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Extracts from *Artemisia vulgaris* L. in Potato Cultivation—Preliminary Research on Biostimulating Effect

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Abstract: Nowadays the size and quality of potato yields is very important aspect in agriculture, due to the continuous climate change. Plants exposed to abiotic stress need new protection tools such as plant biostimulant. The new definition of this product include plant extracts as novel biostimulants. The aim of the study was to assess whether the extracts from *Artemisia vulgaris* L. would act as classic biostimulants, by affecting metabolic pathways. Since these are pilot studies, the content of chlorophyll, carotenoids, proline and polyphenols was chosen as indicators of changes in plants. The experiment was carried out under controlled environmental conditions on a very early cultivar Irys. The obtained results showed that foliar treatment of plants with extracts from *Artemisia vulgaris* L. had a positive effect on the increase of chlorophyll a and chlorophyll b content and its total concentration in potato leaves. The highest increase in the total chlorophyll content, amounting to 26.27% on average, was observed in plants sprayed with macerate at the dose of 0.6 mL· plant^{-1}. Additionally, an increase in the carotenoids content was observed in plants sprayed with macerate. The study demonstrated that the polyphenols level was largely dependent on the method of extracts production and the dose of the tested extracts. Macerate and infusion applied in a higher dose induced in plants the changes in the concentration of polyphenols. The overall evaluation of the effectiveness of the tested preparations showed higher effectiveness of the macerate for all the analyzed traits.

Keywords: mugword; extract; biostimulant; potato; antioxidant; proline

1. Introduction

For many years, a reduction in the field of potato cultivation has been observed in Poland, which is mainly caused by a decrease in its importance as a distillery, feed and starch raw material [1]. Moreover, large price fluctuations related to temporary occurrence of a deficit or overproduction of potatoes affect the profitability of cultivation [2]. Its difficult cultivation, high production costs and high quality requirements for tubers also affect farmers' resignation from growing this plant species [3].

Interestingly, however, despite the decrease in the field of potato cultivation in Poland, its production is increasing. According to Dzwonkowski [4], the average five-year yield level in 2000 was 18.4 t·ha^{-1}, and by 2015 the yield increased by 32.4%, i.e., to 24.3 t·ha^{-1}.

The key factor determining the size and quality of potato yields is the proper cultivation technology [5]. This aspect becomes even more important in the era of continuous climate changes, which make agricultural plants, including potatoes, exposed to abiotic stress factors. The effects of water stress, salt stress, drought, etc., can be mitigated by using appropriate methods of soil tillage (e.g., ploughed tillage) or by using during tillage measures stimulating the defensive mechanisms of plants. However, increasing pressure from society is forcing farmers to restrict the use of chemically synthesized preparations in favour of products that are safe for humans, animals and the environment [6–9].

These requirements are met by natural preparations such as plant extracts, which are obtained by extraction with alcoholic solvents, acetone, or water [10,11]. These extracts are most often called biostimulants. There are many definitions of biostimulants in the literature [12–15]. The most recent is presented in Regulation (EU) 2019/1009 of the European Parliament and Council (EC) defines plant biostimulants as "EU fertilizing product able to stimulate plant nutrition processes independently of the product's nutrient content with the sole aim of improving one or more of the following characteristics of the plant or the plant rhizosphere: (1) nutrient use efficiency, (2) tolerance to abiotic stress, (3) quality traits, or (4) availability of confined nutrients in the soil or rhizosphere" [16,17]. The literature reports show that these extracts obtained from various plant species and their parts in vitro tests have the ability to inhibit surface growth, biomass growth and limit the processes of pathogenic fungi sporulation [18–21]. Moreover, it was observed that extracts from marine algae have fungistatic properties and contribute to the increase in the activity of defence enzymes in such plants as barley, peas, corn, wheat, aubergine and pepper. Additionally, algae extracts have the properties of bioregulators, i.e., they contribute to increasing the resistance of plants to abiotic stress [22–27].

Not only can seaweed extracts stimulate the growth and development of cultivated plants but also preparations obtained from other plant species. This is mainly due to the presence of secondary metabolites which are produced by plants to combat attacks by adverse micro-organisms or herbivorous insects. These metabolites are released to the environment through foliar leaching, decomposition of dead parts or through the root zone [28]. Some of these compounds can have a positive or negative effect on surrounding plants. Allelochemical interactions between plants play a key role in plant dominance or crop yield [29]. It should be remembered that the allelopathic potential of some plants may vary depending on their stage of development [30].

The allelopathic properties are shown by plants of the genus *Artemisia vulgaris* L., which by colonization of the area, reduce diversity of the native flora [31]. The plant is a common weed [26], both in the fields and on roadsides or unused land [32]. Composition analyses of mugwort extracts have shown that they are rich in phenolic compounds, including chlorogenic acid derivatives, flavonoids, phenolic acid and ligands [33]. In addition, they have monoterpenic compounds, including: α-pinen, limonene, camphor, β-pinen and camphene. Barney et al. [31] in their research demonstrated the phytotoxic effect of terpenes obtained from the leaves of *Artemisia vulgaris* L. depending on the concentration used. However, some of these compounds, such as alpha-pinen, have been shown to stimulate the development of *Lepidium sativum* at all tested concentrations.

Therefore, the aim of this study was to evaluate the possibility of using water extracts obtained from dried leaves of *Artemisia vulgaris* L. in potato cultivation as a preparation stimulating the overproduction of proline, chlorophyll, carotenoids and polyphenols in the aboveground part of the plant. The studies were carried out in the form of a pot experiment under controlled environmental conditions. In the current study, it was hypothesised that extracts from *Artemisia vulgaris* could contribute to the increase of the chlorophyll, carotenoids, polyphenols, and proline content. The assumption was also made that the response to the application of these extracts depends on the methods of extraction.

2. Materials and Methods

2.1. Plant Materials

The research material consisted of potatoes of the very early variety Irys, from which small fragments (meshes) were cut out, natural resting was interrupted according to the method given by Wróbel et al. [34], and they were lined on acid peat with the mesh facing down, in order to sprout them. In the next stage, the germinated meshes were placed in 10 × 10 × 10 cm pots. Peat substrate for vegetable and peat plants was used for potato cultivation. Quality parameters of soil: pH 5.4–6.8; salinity below 2.5 g NaCL/L. Loose solid form, fractions 0–20 mm. The plants were grown in a room with an ambient temperature of 22 °C with LED light the red to blue ratio of which was 5:2 and photoperiod 16/8 (day/night). The plants were watered every other day with 80 mL of water to obtain the same soil moisture conditions in all pots.

Three weeks after planting, when the average plant height was 15 cm, the first foliar spray with infusion and macerate obtained from *Artemisia vulgaris* L. was applied. The preparations were piled every seven days at the dose of 0.6 and 1.2 mL·plant^{-1}. The controls consisted of the plants treated with water in the same doses and the plants not treated. The experiment was developed in three independent replications. Each replication consisted of 100 meshes. Twenty plants were randomly selected for the study. The results presented in the tables and figures are the mean values of three independent replications (n = 60), together with the values of standard deviation (SD).

2.2. Preparing the Extracts

Natural preparations of mugwort were obtained according to the method given by Sas-Piotrowska et al. [19]. In the experiment dried leaves of *Artemisia vulgaris* L. (Flos, Poland) were used.

The infusion was obtained by weighing 5 g of dried herbs, which were flooded with 250 mL of water at about 100 °C and left under cover for 30 min at 20 °C. After it had cooled down, the material was washed using semi-hard perforated filter bags.

Macerate was prepared from 5 g of dried material, which was then poured with 100 mL of water at about 20 °C and left for 24 h at the ambient temperature. After this time, the material was filtered in the same way as for the infusion.

2.3. Spectrophotometric Characterization of the Extracts

2.3.1. Total Polyphenol Content

The content of total phenolic compounds (TPC) in extracts was determined by Singleton and Rossi [35] method using Folin–Ciocalteau reagent. To 0.5 mL of extract, 0.5 mL of water was added, followed by 2 mL of Folin–Ciocalteau reagent (1:5 H_2O). After 3 min, 10 mL of 10% sodium carbonate was added to the mixture. After 30 min, the absorbance of samples at 724 nm was measured with UV-VIS spectrophotometer. The total phenolic compounds content was calculated as gallic acid equivalent (GAE).

2.3.2. Total Flavonoid Content

The total flavonoid content in extracts was determined based on the method proposed by Lamaison and Carnet [36]. 1 mL of extract was added to one millilitre of 2% $AlCl_3 \times 6H_2O$. The whole was mixed and then incubated at the ambient temperature for 10 min. In the next stage, absorbance was measured at 430 nm. The total flavonoid content was calculated as quercetin (QE) equivalent in mg per gram of dry matter (DM). The total content of phenolic compounds was determined identically as in the case of extracts obtained from potato leaves.

2.3.3. Total Anthocyanins Content

Based on the method given by Fuleki and Francis [37] the content of anthocyanins in the extracts obtained from *Artemisia vulgaris* L. was determined. In separate vessels, the plant extracts were mixed with KCl in the ratio 1:20 (*v*/*v*). Then, sodium acetate buffer with pH 1.0 was added to one of the vessels and the same buffer, but with pH 4.5, was added to the other one. After 15 min absorbances were measured at 520 and 700 nm in each of the obtained mixtures. The corrected absorbance value was calculated as [(A520–A700) pH 1.0–(A520–A700) pH 4.5]. The content of anthocyanins was calculated using the molar coefficient of absorbance (C) and molecular weight (MW) of 3-glucoside cyanidin (C = 26,900, MW = 449.2). The results were expressed as equivalent of 3-glucoside cyanidin (Cy3-GE) in mg per g of dry matter (DM).

2.3.4. Reduction Power

To determine the reduction power, 2.5 mL of each extract was used, which was then mixed with 2.5 mL 200 mM phosphate buffer with pH 6.6 and 2.5 mL 1% K_3 solution [Fe (CN_6)]. Mixtures were incubated at 50 °C for 20 min. The reaction was stopped by adding 0.5 mL of 10% TCA. The whole was centrifuged for 10 min at 6800× *g*. In the next stage, 2.5 mL of a thick layer of solution was taken and mixed with the same amount of distilled water and 0.5 mL 0.1% $FeCl_3$. Absorbance was measured at 700 nm. The reduction in power was expressed as Trolox equivalent (mg g^{-1} DW) [38].

2.4. Extraction of Potato Leaves

Potato leaf extraction was performed by homogenization of 50 mg of fresh leaf tissue flooded with 5 mL of methanol [39]. The samples were then filtered and stored at −20 °C until the proline content was determined. The determination of plant dyes was performed immediately after extraction.

2.5. Determination of Proline Content

The proline content was tested according to Carillo and Gibon [40]. The obtained potato leaf extract was mixed with the reaction mixture (1% ninhydrin solution in 60% acetic acid and 20% ethyl alcohol) and incubated at 95 °C for 20 min. Absorbance was measured at 520 nm and the total proline content was expressed in µM/mL.

2.6. Determination of Chlorophyll, Carotenoids and Total Phenolic Compounds Content in Leaves

Spectrophotometric analysis of plant pigments was performed based on the method given by Vicas et al. [39]. For the extraction of chlorophylls (a and b), 50 mg of fresh leaf mass was used and 5 mL of methanol was homogenized. The samples were then filtered, and absorbance measured at 652, 665.2 and 470 nm. The following formulas were used to calculate chlorophyll (mg/mL) and carotenoids (mg/g of fresh leaf mass):

$$[\text{Chl a}] = 16.29 \cdot \text{Abs.665.2} - 8.54 \cdot \text{Abs.652.0}, \quad (1)$$

$$[\text{Chl b}] = 30.66 \cdot \text{Abs.652.0} - 13.58 \cdot \text{Abs.665.2}, \quad (2)$$

$$[\text{Chl a + b}] = 22.12 \cdot \text{Abs. } 652.0 + 2.71 \cdot \text{Abs.665.2}, \quad (3)$$

$$\text{Total carotene} = ((1000 \cdot \text{Abs.470}) - (2.860 \cdot [\text{Chl a}]) - (129.2 \cdot [\text{Chl b}]))/245. \quad (4)$$

2.7. Statistical Analysis

Analyses were performed in three replications for each growing season. The Shapiro–Wilk test was used to evaluate the normal distribution of data. Results were analysed using the one-way analysis of variance (ANOVA). The significance of differences between mean values was estimated based on

Tukey confidence intervals, at a significance level of $p < 0.05$. The statistical analysis was performed using Statistica 13.3 software (TIBCO Software Inc., Palo Alto, CA, USA).

3. Results and Discussion

When evaluating the total content of polyphenolic compounds present in the extracts obtained from *A. vulgaris* L. (Table 1), it was observed that hot extraction resulted in a higher amount of these compounds compared to the extracts obtained by the cold method. A different trend was observed in the case of the flavonoid content, where hot extraction resulted in more than four times lower content of these compounds in relation to cold extraction. No anthocyanins were found in both the macerate and the infusion.

Table 1. Antioxidant potential of extracts from *A. vulgaris* L.

Total Phenolic Compounds		Total Flavonoid Content		Total Content of Anthocyanins		Reducing Power	
mg g^{-1} DM						mg TE g^{-1} DM	
Infusion	Macerate	Infusion	Macerate	Infusion	Macerate	Infusion	Macerate
48.093 ± 0.978	35.274 ± 0.411	4.017 ± 0.024	16.418 ± 0.033	nd	nd	9.170 ± 0.053	6.447 ± 0.029

nd—not detected.

Analysis of the reduction strength of the obtained plant extracts showed that it depended on the extraction method. The infusions were characterized by a higher reduction force by about 42%. The antioxidant activity of plant extracts was studied by many authors [41–44]. These studies have shown that the antioxidant activity of plant extracts is determined by the total content of phenolic compounds. A positive correlation between the content of these compounds and the ability to remove or reduce radicals was proved [44]. Moreover, flavonoids by chelating free radicals influence the general antioxidant character of plant extracts [45]. The literature also contains information on the lack of positive correlation between the polyphenol content and antioxidant activity [46].

The application of plant extracts obtained from *Artemisia vulgaris* L. as foliar spray differentiated the content of plant pigments in the aboveground part of potatoes of Irys cultivar. Regardless of the amount applied and the extraction method, an increase in the chlorophyll a (Figure 1) content was observed in all combinations compared to the control plants. The highest chlorophyll a content was obtained in the combination where foliar macerate spraying was applied in a smaller amount (increase by about 43.7% in relation to the control plants). Foliar application of macerate and infusion in the dose of 1.2 mL·plant^{-1} resulted in similar values of the discussed plant dye.

In the case of concentration of chlorophyll b (Figure 1), in potato leaves in almost all combinations where the natural preparation was applied, a reduction in its content was noted. The exception was macerate applied in the amount of 0.6 mL·plant^{-1}, where a slight increase in chlorophyll b concentration was obtained, compared to the control, amounting to 1.85% on average. The greatest reduction was observed in leaves, whose plants were treated only with distilled water in a lower dose (the decrease in concentration in comparison with the unsprayed plants was 23.15%). The highest decrease in chlorophyll b content after application of plant extracts was obtained in the combination where plants were treated with macerate in a larger amount (decrease in concentration by 12.07% compared to the control).

When determining the ratio of chlorophyll a to chlorophyll b, an increase in the ratio of chlorophyll a to chlorophyll b was recorded in all tested samples in relation to the control facility. The smallest growth of chlorophyll a/b (Table 2) ratio was observed in plants treated with macerate in smaller amounts and infusion in the dose of 0.6 and 1.2 mL·plant^{-1}. Spraying the plants with distilled water resulted in the highest chlorophyll a/b ratio in comparison to control plants.

When evaluating the effect of the dose and type of plant extract on the total chlorophyll content in potato leaves, it was observed that treating plants with macerate in the amount of 0.6 mL·plant^{-1} in the most beneficial way affected its content. In this combination, its content was the highest in comparison

with the other samples analysed. The use of infusions and macerate in the amount of 1.2 mL·plant^{-1} caused the discussed feature to be at a similar level.

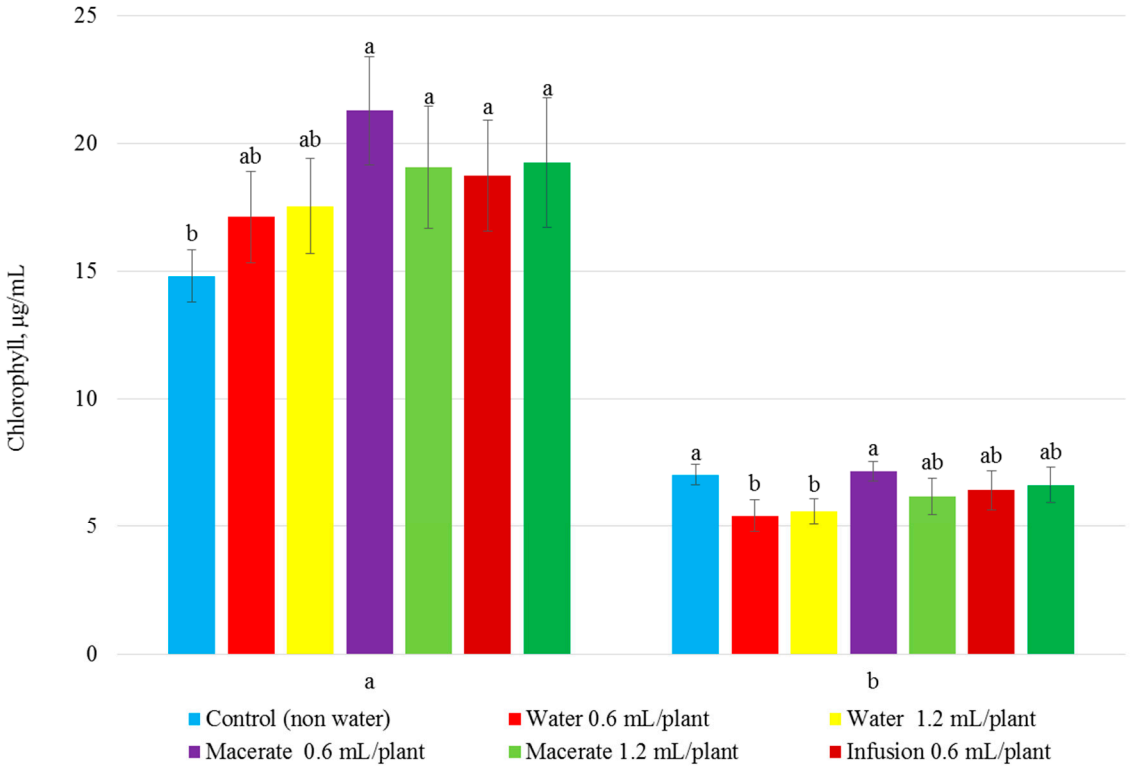

Figure 1. Concentration of chlorophyll a and b in potato leaves treated by extracts form *Artemisia vulgaris* L. (average of three independent experiments and SD). Values followed by different small letters are significantly different at $p < 0.05$.

Table 2. Plant pigments content depending on the plant extract used (average of three independent experiments ± SD).

Combination	a + b	a/b
	µg·mL^{-1}	
Control	21.86 ± 1.53 b *	2.11 ± 0.07 b
Water 0.6 mL·plant^{-1}	22.54 ± 1.88 b	3.17 ± 0.14 a
Water 1.2 mL·plant^{-1}	23.12 ± 1.61 b	3.14 ± 0.08 a
Macerate 0.6 mL·plant^{-1}	28.46 ± 1.68 a	2.97 ± 0.03 a
Macerate 1.2 mL·plant^{-1}	25.27 ± 3.28 ab	3.08 ± 0.09 a
Infusion 0.6 mL·plant^{-1}	25.18 ± 3.13 ab	2.93 ± 0.11 a
Infusion 1.2 mL·plant^{-1}	25.89 ± 3.83 ab	2.95 ± 0.28 a

* Values followed by different small letters are significantly different at $p < 0.05$.

An increase in chlorophyll content in plants where natural preparations were applied during cultivation was noted by many researchers [47–49]. Blunden et al. [50] observed that the increase in the chlorophyll content in bean, barley, wheat, and maize leaves was caused by the presence of betaine in seaweed extracts. The effect on the overall chlorophyll content in the potato leaves studied may result from a slower degradation process of this compound than from its increased synthesis [51].

Chlorophyll a and chlorophyll b are the main plant dyes playing an important role in photochemical reactions of photosynthesis. In turn, carotenoids play an important role in the protection of chlorophylls against destruction under stress conditions [52,53]. Therefore, in this study the content of carotenoids in potato leaves whose plants were treated with extracts from *A. vulgaris* L. was evaluated.

There was no decrease in the carotenoid content (Figure 2) compared to controls in any of the analysed combinations. The smallest increase of this compound was observed after macerate and infusion in higher doses. The increase was 30.23% and 39.07%, respectively. The highest over 1.6-fold increase in carotenoids occurred when foliar macerate was applied in smaller doses.

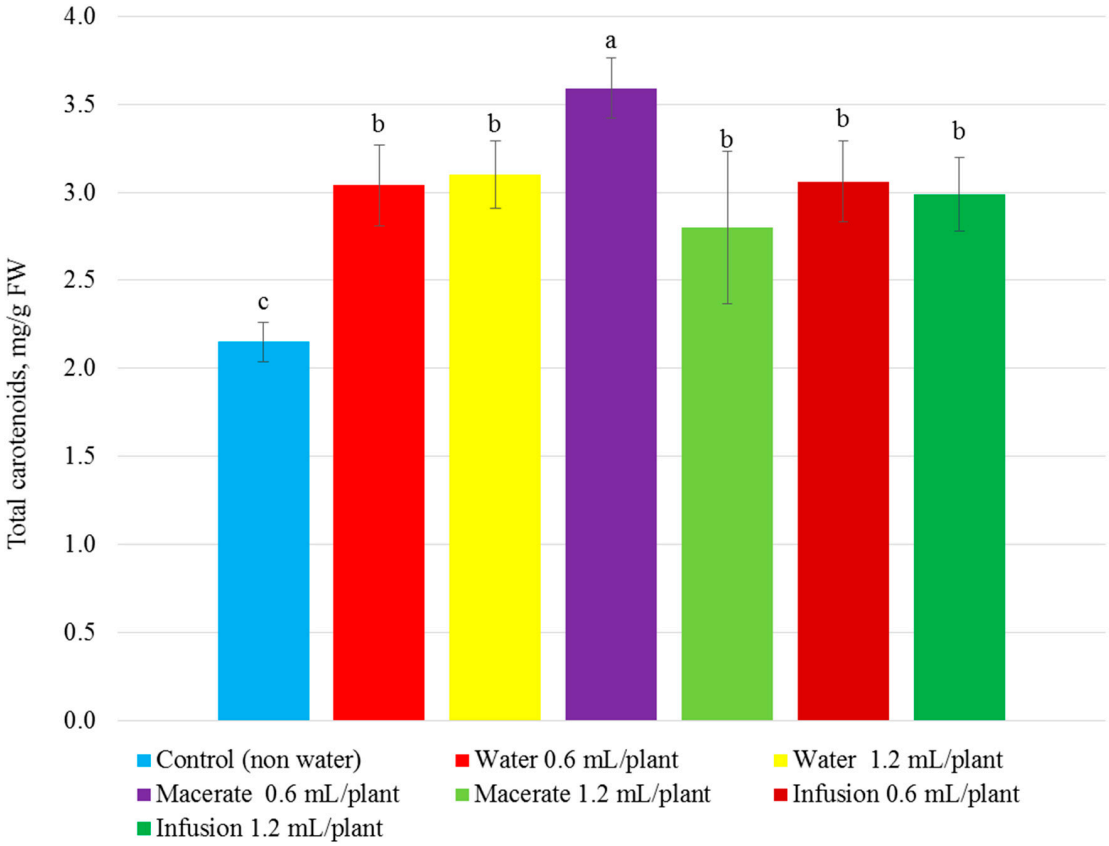

Figure 2. Concentration of carotenoids in potato leaves treated with extracts form *Artemisia vulgaris* L. (average of three independent experiments and SD). Values followed by different small letters are significantly different at $p < 0.05$.

Analysing the overall effect of plant extracts and the amount of foliar spraying applied, it can be observed that the application of macerate in the amount of 0.6 mL·plant^{-1} had the most beneficial effect on plant dyes including carotenoids in relation to other combinations where extracts from *Artemisia vulgaris* L. were applied.

Proline is synthesized by many plant species [54]. This amino acid has many functions in the proper development of plants. It acts as a strong non-enzymatic antioxidant involved in the removal of reactive oxygen species, is a source of energy, carbon and nitrogen, and maintains the cytoplasmic pH at an appropriate level [55,56].

The use of natural plant preparations in the form of foliar spray during the vegetation of potato plants affected differently the concentration of proline in the leaves (Figure 3). Spraying the plants with macerate caused an increase in this amino acid content. The highest increase of 0.957 µg·mL^{-1} compared to the control was recorded in samples where 1.2 mL·plant^{-1} macerate was applied. On the other hand, combinations where infusions were applied were characterized by a decrease in L-proline content in relation to non-traditional plants with plant extract. The highest reduction of the proline content occurred when the infusion was used in larger amounts (the average reduction compared to the control object was 9.61%). Treatment of plants with 0.6 mL·plant^{-1} distilled water resulted in a decrease in the concentration of the amino acid in question from 4.653 (control object) to 3.588 µg·mL^{-1}.

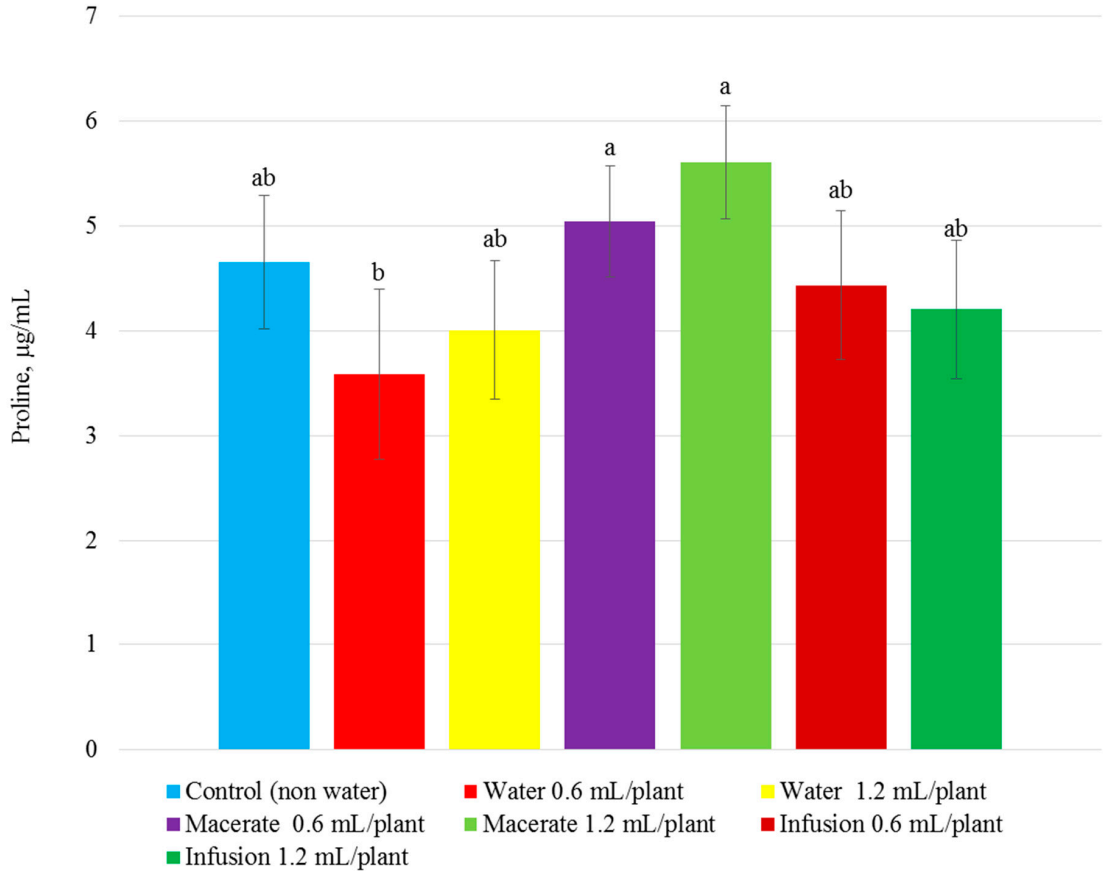

Figure 3. Proline concentration in potato leaves treated with extracts form *Artemisia vulgaris* L. (average of three independent experiments and SD). Values followed by different small letters are significantly different at $p < 0.05$.

The content of polyphenolic compounds in potato leaves mainly depended on the amount of natural preparation used (Figure 4). The application of macerate and infusion in larger quantities resulted in the highest values of these compounds in relation to the other combinations. On the other hand, spraying plants with these preparations in smaller amounts caused that the polyphenol content slightly differed from the control object. In the case of macerate, the value was 0.133 mg·mL^{-1} and in the case of infusion 0.122 mg·mL^{-1}. A significant increase of these compounds amounting to 18.4% (compared to control samples) was also recorded in combinations where distilled water in smaller amounts was used.

Extracts obtained from *Artemisia vulgaris* L. have a high total content of polyphenols [57]. In Melguizo-Melguizo et al.'s study [33], 22 compounds were identified, 15 of which were phenolic compounds. The use of larger amounts of extracts in own studies may have resulted in an increase in plant exposure to these substances, thus inducing the metabolism of phenylpropanoids and an increase in the accumulation of polyphenols in these combinations [58]. An increase in the content of polyphenolic compounds in plant tissues has a positive effect on increasing plant resistance to stress conditions [59].

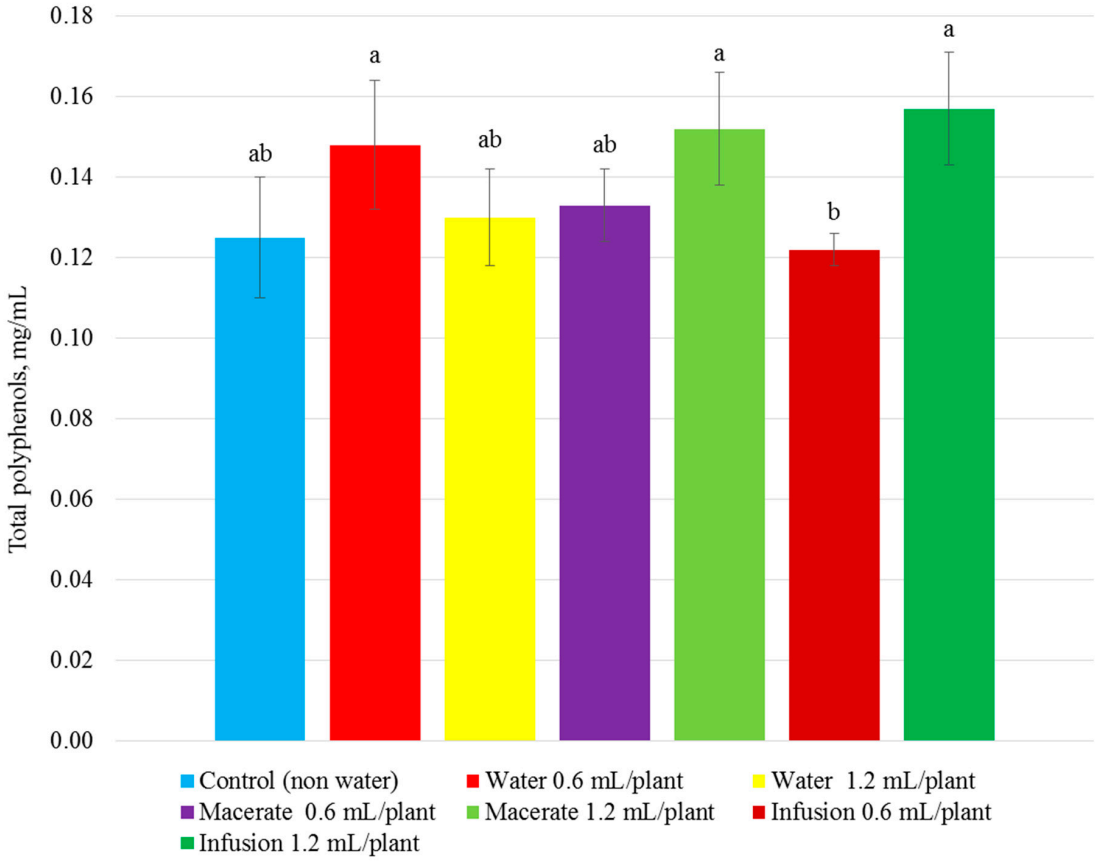

Figure 4. Total concentration of polyphenols in potato leaves treated with extracts form *Artemisia vulgaris* L. (average of three independent experiments and SD). Values followed by different small letters are significantly different at $p < 0.05$.

Nowadays, one of the biggest challenges facing agriculture is to develop sustainable and ecosystem friendly techniques to meet the nutritional needs of a growing world population [60]. A continuous climate change makes it even more difficult to meet this challenge. As recent years have shown, the climate change is most visible due to the occurrence of long-term drought periods not only in Poland but also in a large part of Europe.

Abiotic stress is one of the main reasons of crop losses worldwide reducing average yields of most crucial crops by more than 50% [55]. The reduction in yields under abiotic stress primarily results from the energy that plants need to use to adapt. To increase the plant tolerance under adverse environmental conditions, several approaches have been used. However, some of these approaches are considered time consuming. For example, conventional cultivation requires laborious selection and processing of several generations of crossings and tolerance testing of plants [61]. Cultivation of genetically modified crops is a less time-consuming and labour-intensive process, but currently their cultivation is prohibited in many countries [62]. An interesting and sustainable alternative may be the use of plant extracts, which can be classified as biostimulators, which strengthen the defence mechanisms of plants in order to tolerate future conditions of abiotic stress more effectively [55].

In the conducted tests, an increase in the content of chlorophyll a and b and its total concentration in potato leaves after treatment of plants with *A. vulgaris* L. extracts was noted. An increase in chlorophyll content was also noted by Matysiak et al. [63] in the leaves of winter rape in cultivation, foliar application of the extract from the sea algae *Ecklonia maxima* (Kelpak SL). Rouphael et al. [64] suggest that higher values of assimilation dyes may be conducive to the increase of the nitrogen uptake efficiency thus improving the crop efficiency.

Moreover, a decrease in chlorophyll a to b ratio after foliar application of plant extracts was observed in the conducted studies. This ratio in chloroplasts of higher plants is often used as an indicator of plant ageing [39]. As it results from the performed analysis, the control sample was characterized by a higher ageing index in relation to plants treated with natural preparations.

Foliar application of plant extract in the form of macerate contributed to an increase in the concentration of proline in potato leaves. It is well known that this amino acid is synthesized by plants to maintain an osmotic and ionic balance in cells [65]. Maintaining this balance reduces the negative consequences caused by abiotic stressors in crops. In addition to its osmotic properties, proline performs several other important functions such as: stabilization of protein structures carbon and nitrogen storage protection of membrane integrity and scavenger of reactive oxygen species [66,67].

According to Ali et al. [68], the exogenous use of proline contributes to the increase of the proline content in maize cells, resulting in the increased tolerance in water-deficient environments. Other studies showed an increased resistance to salt stress [66] and the presence of heavy metals in soil [69] in crops with a higher proline content. In our own research, we also observed an increase in the concentration of this amino acid after macerate application which may suggest that the obtained plants may have higher tolerance indexes for abiotic stress factors.

The results of the research conducted by Melguizo-Melguizo et al. [33] showed that most abundant compound was 3.5-*O*-dicaffeoylquinic acid. Generally, chlorogenic acid derivatives were the most abundant compounds. Protocatechuic acid and quinic acid were also present in high amounts. The research results presented in the article indicate that the use of plant extracts, due to the content of polyphenols in them, can ensure the increased tolerance of potato plants to stress factors, due to their multifaceted action at the biochemical and physiological level. Substantially different plant responses were found when using both test extracts compared to control combinations. However, it should be emphasized that the biological effects exerted by the active compounds contained in them are clearly different from those associated with conventional fertilizers [51]. According to Rouphael et al. [70] it can be attributed primarily to higher absorption of nutrients, osmotic regulation, as well as an increase in the concentration of many secondary metabolites. This was also the case in the present study since the main biochemical changes in particular, the concentration of polyphenols, proline or dyes were induced by the use of plant extracts. The results of own research indicate that applying extracts resulted in changes at the biochemical level associated with an increase in the production of secondary metabolites. Shahabivand [71] even concluded that the higher total antioxidant capacity in plants treated with preparations containing active compounds helps plants to scatter photosynthetically produced electrons and alleviate probabilistic oxidative damage.

The results of our study showed that extraction methods influenced the efficacy of the tested extracts. Differences in their activity could be due to the extraction process parameters that have various effects on the softening and destruction of a plant cell wall and the release of soluble phytochemicals. The results of our study also indicate that tested plant extracts were rich in aromatic secondary metabolites that exhibit biostimulating activity as many authors have emphasized that components with phenolic structures are highly active in plant growth. However, such research has just begun. It will be necessary to standardize extraction methods and the methods used to analyse the biostimulating effectiveness to assess the effectiveness of these products in stimulation plant phenological development.

4. Conclusions

The studies have shown differences in the antioxidant potential of extracts, depending on the extraction method. A higher concentration of phenolic compounds was found for infusions. In contrast, macerates were richer in flavonoids. These differences could have been the reason for the different effects of the extracts on potato plants.

The treatment of potato plants with natural extracts from *Artemisia vulgaris* L. in the form of foliar application resulted in an increase in the chlorophyll content a, b and the total concentration

in comparison to the control samples. This increase was differentiated depending on the method of preparation of the plant extract and its dose. Additionally, an increase in the proline and carotenoids content was observed in plants sprayed with macerate. The study demonstrated that the polyphenols level was largely dependent on the method of extracts production and the dose of the tested extracts. Macerate and infusion applied in a higher dose induced in plants the changes in the total polyphenols concentration. The overall evaluation of the effectiveness of the tested preparations showed higher effectiveness of the macerate for the analysed traits.

Due to the lack of toxicity, the use of macerate in potato cultivation is conducive to the development and idea of sustainable agriculture. The increase in the proline content promoting the resistance of plants to abiotic stress factors may bring many measurable benefits to the farmer. However, to confirm the results obtained it is necessary to carry out a field experiment under natural environmental conditions.

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