivity to stress factors (every 3 weeks drought alternating with flooding).

A beneficial effect of the extract based on diatoms was also observed during the analysis of the physicochemical parameter—chlorophyll content index (CCI). Plants grown in different temperature conditions compared to the control series were characterized by a higher content of chlorophyll

Fig. 3 Growth dynamics of plants (I) Virginia mallow (*Sida hermaphrodita*), (II) willow (*Salix viminalis*) and (III) Jerusalem artichoke (*Helianthus tuberosus*) grown in field conditions with variable

temperature and variable soil moisture. A mixture of diatom monocultures applied in the form of watering and spraying every 3 weeks

in the leaves (Fig. 4). Similarly to the analysis of growth dynamics, the CCI index was the highest in the series of plants treated with the mixture by both watering and spraying (Figs. 5, 6). The plants were characterized by a faster and more decisive response to stressors—sudden and

extreme temperature changes as well as extreme drought and flooding.

The consequence of the beneficial effect of the *Navicula* sp. monoculture extract on the tested plant species was the increase in the integrity of cytoplasmic membranes, which

Fig. 4 Index of chlorophyll content in the leaves of (I) Virginia mallow (*Sida hermaphrodita*), (II) willow (*Salix viminalis*) and (III) Jerusalem artichoke (*Helianthus tuberosus*) grown in a phytotron room with variable temperature and constant soil humidity. A mixture of diatom monocultures applied in the form of watering and spraying every 3 weeks. Variable temperature: 20/0/20 °C—plants grown at 20 °C were lowered three times every 14 days to 0 °C for 24 h; 20/40/20 °C—plants grown at 20 °C were raised three times to 40 °C

for 24 h every 14 days; 20/0/40/20 °C—plants grown at 20 °C were lowered to 0 °C twice for 24 h after 14 and 42 days of cultivation and one temperature increase to 40 °C for 24 h on the 28th day of the experiment; 10/-5/20/40/20 °C—the plants for the first 14 days of the experiment were grown at 10 °C, then the temperature was lowered to -5 °C for 24 h, for the next 42 days, the plants were grown at 20 °C, meanwhile, the 28th day the temperature was raised to 40 °C for 24 h

was demonstrated by the outflow of smaller amounts of electrolytes from tissues. Unfavorable conditions for plant development, such as high and low temperatures, significantly increase the permeability of cell membranes. Measurements in the experimental variant carried out in variable temperature in the phytotron room indicated a beneficial effect of diatom extract on plant tissues (Fig. 7). Compared with the control series, plants grown under high-temperature conditions had a lower degree of

cytoplasmic membrane integrity, which was demonstrated by electrolyte leakage both after 2 h and 4 h. The same relationship was confirmed in the variants grown in the greenhouse (Fig. 8) and in the field (Fig. 9). All three plant species tested, Virginia mallow, willow and Jerusalem artichoke were characterized by lower (by 10%) leakage of electrolytes from tissues compared to the control sample, which proves the increased integrity of cytoplasmic membranes.

Fig. 5 Index of chlorophyll content in the leaves of (I) Virginia mallow (*Sida hermaphrodita*), (II) willow (*Salix viminalis*) and (III) Jerusalem artichoke (*Helianthus tuberosus*) grown in a greenhouse with a

variable temperature (depending on weather) and variable soil moisture 20–60% (drought and flooding). A mixture of diatom monocultures applied in the form of watering and spraying every 3 weeks

Fig. 6 Index of chlorophyll content in the leaves of (I) Virginia mallow (*Sida hermaphrodita*), (II) willow (*Salix viminalis*) and (III) Jerusalem artichoke (*Helianthus tuberosus*) grown in field conditions

with variable temperature and variable soil moisture. A mixture of diatom monocultures applied in the form of watering and spraying every 3 weeks

Fig. 7 Electrolyte leakage from the leaves of (I) Virginia mallow (*Sida hermaphrodita*), (II) willow (*Salix viminalis*) and (III) Jerusalem artichoke (*Helianthus tuberosus*) grown in a phytotron room with variable temperature and constant soil humidity. A mixture of diatom monocultures applied in the form of watering and spraying every 3 weeks. Variable temperature: 20/0/20 °C—plants grown at 20 °C were lowered three times every 14 days to 0 °C for 24 h; 20/40/20 °C—plants grown at 20 °C were raised three times to 40 °C

for 24 h every 14 days; 20/0/40/20 °C—plants grown at 20 °C were lowered to 0 °C twice for 24 h after 14 and 42 days of cultivation and one temperature increase to 40 °C for 24 h on the 28th day of the experiment; 10/-5/20/40/20 °C—the plants for the first 14 days of the experiment were grown at 10 °C, then the temperature was lowered to -5 °C for 24 h, for the next 42 days the plants were grown at 20 °C, in meanwhile the 28th day the temperature was raised to 40 °C for 24 h

Increased plant development was also the consequence of the increased activity of enzymes responsible for the regulation of phosphorus management—acid (APs) and alkaline phosphatases (ALP), as well as RNase (enzymes responsible for breaking down phosphodiester bonds

in ribonucleic acids into mono- and oligonucleotides) (Table 2). The results of the analyzes indicated that in the plants treated with the diatom extract biochemical activity was by 25% higher compared to the control series.

Fig. 8 Electrolyte leakage from the leaves of (I) Virginia mallow (*Sida hermaphrodita*), (II) willow (*Salix viminalis*) and (III) Jerusalem artichoke (*Helianthus tuberosus*) grown in a greenhouse with

variable temperature (depending on weather) and variable soil moisture 20–60% (drought and flooding). A mixture of diatom monocultures applied in the form of watering and spraying every 3 weeks

Fig. 9 Electrolyte leakage from the leaves of (I) Virginia mallow (*Sida hermaphrodita*), (II) willow (*Salix viminalis*) and (III) Jerusalem artichoke (*Helianthus tuberosus*) grown in field conditions with

variable temperature and variable soil moisture. A mixture of diatom monocultures applied in the form of watering and spraying every 3 weeks

Table 2 Physiological activity in the leaves of Virginia mallow (*Sida hermaphrodita*), willow (*Salix viminalis*) and Jerusalem artichoke (*Helianthus tuberosus*) grown in conditions of changing soil tempera-

ture and humidity with every 3 weeks of application with a mixture of diatom monocultures

Experimental variant	APs (pH 6.0), U/g f.w	ALP (pH 7.5), U/g f.w	RNase mU/g f.w	APs (pH 6.0), U/g f.w	ALP (pH 7.5), U/g f.w	RNase mU/g f.w	APs (pH 6.0), U/g f.w	ALP (pH 7.5), U/g f.w	RNase mU/g f.w
Variable temperature, 30% soil moisture									
	<i>Sida hermaphrodita</i>			<i>Salix viminalis</i>			<i>Helianthus tuberosus</i>		
Control	0.5cd	0.15b	3.1c	0.5cd	0.15b	3.0c	0.6dc	0.2d	4.1c
20/0/20 °C	0.6bc	0.2b	2.2b	0.65b	0.2a	3.2b	0.5ba	0.3b	3.2a
20/40/20 °C	1.3h	0.3e	5.3e	0.7h	0.5e	5.2e	1.6de	0.5e	6.3d
20/0/40/20 °C	0.7de	0.3b	3.2c	1.2de	0.3b	3.8c	0.9bd	0.45d	4.2b
10/-5/20/40/20 °C	0.2a	0.05a	0.7a	0.1a	0.05a	0.04a	0.15a	0.05a	0.8a
Variable temperature, 20–60% soil moisture									
Control	0.6bc	0.2b	1.9c	0.9cd	0.5c	5.3d	0.7cd	0.3b	5.1c
Diatoms watering	0.5ef	0.2c	2.2c	0.8b	0.6a	5.8b	1.3b	0.4c	4.7c
Diatoms spraying	0.8ab	0.25b	3.2f	0.8ef	0.3c	4.8d	2.1ef	0.3e	4.7f
Diatoms watering and spraying	1.0a	0.4a	3.2g	1.2dc	1.2c	5.1c	2.2a	1.2b	5.6c
Field—weather-dependent conditions									
Control	0.5cd	0.25c	3.0d	1.3bc	1.3b	5.2c	0.9bc	0.4b	4.3c
Diatoms watering	0.8b	0.3f	3.1c	0.9ef	1.5e	4.9f	1.4ef	0.6d	5.1d
Diatoms spraying	0.8c	0.2bc	3.9b	1.4ef	1.4e	4.3f	1.1ed	1.05e	5.5d
Diatoms watering and spraying	0.75ef	0.4fg	4.1f	1.3f	0.9c	5.8c	1.2 g	0.9c	6.05c
LSD _{0.05}	0.9	0.09	0.9	0.1	0.1	0.9	0.2	0.1	0.9

^aMeans marked with the same letters within individual columns do not differ significantly according to Duncan's test at significance level $p=0.05$

The applied extract from *Navicula* sp. monocultures had a positive effect on the gas exchange process in the tissues of the tested plant species (Tables 3, 4 and 5). Analysis of individual parameters using modern equipment (TPS-2-PP Systems analyzer, USA) proved the relationship between the form of application of the tested stimulator and increased values of net photosynthesis [$\text{mmol}/(\text{m}^2 \cdot \text{s})$], transpiration [$\text{mmol}/(\text{m}^2 \cdot \text{s})$], stomatal conductivity [$\text{mmolH}_2\text{O}/\text{m}^2 \text{s}^{-1}$] and lower intercellular CO_2 content [$\mu\text{molCO}_2/\text{mol} \cdot \text{air}$]. Simultaneous watering and application in the form of an aerosol caused the best effect compared to the control series in all variants of the experiment. High indices of individual gas exchange parameters translated directly into better vigor of plants and increased biomass yield.

The methods used in the experiment and the analysis of the physiological and biochemical activity of plants are widely used and recognized as tools to obtain useful markers of metabolic processes which are also recommended as indicators of plant responses to external stimuli, in particular responses to stressors caused by adverse climatic conditions.

Discussion

The experiment and analysis of individual physicochemical parameters confirmed the legitimacy of using the mixture of *Navicula* sp. monocultures in energy crops, both in optimal and unfavorable conditions, characterized by extreme temperatures and soil moisture. The scale of the positive effect depended on the form of application of the tested preparation. The preparation used significantly alleviated the symptoms of environmental stress and increased plant metabolic activity, which translated into higher biomass. The high content of silicon and other necessary macro- and micronutrients found in the diatom cells can directly translate into faster growth and development of cultivated plants (Kaya et al. 2006). Research to date has shown a large variety of biologically active substances and a high content of antioxidant compounds found in the diatom cells (Fimbres-Olivarria et al. 2018). Foliar application and direct watering of plants with the mixture of monocultures at the same time caused faster plant growth, intensified plant gas exchange processes, had a positive effect on water and enzymatic management, and improved the absorption of nutrients from soil. All of the above-mentioned effects contributed to obtaining higher plant biomass. In addition, due to the influence of natural stimulators on a number of metabolic processes, the

Table 3 Gas exchange in the leaves of *Sida hermaphrodita* grown under of changing temperature and soil moisture (every 3 weeks application of diatom extract)

<i>Sida hermaphrodita</i>				
Experimental variant	Netto photosynthesis [$\text{mmol}/\text{m}^2 \text{s}$]	Transpiration [$\text{mmol}/\text{m}^2 \text{s}$]	Stomatal conductance [$\text{mmol H}_2\text{O}/\text{m}^2 \text{s}^{-1}$]	Concentration intercellular CO_2 [$\mu\text{mol CO}_2 / \text{mol air}$]
Variable temperature, 30% soil moisture				
Control	4.1cd ^a	1.29c	456c	296d
20/0/20 °C	4.2b	1.02b	378b	322e
20/40/20 °C	5.3 g	1.62e	564e	276bc
20/0/40/20 °C	4.0bc	1.34c	478cd	324de
10/−5/20/40/20 °C	3.2a	0.22a	59a	54a
Variable temperature, 20–60% soil moisture				
Control	4.3cd	1.32c	451c	288d
Diatoms watering	4.3b	1.30b	441c	287d
Diatoms spraying	4.4e	1.43e	452e	292d
Diatoms watering and spraying	4.5f	1.54e	477ef	298f
Field—weather-dependent conditions				
Control	4.2fg	1.45g	438g	284g
Diatoms watering	4.2e	1.40bc	411e	267e
Diatoms spraying	4.3bc	1.38de	422d	271e
Diatoms watering and spraying	4.4e	1.35f	438g	284g
LSD _{0.05}	0.2	0.05	33	21

^aMeans marked with the same letters within individual columns do not differ significantly according to Duncan's test at significance level $p=0.05$

Table 4 Gas exchange in the leaves of *Salix viminalis* grown under of changing temperature and soil moisture (every 3 weeks application of diatom extract)

<i>Salix viminalis</i>				
Experimental variant	Netto photosynthesis [mmol/m ² s]	Transpiration [mmol/m ² s]	Stomatal conductance [mmol H ₂ O/m ² s ⁻¹]	Concentration intercellular CO ₂ [μmol CO ₂ / mol air]
Variable temperature, 30% soil moisture				
Control	4.0cd ^a	1.24c	446d	287d
20/0/20 °C	4.1b	1.08bc	388c	342c
20/40/20 °C	4.8e	1.68d	584d	226bc
20/0/40/20 °C	3.1f	1.37e	488e	314c
10/−5/20/40/20 °C	3.1a	0.22a	51a	51a
Variable temperature, 20–60% soil moisture				
Control	4.1cd	1.42d	441d	298c
Diatoms watering	4.1e	1.37d	431d	281bc
Diatoms spraying	4.2de	1.33f	462f	289ef
Diatoms watering and spraying	4.5f	1.44e	467e	271b
Field—weather-dependent conditions				
Control	4.1cd	1.35d	428c	294c
Diatoms watering	4.1e	1.30d	416d	281c
Diatoms spraying	4.2f	1.25cd	432d	279c
Diatoms watering and spraying	4.4ef	1.32e	448e	274d
LSD _{0.05}	0.2	0.06	31	19

^aMeans marked with the same letters within individual columns do not differ significantly according to Duncan's test at significance level $p=0.05$

Table 5 Gas exchange in the leaves of *Helianthus tuberosus* grown under of changing temperature and soil moisture (every 3 weeks application of diatom extract)

<i>Helianthus tuberosus</i>				
Experimental variant	Netto photosynthesis [mmol/m ² s]	Transpiration [mmol/m ² s]	Stomatal conductance [mmol H ₂ O/m ² s ⁻¹]	Concentration intercellular CO ₂ [μmol CO ₂ / mol air]
Variable temperature, 30% soil moisture				
Control	4.2cd ^a	1.49c	446c	301e
20/0/20 °C	4.2b	1.12a	387b	312bc
20/40/20 °C	4.9g	1.52e	490c	256b
20/0/40/20 °C	4.2bc	1.64e	468cd	334d
10/−5/20/40/20 °C	3.1a	0.29a	56a	51a
Variable temperature, 20–60% soil moisture				
Control	4.2cd	1.42c	459c	298d
Diatoms watering	4.3a	1.36b	451bc	297de
Diatoms spraying	4.4bc	1.53c	442d	272b
Diatoms watering and spraying	4.5f	1.64c	467d	278bc
Field—weather-dependent conditions				
Control	4.1cd	1.35c	458c	294d
Diatoms watering	4.2b	1.45b	451d	277bc
Diatoms spraying	4.1e	1.48d	442b	281d
Diatoms watering and spraying	4.4f	1.39bc	448bc	294e
LSD _{0.05}	0.3	0.06	35	25

^aMeans marked with the same letters within individual columns do not differ significantly according to Duncan's test at significance level $p=0.05$

plants respond with a much lower oxidative stress, as confirmed by (Anyszka et al. 2008; Gawrońska et al. 2008). The use of a mixture of diatoms in modern, innovative economy can become an alternative and significantly reduce the use of chemical fertilizers that are harmful to the environment (Simental et al. 2003). Low production costs are another aspect that indicates the legitimacy of using sonicated diatom cells as natural biostimulants. In 1992, McAnally-Salas et al. (1992) in their research showed that the production of diatomaceous fertilizers compared to standard conventional fertilizers can be cheaper by almost 98%. Valenzuela-Espinoza (1997) came to similar conclusions, his natural agricultural preparation had eight times lower production costs.

The conducted experiment confirmed the legitimacy of the use of diatomaceous extracts and their beneficial effect on the growth, development and physiological activity as well as vigor of energy plants in adverse conditions of climate change. Such pro-ecological activities create a chance to significantly reduce chemical fertilizers dangerous for the environment and humans, while reducing the production costs of energy crops.

Conclusion

The obtained results confirmed the positive effect of the diatom monoculture extract on the growth and development of the cultivated plants. The analyzed physicochemical parameters were 15–20% higher in comparison with the control sample. The extract used stimulated plant growth and development, which was confirmed by their increased height and biomass yield as well as metabolic activity. The use of natural extracts from *Navicula* sp. diatoms as plant growth stimulants can be an alternative to chemical fertilizers and is considered one of the promising strategies in organic and integrated plant cultivation.

Author contributions statement KP was responsible for investigations and writing the original draft and editing of the manuscript; ZRD was responsible for review and editing of the manuscript; KP, ZRD, BM were responsible for methodology; KP was responsible for formal analysis; KP was responsible for visualization of figures and tables; ZRD was responsible for the resources, supervision; KP, ZRD, BM, were responsible for review and editing of the manuscript.

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

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