horticulturae

MDPI

Article

Initial Physiological, Biochemical and Elemental Garlic (*Allium sativum* L.) Clove Responses to *T. vulgaris* and *S. aromaticum* Extract Application

Lina Dėnė *, Kristina Laužikė, Danguolė Juškevičienė, Alma Valiuškaitė and Rasa Karklelienė

Institute of Horticulture, Lithuanian Research Centre for Agriculture and Forestry, Kaunas District, 54333 Babtai, Lithuania; kristina.lauzike@lammc.lt (K.L.); danguole.juskeviciene@lammc.lt (D.J.); alma.valiuskaite@lammc.lt (A.V.); rasa.karkleliene@lammc.lt (R.K.)
* Correspondence: lina.dene@lammc.lt

Abstract: Plant extracts are getting attention for their sterilizing, growth-promoting properties in the agricultural field. No exception exists when it comes to the treatment of propagating material with these natural components. Plant extracts may have complex compositions which affect plants or seeds in a positive way. However, they could also cause negative effects, like decreased germination, secondary metabolite contents or biomass gain. As the agricultural field requires sustainable techniques for plant growth and quality assurance in production, it is important to evaluate the potential effects of every alternative natural compound that shows promise for future plant treatment. Garlic (*Allium sativum* L.) is a popular spice crop grown in various regions of the world. Therefore, our study focused on an investigation of the early physiological and biochemical changes in garlic cloves (cv. 'Jarus' and 'Vasariai') treated with *Thymus vulgaris* essential oil and *Syzygium aromaticum* extract. Experiments were carried out in controlled climate conditions, in which the treated cloves were kept for four weeks. Results show that *S. aromaticum* extract increased germination in cv. 'Jarus' compared to untreated garlic cloves. Meanwhile, *T. vulgaris* caused lower germination of both cultivars compared to untreated cloves. None of the extracts increased biomass gain in garlic cloves. ABTS antioxidant activity was decreased by both extracts in cv. 'Jarus' (~7–47%) and 'Vasariai' (~22–32%) compared to untreated garlic cloves. Antioxidant DPPH and FRAP activities were ~6–11% and ~14–15% higher after 'Vasariai' treatment with extracts. Meanwhile, in 'Jarus' only, *S. aromaticum* extract caused an increase in DPPH and FRAP antioxidant activities. The elemental content was the highest in garlic cloves treated with *T. vulgaris* essential oil. Total phenolic compounds (TPC) and flavonoids (TF) were 1.2 times higher after *S. aromaticum* treatment of 'Jarus' compared to untreated cloves, while slightly increased TPC and two times higher TF values were found after treatment with *T. vulgaris*. Around 10% higher TPC and 1.5–3 times lower TF values were observed after the plant extract treatment of 'Vasariai' garlic cloves. Our study demonstrates initial changes in garlic cloves dependent on the plant extract treatment and cultivar. This provides important information for the future utilization of such technology, either alone or in combination with others.

Keywords: garlic; germination; antioxidant response; physiological response; plant extracts

Citation: Dėnė, L.; Laužikė, K.; Juškevičienė, D.; Valiuškaitė, A.; Karklelienė, R. Initial Physiological, Biochemical and Elemental Garlic (*Allium sativum* L.) Clove Responses to *T. vulgaris* and *S. aromaticum* Extract Application. *Horticulturae* **2024**, *10*, 99. https://doi.org/10.3390/horticulturae10010099

Academic Editor: Charalampos Proestos

Received: 20 December 2023
Revised: 11 January 2024
Accepted: 17 January 2024
Published: 20 January 2024

1. Introduction

Assurance of a sufficient amount of food by improving cultivation technologies is one of the key factors in connection with the growing population. One of the disease inoculum sources in plants could be propagation material; therefore, it is important to guarantee healthy seeds or effective protection against possible disease. Treatment of seeds can have several positive effects, like reducing seedborne pathogens and increasing seedling emergence [1]. Current global goals aim to ensure food security in sustainable ways; moreover, organic farming is being encouraged. As a result, propagation material treatment technologies must adapt or change to obtain not only their primary aims, but also to be favorable to the climate and the environment.

Garlic (*Allium sativum* L.) is a valuable spice, rich not only in taste but also in medicinal properties [2]. It has a high nutritional content of protein, calcium, magnesium, iron, zinc, potassium, arginine, selenium [3], polyphenols, alkaloids and vitamins. However, sulfur is the major element in garlic bulbs [4]. Garlic has anticancer, antioxidant, anti-inflammatory, antimicrobial, antithrombotic, hypocholesterolemic, hypoglycemic and hypotensive properties, and is used to treat various diseases and illnesses [5]. Garlic is also a source of organosulfur compounds, flavonoids and polyphenols [6]. Regarding the mentioned properties, the value of garlic is only increasing, and cultivation of this crop will be relevant globally.

The beginning of vegetable vegetation starts with seeds, or, in the case of garlic, cloves. Propagation material treatment may promote plant growth, resistance to diseases, survival in unfavorable conditions, etc. As garlic is propagated with cloves, it is crucial to sow healthy garlic cloves and protect plantlets during the early growth stages, as disease symptoms often become visible only later [7]. Fungi could significantly decrease garlic clove germination and lower other important parameters [8]. Seed treatment with essential oils can be used not only to prevent infections but also to protect seeds during seedling germination, emergence and initial development [9]. Several studies on seed treatment with plant extracts are present in the literature [1,10–18]. Extracts from *A. vera*, *C. arabica* and *Y. schidigera* were tested on tomato seeds of different cultivars for bacterial leaf spot control, seedling vigor, height and weight investigation [13]. *S. aromaticum* and *T. vulgaris* essential oils were investigated as carrot seed pretreatments to control *Alternaria radicina* both in combination with hot water treatment and individually [15]. Pearl millet seed germination and seedling vigor were enhanced, and downy mildew disease was decreased by plant extracts, especially by 10% *V. album* extract [11]. Essential oils of *H. marrubioides*, *C. verbenacea* and *A. gratissima* reduced *Colletotrichum truncatum* infection in inoculated soybean seeds and increased the development of seedlings compared to non-treated seeds [12]. However, only germination parameters are available together with inhibition of fungus, and no evaluation of the initial internal response of seeds to the plant extract applications could be found.

Various innovative technologies were applied as garlic clove treatments. For example, garlic cloves were treated with weakly ionized, low-pressure oxygen plasma [19] and gibberellic acid [20]. Chemical and morphological changes occurring at the garlic skin surface were analyzed after the application of gaseous plasma to improve germination [21]. Edible coatings were used to increase minimally processed green garlic quality during storage [22]. Ben-Jabeur et al. [17] found that coating wheat seeds with thyme oil enhanced the water-stress avoidance strategies under stress conditions. Nanoparticle solutions increased the germination of wheat, maize, peanut and garlic [23]. Thermal treatment was used to enhance the growth and yield of garlic [24,25] and to reduce sprout and root growth in peeled or unpeeled cloves [26]. For protection from diseases, gaseous ozone flow was investigated [27]. Antioxidant levels in peeled garlic cloves were preserved under the influence of UV-C [28].

Plant extracts are often investigated as growth promoters [29,30], antifungal compounds [17,31–37] and phytotoxic agents against weeds [38] in the horticultural sector. *S. aromaticum* essential oil and extract were found to have strong antifungal activity in vitro against *Fusarium* spp. [31,36] and *B. cinerea* [37], which was also expressed on plant leaves in vitro [35]. Meanwhile, *T. vulgaris* essential oil had strong antifungal activity against *C. acutatum* [39]. *T. vulgaris* essential oil-treated carrot seeds exhibited reduced *A. radicina* germination and a reduced infection percentage in the plants [15]. Furthermore, this essential oil was very effective for onion, tomato and carrot seed disinfection against *Alternaria* spp. [40]. In other research, *S. aromaticum* and *T. vulgaris* essential oils demonstrated promising disinfection effects against seedborne pathogens on cabbage seeds [10]. Seed treatment with antifungal agents as plant extracts could reduce the risk of soil-borne pathogen resistance and reduce usage of conventional pesticides [9]. Compounds in garlic change during storage [6]; thus, when applying new measures, for example, plant extracts, it is relevant to identify whether changes are positive or not. Antioxidant compounds in plants can help to fight infections. Furthermore, this could be an indicator of plant response to changing conditions, like stress occurred after the application of natural compounds. There are studies indicating plant extracts' impact on antioxidant activity and compounds in plants and fruits. Coriander seed essential oil and extract decreased DPPH, ABTS antioxidant activity and total phenolic compounds in inoculated strawberry leaves, but only with lower applied concentrations [41]. A dose-dependent effect was observed with *Lawsonia inermis* L. extract, which increased wheat shoot and root system growth and accumulated 90% more total phenolic compounds in wheat compared to untreated plants [42]. The total concentration of polyphenols in potato leaves treated with extracts from *Artemisia vulgaris* L. increased by up to 20% compared to untreated plants and was dependent on the amount of the natural preparation used and the extraction method [43]. An *Aloe vera* gel coating and a *Fagonia indica* plant extract preserved a reduction in total flavonoids, strongly inhibited the loss of total phenolics and increased DPPH antioxidant activity in sapodilla fruit during storage [44].

As is shown in the literature, various technologies are applied in garlic to protect plants from diseases or to promote growth. There are no scientific data regarding the evaluation of the early antioxidant response of garlic cloves and changes in elemental composition, which could help to identify the eligibility of plant extracts as propagation material treatments. Furthermore, there is no study investigating plant extracts for such an application. Treatment of garlic cloves with a plant extract instead of a chemical one could decrease synthetic compound consumption in food production, guarantee an increase in physiological properties and promote future resistance to diseases. Thus, in this study, we evaluated the initial physiological, biochemical and elemental composition changes in garlic cloves of two garlic cultivars, 'Vasariai' and 'Jarus', treated with plant extracts: *T. vulgaris* essential oil and *S. aromaticum* extract. The germination, biomass gain, antioxidant capacity, total phenolic compounds, total flavonoids, and elemental composition of garlic cloves were investigated.

2. Materials and Methods

2.1. Plant Extracts

The experimental design contained three treatments of garlic cloves: (1) untreated, (2) 0.01% *Thymus vulgaris* essential oil (THY) and (3) 0.75% *Syzygium aromaticum* extract (SYZ). These specific concentrations of the extract were used based on antifungal activity studies performed at the Institute of Horticulture. The chosen concentrations had high antifungal activity against horticultural pathogens; therefore, they have potential to be used for garlic clove treatments as antifungal agents. Based on that, it was appropriate to investigate them in this experiment. THY contained 41.35% thymol in its volatile fraction [39] and was produced from dried *T. vulgaris* leaves collected and prepared as described in our previous study [39]. SYZ contained 52.88% eugenol, and determined volatile compounds of the used extract were presented in our previous study [35]. SYZ was prepared from dried *S. aromaticum* buds, and the sources of the material and the extraction conditions were described in previous research [37]. In the further text, THY and SYZ together will be referred to as 'plant extracts'.

2.2. Treatment of Garlic Cloves

Garlic cloves (*Allium sativum* L.) were provided from the propagation material collection of the LAMMC Institute of Horticulture; the cultivars were 'Vasariai' (LAMMC Institute of Horticulture, Lithuania) and 'Jarus' (Polan, Zabno, Poland). 'Vasariai' and 'Jarus' are mid-early garlic cultivars, representing softneck morphotypes. Both cultivars are suitable for storage and for spring planting. Non-peeled garlic cloves were surface sterilized using 1% NaOCl solution for 3 min. in a sterile chamber and dried well. The garlic cloves were put in a flask, into which 250 mL of each treatment solution (plant extract + sterile water) was poured separately, and left on a rotary shaker for 30 min. After treatment, the garlic cloves were dried well in a laminar flow cabinet, weighed on scales and put in sterilized aluminum boxes with two layers of sterile filter paper with 30 mL of sterile water. The boxes were covered with food film and transferred to a controlled climate chamber to be kept for four weeks under the following conditions: 21 °C temperature, 60% moisture, 16/8 day/night period. The treatment contained 4 replicates, 8 cloves each. The experiment was performed in randomized blocks and was repeated twice.

2.3. Germination and Physiological Parameters of Garlic Cloves

After 3, 7, 14 and 21 days, the germination percentage of the garlic cloves was evaluated and calculated: germination percentage (%) = germinated cloves/cloves overall. At the end of the experiment, the fresh garlic cloves were weighed on the scales again and the biomass gain was calculated: biomass gain (g) = mass of garlic cloves at the end of the experiment–mass of garlic cloves at the beginning of the experiment. The dry matter of the garlic cloves was evaluated first by weighing the fresh garlic cloves (FW). The garlic cloves collected at the end of the experiment were frozen in liquid nitrogen and freeze-dried using an FD-7 Vacuum Freeze Drying Chamber (SIA Cryogenic and Vacuum Systems, Ventspils, Latvia). After the freeze-dry process, all the material was weighed again and ground. The dry (DW) and fresh weight ratio (DW/FW $\times$ 100) was calculated and expressed as dry matter (%).

2.4. Determination of Macro- and Microelements

The garlic macro- and microelement contents were determined by the microwave digestion technique combined with inductively coupled plasma optical emission spectrometry (ICP-OES). A complete digestion of dry plant material (0.2 g) was achieved with 65% HNO_3 using a microwave digestion system, the Multiwave GO (Anton Paar GmbH, Graz, Austria). The digestion program was as follows: (1) 170 °C reached within 5 min, digested for 10 min; (2) 180 °C reached within 10 min, digested for 10 min. The mineralized samples were diluted to 50 mL with deionized water. The elemental profile was analyzed by means of an ICP—OES spectrometer (Spectro Genesis, SPECTRO Analytical Instruments, Kleve, Germany). The operating conditions employed for the ICP-OES determination were: 1300 W RF power, 12 L min^{-1} plasma flow, 1.0 L min^{-1} auxiliary flow, 0.8 L min^{-1} nebulizer flow, and 1.0 mL min^{-1} sample uptake rate. Calibration standards were prepared by diluting a stock multi-elemental standard solution (1000 mg L^{-1}) in 6.5% (v/v) nitric acid and by diluting stock phosphorus and sulfur standard solutions (1000 mg L^{-1}) in deionized water. The calibration curves for all the studied elements were in the range of 0.01–400 mg L^{-1}.

2.5. Antioxidant Activity Determination

The ABTS antioxidant activity was determined using the radical cation ABTS (2,2′-azino-di-[3-ethylbenzthiazoline sulphonate]) generated following Re et al. [45]. The radical solution was diluted to reach an absorbance value of 0.70 $\pm$ 0.05 at 734 nm, and 10 µL of the garlic extract was added to perform the analysis. Trolox standard solutions were used for comparison, and activity was expressed as mM Trolox equivalent antioxidant capacity (TE g^{-1} fresh weight (FW)) of plant material.

The DPPH antioxidant activity of the methanolic garlic extracts was evaluated spectrophotometrically [46]. The absorbance was scanned at 515 nm after 16 min from the beginning of the reaction, and the DPPH antiradical activity of the garlic cloves was calculated as mM Trolox equivalent antioxidant capacity (TE g^{-1} FW) of plant material.

The FRAP method is based on reducing ferric ion (Fe^{3+}) to ferrous ion (Fe^{2+}). A fresh working solution was prepared by mixing 300 mM, pH 3.6 acetate buffer, 10 mM TPTZ (2,4,6-tripyridyl-s-triazine) solution in 40 mM HCl, and 20 mM $FeCl_3 \times 6H_2O$ at 10:1:1 ($v/v/v$) [47]. Readings of the colored product (ferrous tripyridyl-triazine complex) were then taken at 593 nm after 30 min. A calibration curve was determined using $Fe_2(SO_4)_3$ (Iron (III) sulfate; 97% purity; Sigma-Aldrich, Burlington, MA, USA) as an external standard with a range of concentrations from 0.005 to 0.5 mM (R^2 = 0.99). The antioxidant power was expressed as Fe^{2+} antioxidant capacity (Fe^{2+} µmol g^{-1} FW).

2.6. Total Phenolic Compounds and Flavonoids

The total phenolic compounds in the methanolic garlic extracts were analyzed as reported by [48], using the Folin–Ciocalteu method. The absorbance was scanned at 765 nm after 20 min. from the beginning of the reaction. A calibration curve was prepared using Gallic acid as a standard. Total phenols were expressed as gallic acid equivalents (GAE) g^{-1} FW.

The total flavonoid content in the methanolic garlic extracts was analyzed based on Engida et al.'s report [49]. A calibration curve was prepared using Gallic acid as a standard. The absorbance was scanned at 510 nm after 20 min. from the beginning of the reaction. Total flavonoids were expressed as gallic acid equivalents (GAE) g^{-1} FW.

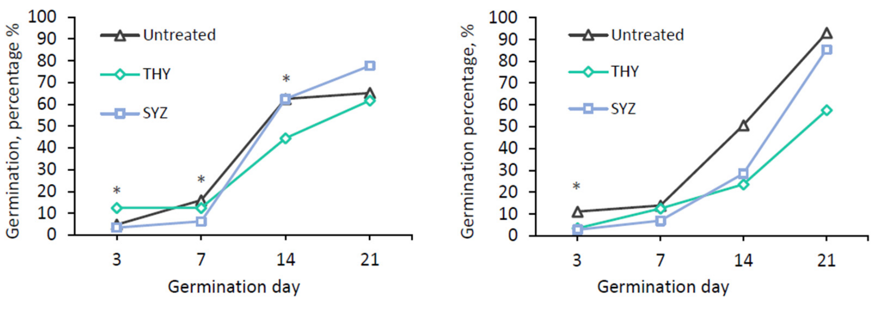
2.7. Statistical Analysis

MS Excel Version 2310 and XLStat 2023 Data Analysis and Statistical Solution for Microsoft Excel (Addinsoft, Paris, France) statistical software were used for data processing. Analysis of variance (ANOVA) was carried out along with the Tukey multiple comparisons test for statistical analyses, $p < 0.05$, with three biological replicates, and conjugated samples (from ten garlic cloves) for biochemical analysis, with three analytical replicates.

3. Results

3.1. Germination and Physiological Parameters of the Garlic Cloves

The plant extracts' impact on the germination of the garlic cloves is presented in Figure 1. The germination peak of both cultivars was from day 7 to day 14. SYZ-treated 'Jarus' garlic cloves had the highest germination percentage, 77.8%; THY-treated cloves had a germination percentage of 61.8%; and the untreated cloves had a germination percentage of 65.3%. While in 'Vasariai' none of the extracts caused higher germination than in the untreated cloves (93.1%), the THY-treated cloves had a germination percentage of 57.4% and the SYZ-treated cloves had a germination percentage of 85.4%. SYZ treatment increased germination in 'Jarus' the most only in the last quarter of the investigation (from day 14 to 21). THY caused slower but constant germination of both cultivars' garlic cloves. In cv. 'Jarus', it was 12.5% on days 3 and 7, 44.4% on day 14 and 61.8% on day 21, and in cv. 'Vasariai', it was 3.5% on day 3, 12.5% on day 7, 23.6% on day 14 and 57.6% on day 21. SYZ treatment of garlic cloves resulted in a higher germination percentage compared to THY in both cultivars. Statistically significant differences were determined on germination days 3, 7 and 14 for 'Jarus' garlic and on germination day 3 for 'Vasariai' garlic.



(a) (b)

Figure 1. Germination percentage (%) of 'Jarus' (**a**) and 'Vasariai' garlic cloves (**b**) with no additional treatment (untreated) and treated with *T. vulgaris* essential oil (THY) and *S. aromaticum* extract (SYZ). "*" sign indicates significant differences between the means according to Tukey (HSD) analysis ($p < 0.05$).

Biomass gain results cover germinated sprouts and root growth together with loss or absorption of moisture, which may affect germination results. To evaluate this parameter correctly, fresh and dried weights of the garlic cloves were registered and dry matter (%) values were calculated. The obtained results are presented in Table 1.

Table 1. Physiological parameters of garlic cloves with no additional treatment (untreated) and treated with *T. vulgaris* essential oil (THY) and *S. aromaticum* extract (SYZ).

Cultivar	Treatment	Biomass Gain, g	Dry Matter, %
Jarus	Untreated	1.332 ± 0.20 a	33.039 ± 0.62 b
	THY	0.543 ± 0.32 b	35.613 ± 0.35 a
	SYZ	0.734 ± 0.27 ab	30.391 ± 0.71 c
Vasariai	Untreated	2.558 ± 0.52 a	28.006 ± 0.79 c
	THY	0.477 ± 0.42 b	37.215 ± 0.55 a
	SYZ	0.914 ± 0.12 b	35.078 ± 0.52 b

Data are presented as means ± standard deviations. Different letters indicate significant differences between the means according to Tukey (HSD) analysis ($p < 0.05$).

Although the germination of 'Jarus' garlic cloves treated with extracts was conditionally equal on the 21st day of germination, biomass gain reveals which extract caused more productive sprouts and root sizes. As can be seen (Table 1, Figure 2), SYZ treatment resulted in a higher biomass gain than THY, almost 0.2 g in 'Jarus' and 0.5 g in 'Vasariai'. Values differed statistically only in 'Vasariai' between extract treatments. Meanwhile, the SYZ treatment biomass gain was around 45% lower compared to untreated cloves in 'Jarus' and almost 65% lower in 'Vasariai'. SYZ-treated garlic had significant differences from untreated garlic in cv. 'Vasariai'. Even though the THY treatment influence is not that noticeable visually (Figure 2), it caused a slightly lower biomass gain in 'Jarus' garlic cloves compared to SYZ. Overall, it was around 60% lower in 'Jarus garlic' and 82% lower in 'Vasariai' garlic compared to untreated cloves. Significant differences from untreated garlic were observed in both cultivars. SYZ-treated cloves had the lowest dry matter percentage (30.39%) in 'Jarus', which suggests that these cloves absorbed more moisture than the others, which could affect higher biomass gain. Meanwhile, THY-treated garlic cloves had the highest dry matter in both cultivars, which was ~8% higher in 'Jarus' and ~33% higher in 'Vasariai' garlic.

Figure 2. 'Jarus' and 'Vasariai' garlic cloves with no additional treatment (untreated) and treated with *T. vulgaris* essential oil (THY) and *S. aromaticum* extract (SYZ) on the 21st day of storage.

'Vasariai' garlic cloves after SYZ treatment had a higher biomass gain than those treated with THY. Although the biomass gain in the THY- and SYZ-treated 'Vasariai' garlic cloves was low, the dry matter was high, which suggests the dryness of these garlic cloves. Overall, none of the extract treatments produced higher biomass than in the untreated garlic cloves. However, the dry matter in the untreated cloves was lower than in the THY-treated 'Jarus' cloves and the THY- and SYZ-treated 'Vasariai' cloves. It is possible that high absorbance of moisture probably influenced high germination, with consequential high biomass gain.

3.2. Macro- and Microelements in the Plant Extract-Treated Garlic Cloves

At the end of the experiment, the macro- and microelements in the garlic cloves were evaluated (Table 2). An interesting tendency is visible in THY-treated garlic cloves of both cultivars, where the majority of determined elements were in higher amounts than in the other garlic cloves. Statistically significant increases in phosphorus, potassium, sulfur, copper, magnesium, manganese and zinc were detected in 'Jarus' garlic cloves, while an increase in phosphorus, sulfur, calcium, copper, magnesium and manganese was found in 'Vasariai'. Lower macro- and microelement contents in the cultivar 'Jarus' were found in the garlic cloves treated with SYZ, and only several macro- and microelements were found in higher contents than in the untreated garlic cloves. In contrast, in 'Vasariai', SYZ treatment resulted in higher elemental contents than in the untreated garlic cloves for the majority of elements, which were close to the THY treatment results.

Table 2. Macro- and microelements (mg L^{-1}) in garlic cloves with no additional treatment (untreated) and treated with *T. vulgaris* essential oil (THY) and *S. aromaticum* extract (SYZ).

Cultivar	Treatment	P	K	S	Ca	Cu	Fe	Mg	Mn	Zn
Jarus	Untreated	0.131 ± 0.002 b	0.005 ± 0.001 b	0.291± 0.002 c	0.014 ± 0.000 a	0.370 ± 0.007 b	0.004 ± 0.000 a	3.167 ± 0.093 b	0.254 ± 0.001 b	0.046 ± 0.003 c
	THY	0.230 ± 0.027 a	0.011 ± 0.003 a	0.358 ± 0.014 a	0.012 ± 0.001 b	0.577 ± 0.052 a	0.002 ± 0.001 b	4.651 ± 0.302 a	0.416 ± 0.036 a	0.124 ± 0.013 a
	SYZ	0.149 ± 0.001 b	0.005 ± 0.001 b	0.292 ± 0.004 b	0.014 ± 0.000 a	0.356 ± 0.049 b	0.004 ± 0.000 a	3.490 ± 0.005 b	0.276 ± 0.000 b	0.082 ± 0.010 b
Vasariai	Untreated	0.123 ± 0.002 c	0.005 ± 0.001 a	0.194 ± 0.002 c	0.010 ± 0.000 c	0.285 ± 0.016 b	0.003 ± 0.000 b	2.753 ± 0.024 c	0.226 ± 0.003 c	0.089 ± 0.006 a
	THY	0.153 ± 0.000 a	0.005 ± 0.000 a	0.277 ± 0.001 a	0.016 ± 0.001 a	0.410 ± 0.011 a	0.004 ± 0.001 a	3.601 ± 0.026 a	0.277 ± 0.002 a	0.088 ± 0.003 a
	SYZ	0.148 ± 0.002 b	0.003 ± 0.001 b	0.249 ± 0.003 b	0.014 ± 0.000 b	0.290 ± 0.001 b	0.004 ± 0.000 a	3.491 ± 0.000 b	0.252 ± 0.001 b	0.059 ± 0.006 b

Data are presented as means ± standard deviations. Different letters indicate significant differences between the means according to Tukey (HSD) analysis ($p < 0.05$).

3.3. Antioxidant Response, Total Phenolic Compounds and Total Flavonoids in the Plant Extract-Treated Garlic Cloves

Antioxidant activity in the garlic cloves is presented in Figure 3a–c. In both cultivars, THY- and SYZ-treated cloves had lower ABTS antiradical activity compared to untreated cloves (24.64 mM TE g^{-1} FW in 'Jarus' and 40.83 mM TE g^{-1} FW in 'Vasariai'). ABTS was ~47% lower after THY treatment (13.05 mM TE g^{-1} FW) and ~7% lower after SYZ treatment (22.92 mM TE g^{-1} FW) in 'Jarus'. Only the THY treatment showed significant differences from untreated garlic with respect to ABTS antiradical activity in this cultivar. In 'Vasariai', ABTS was ~32% lower after THY treatment (27.90 mM TE g^{-1} FW) and ~22% lower after SYZ treatment (31.87 mM TE g^{-1} FW). Moreover, both extract treatments differed significantly from untreated garlic. In contrast, 'Vasariai' cloves treated with THY and SYZ had higher antiradical DPPH (73.54 and 77.09 mM TE g^{-1} FW, respectively) and FRAP (57.06 and 57.37 Fe^{2+} µmol g^{-1} FW, respectively) activities than the untreated cloves (DPPH: 69.63 mM TE g^{-1} FW and FRAP: 50.14 Fe^{2+} µmol g^{-1} FW, respectively). THY treatment resulted in the lowest ABTS, DPPH and FRAP activities in 'Jarus' garlic cloves, which were also lower than in the untreated cloves. No significant differences were observed between DPPH and FRAP antioxidant activities comparing the untreated garlic and plant extract treatments.

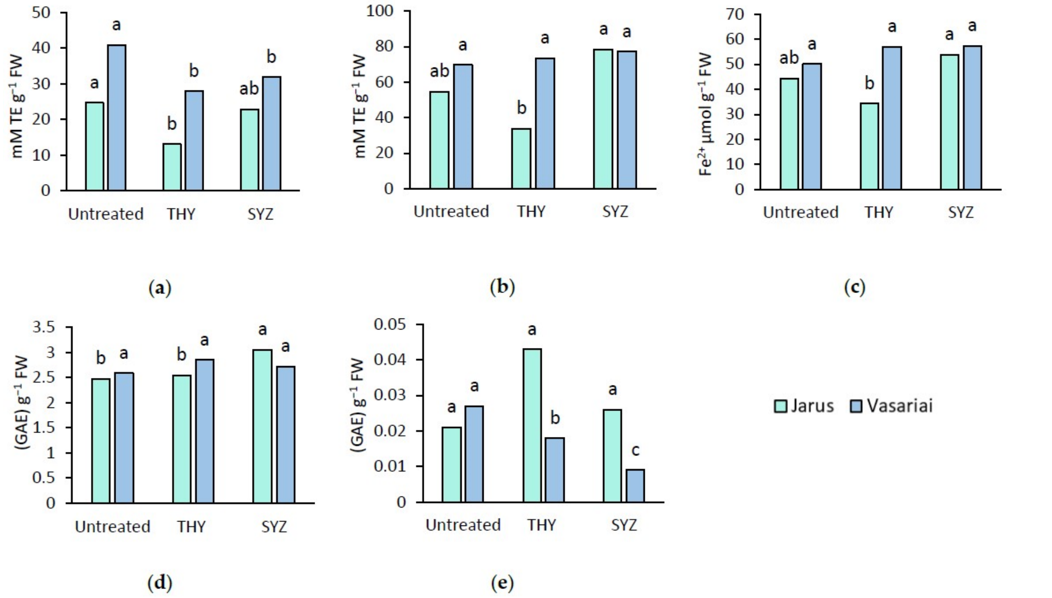


Figure 3. ABTS (**a**), DPPH (**b**) and FRAP (**c**) antioxidant activities; total phenolic compounds (**d**); and total flavonoids (**e**) of 'Jarus' and 'Vasariai' garlic cloves with no additional treatment (untreated) and treated with *T. vulgaris* essential oil (THY) and *S. aromaticum* extract (SYZ). Different letters indicate significant differences between the means according to Tukey (HSD) analysis ($p < 0.05$).

The total phenolic compound (TPC) determinations are presented in Figure 3d. The results were similar between the treatments; however, the extract treatments of both cultivars' garlic cloves resulted in higher total phenolic compound amounts overall. SYZ treatment of 'Jarus' garlic resulted in a TPC value of 3.05 GAE g^{-1} FW, which was significantly higher than that for the untreated garlic cloves (2.47 GAE g^{-1} FW), while the THY treatment resulted in a TPC value for 'Vasariai' of 73.54 GAE g^{-1} FW. Figure 3e demonstrates total flavonoids (TF) in garlic cloves. In 'Vasariai', both THY- and SYZ-treated garlic cloves had significantly lower contents of flavonoids (0.018 and 0.009 GAE g^{-1} FW, respectively) compared to untreated cloves (0.027 GAE g^{-1} FW). In contrast, in 'Jarus', both extracts resulted in 1.2–2 times higher contents of total flavonoids compared to the untreated cloves.

4. Discussion

The treatment of garlic cloves with plant extracts is a prospective, albeit not frequently investigated, method of processing garlic propagation material. Other studies have revealed the important antifungal activity of plant extracts in various plant seeds, with no negative effect on seed germination [10–12,14]. The current study showed that *T. vulgaris* essential oil (THY) and *S. aromaticum* extract (SYZ) exerted versatile effects on garlic clove germination. Statistically significant differences were mostly observed in the germination of cv. 'Jarus' garlic. Despite previous reports of *S. aromaticum*'s phytotoxicity on the germination of weed seeds [50], it did not demonstrate a negative effect on garlic clove germination in the cultivars 'Jarus' and 'Vasariai'. In contrast, *T. vulgaris* decreased garlic clove germination and was high in dry matter, which suggests that this essential oil may increase dryness, for example, through osmotic processes. Uncoated, minimally processed garlic cloves lost approximately 1% of their initial mass after 9 days of storage [22]; however, in our study, we did not experience biomass loss. THY did not completely stop the germination but slowed it down, as we still registered biomass gain, only much lower than in the untreated garlic cloves in both cultivars. A similar effect was observed in carrot seeds with *T. vulgaris*, where mild phytotoxicity was detected in fresh biomass of plants from essential oil-treated seeds [15]. In another study involving cabbage seeds, *T. vulgaris* essential oil exhibited relatively low phytotoxicity and high activity against seed fungal infections [10]. Summing up, both investigated extracts caused initial changes in garlic cloves' germination and physiological parameters, with SYZ being more effective in the case of garlic cloves. Considering the antifungal and antibacterial activity of *S. aromaticum* [31,35,36], it demonstrates a higher potential for application in garlic cloves (or other plant seeds) than *T. vulgaris* essential oil. However, these findings could be proved by performing antifungal assays. We should state that in this study of initial changes in garlic cloves, root development was not evaluated and should be performed in the future, together with further study of plant development from treated garlic cloves after planting. Combining different techniques showed positive effects of seed applications [15], and SYZ and THY should be under consideration for use together with other seed treatments.

Interesting findings were observed in elemental analysis of treated garlic cloves. In both garlic cultivars, THY treatments resulted in increased contents of macro- and microelements. During the germination of garlic cloves, plant extracts serve as specific media, with only a small amount of water added to prevent drying. Given the individual compositions of bioactive compounds, plant extracts could influence initial changes in developing garlic cloves, as they have biological differences from seeds and may be more easily affected at the surface by biological compounds from plant extracts. Plasma treatment (time: 60 s) increased the elemental composition of nitrogen by up to 1.9%, calcium by up to 1.2% and potassium by up to 0.6% in peeled garlic cloves, which could be correlated with higher etching of organic material [21]. In this study, THY, a strong organic substance, may have influenced changes in garlic clove surfaces or deeper layers after soaking, which resulted in increased elemental contents. SYZ treatment had a neutral effect on 'Jarus' garlic and a stronger effect on 'Vasariai'. These findings suggest that the biological structure of different cultivars' garlic cloves should also be considered.

Phenolic compounds play a vital role in the protective mechanisms of plants and increase during stress conditions [44]. Increased antioxidant activity and phenolic compound contents after treatment with extracts could provide an effective natural way to enhance the defense response of plants, fruits and vegetables. There are almost no data on the initial changes in germinating garlic cloves' antioxidant compounds. In this study, we observed a tendency for the THY treatment of 'Jarus' garlic cloves to cause lower antioxidant activity in garlic cloves compared to untreated ones. The opposite effect was observed with SYZ, as it increased DPPH and FRAP activities, but not ABTS. We observed statistically significant changes in ABTS antioxidant activity. In cv. 'Jarus', THY significantly decreased ABTS antioxidant activity. It should be stated that no significant differences were observed between values of DPPH and FRAP antioxidant activities in the untreated and extract-treated garlic. In 'Vasariai', both extracts slightly increased DPPH and FRAP activities and lowered ABTS. Initial changes in antioxidant activity were evident after treatment with the extracts and depended on the cultivar. In Skrovankova et al.'s study [51], DPPH antioxidant activity in different types of garlic ranged from 93.2 to 190.6 mg TE 100 g^{-1} of fresh samples, while ABTS ranged from 331.6 to 525.2 mg TE 100 g^{-1}. In a study of long-term storage of garlic, DPPH and FRAP activities increased through time and were highest at 8 weeks [6]. In another study of different types of garlic, ABTS antioxidant activity varied from 0.784 to 1.492 µmol TE g^{-1}, DPPH varied from 0.664 to 1.578 µmol TE g^{-1}, TPC varied intensely from 212.86 to 623.72 µg GAE g^{-1}, and TF varied from 119.56 to 353.58 µg QE g^{-1} [52].

In our case, slightly higher (2.8–23.5%) total phenolic compound (TPC) contents were observed in the plant extract-treated garlic cloves than in the untreated ones. TPC change was statistically significant only in SYZ-treated cv. 'Jarus' garlic. Our obtained values were lower than the range of 17.16–42.53 mg GAE g^{-1} dry weight which was observed in garlic bulbs of 32 different cultivars [53]. Also, higher total polyphenols were determined—in a range from 92.2 to 119.6 mg GAE 100 g^{-1} fresh weight—in various *A. sativum* and *A. ursinum* cultivar samples [51]. Although variability in TPC and total flavonoids (TF) was observed between different cultivars of garlic [53], our results for untreated garlic were similar between the cultivars 'Jarus' and 'Vasariai' for TPC (2.47 and 2.59 GAE g^{-1} FW) and TF (0.021 and 0.027 GAE g^{-1} FW). Similar results were obtained after UV-C treatment in peeled garlic cloves. After 15 days of storage, the total phenolic compound content in garlic treated with irradiation of UV-C was 507.98–559.08 GAE mg kg DW^{-1}, whereas in the untreated garlic, it was 502.60 GAE mg kg DW^{-1} [28]. There is no other study of plant extracts' impact on phenolics in garlic cloves. Based on our results, we suggest that SYZ and THY demonstrate initial phenolic-enhancing effects in short-term germination processes of 21 days. Chen et al. [53] determined TF from 0.15 to 0.60 mg rutin DW g^{-1} in different cultivars of garlic. Flavonoid content increased only in 'Jarus' cultivar garlic cloves after treatment with extracts. In another study, coating with carvacrol chemotype thyme oil activated plant signaling at the early vegetative growth stage and increased flavonoids at a further plant growth stage [17]. Total flavonoid content increased significantly after treatment with 2 kJm^{-2} UV-C and within the first 5 days of storage at room temperature. However, after 15 days of storage in the control treatment, TF deteriorated by around 4% from initial levels, while in the UV-C treatments, the deterioration ranged from approximately 2 to 11%, depending on the dose [28]. We found total flavonoids in the extract-treated 'Vasariai' garlic cloves to be 37–67% significantly lower than in the untreated ones at 21 days of germination. As other authors have stated, flavonoids depend on storage time [28]; in our case, we could also suggest dependence on the cultivar.

In a phytotoxicity study of *S. aromaticum*, *T. vulgaris* and other extracts expressed varied phytotoxic effects, and the authors suggested dependence on the phytochemical composition of the extracts [38]. We could agree with the statement, as, in our results, THY had a slight phytotoxic effect on garlic germination, and a much higher amount of thymol was used [39] than in the Rolli et al. [38] study, where *T. vulgaris* did not express as high a phytotoxic effect on weed seeds as expected. Phytochemical composition could also influence changes in garlic clove organic compounds, resulting in changed secondary metabolites. Another assumption would be that plant extracts have selective phytotoxicity and could affect vegetable seeds, garlic cloves and weed seeds differently.

There are several studies investigating the further development of garlic under field conditions. Chilling treatment significantly improved various biological parameters of garlic grown in soil [24]. Our results indicate initial changes in garlic cloves' physiological parameters, including antioxidant capacity, total phenolic compounds, total flavonoids, and macro- and microelements, which may be dependent on the cultivar, plant extract chemical composition and other environmental factors. Evidently, soaking in SYZ and THY affects garlic cloves during germination, and various techniques involving these extracts should be investigated in the future. Examination of the response in garlic roots and seedlings in the soil or in the field could further extend the findings of the current study.

Author Contributions: Conceptualization, L.D.; methodology, L.D. and K.L.; software, K.L.; validation, L.D. and K.L.; formal analysis, L.D.; investigation, L.D., K.L. and D.J.; resources, L.D., K.L., D.J., A.V. and R.K.; data curation, L.D.; writing—original draft preparation, L.D.; writing—review and editing, L.D., K.L., A.V. and R.K.; visualization, L.D. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data is contained within the article.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Mancini, V.; Romanazzi, G. Seed treatments to control seedborne fungal pathogens of vegetable crops. *Pest Manag. Sci.* **2014**, *70*, 860–868. [CrossRef]
2. Abdel-Rasheed, K.G.; Moustafa, Y.M.M.; Hassan, E.A.; Abdel-Ati, Y.Y.; Gadel-Hak, S.H. Traits under laboratory conditions to identify garlic genotypes suitable for organic agriculture. *Egypt. J. Agric. Res.* **2016**, *94*, 73–88. [CrossRef]
3. Kaur, J.; Sharma, R.; Sharma, M.; Chhabra, R. Evaluation of physiological and biochemical changes in garlic (*Allium sativum* L.) bulbs stored under different temperature conditions. *Plant Physiol. Rep.* **2021**, *26*, 412–418. [CrossRef]
4. Karklelienė, R.; Juškevičienė, D.; Radzevičius, A.; Sasnauskas, A. Productivity and adaptability of the new carrot and garlic cultivars in Lithuania. *Zemdirb.-Agric.* **2018**, *105*, 165–170. [CrossRef]
5. Divya, B.J.; Suman, B.; Venkataswamy, M.; Thyagaraju, K. a Study on Phytochemicals, Functional Groups and Mineral Composition of *Allium sativum* (Garlic) Cloves. *Int. J. Curr. Pharm. Res.* **2017**, *9*, 42–45. [CrossRef]
6. Li, F.M.; Li, T.; Li, W.; Yang, L.D. Changes in antioxidant capacity, levels of soluble sugar, total polyphenol, organosulfur compound and constituents in garlic clove during storage. *Ind. Crops Prod.* **2015**, *69*, 137–142. [CrossRef]
7. Mondani, L.; Chiusa, G.; Battilani, P. Chemical and biological control of Fusarium species involved in garlic dry rot at early crop stages. *Eur. J. Plant Pathol.* **2021**, *160*, 575–587. [CrossRef]
8. Moharam, M.H.A.; Farrag, E.S.H.; Mohamed, M.D.A. Pathogenic fungi in garlic seed cloves and first report of *Fusarium proliferatum* causing cloves rot of stored bulbs in upper Egypt. *Arch. Phytopathol. Plant Prot.* **2013**, *46*, 2096–2103. [CrossRef]
9. Gonçalves, D.C.; de Queiroz, V.T.; Costa, A.V.; Lima, W.P.; Belan, L.L.; Moraes, W.B.; Iorio, N.L.P.P.; Póvoa, H.C.C. Reduction of Fusarium wilt symptoms in tomato seedlings following seed treatment with *Origanum vulgare* L. essential oil and carvacrol. *Crop Prot.* **2021**, *141*, 105487. [CrossRef]
10. Van Der Wolf, J.M.; Birnbaum, Y.; Van Der Zouwen, P.S.; Groot, S.P.C. Disinfection of vegetable seed by treatment with essential oils, organic acids and plant extracts. *Seed Sci. Technol.* **2008**, *36*, 76–88. [CrossRef]
11. Chandrashekhara; Niranjan Raj, S.; Manjunath, G.; Deepak, S.; Shekar Shetty, H. Seed treatment with aqueous extract of *Viscum album* induces resistance to pearl millet downy mildew pathogen. *J. Plant Interact.* **2010**, *5*, 283–291. [CrossRef]
12. da Silva, A.C.; de Souza, P.E.; Machado, J.d.C.; da Silva, B.M.; Pinto, J.E.B.P. Effectiveness of essential oils in the treatment of *Colletotrichum truncatum*-infected soybean seeds. *Trop. Plant Pathol.* **2012**, *37*, 305–313. [CrossRef]
13. Mbega, E.R.; Mortensen, C.N.; Mabagala, R.B.; Wulff, E.G. The effect of plant extracts as seed treatments to control bacterial leaf spot of tomato in Tanzania. *J. Gen. Plant Pathol.* **2012**, *78*, 277–286. [CrossRef]
14. Ahmed, M.; Hossain, M.; Hassan, K.; Kanta Dash, C. Efficacy of Different Plant Extract on Reducing Seed Borne Infection and Increasing Germination of Collected Rice Seed Sample. *Univers. J. Plant Sci.* **2013**, *1*, 66–73. [CrossRef]
15. Lopez-Reyes, J.G.; Gilardi, G.; Garibaldi, A.; Gullino, M.L. In vivo Evaluation of Essential Oils and Biocontrol Agents Combined with Hot Water Treatments on Carrot Seeds against *Alternaria radicina*. *J. Phytopathol.* **2016**, *164*, 131–135. [CrossRef]
16. Masangwa, J.I.G.; Kritzinger, Q.; Aveling, T.A.S. Germination and seedling emergence responses of common bean and cowpea to plant extract seed treatments. *J. Agric. Sci.* **2017**, *155*, 18–31. [CrossRef]
17. Ben-Jabeur, M.; Vicente, R.; López-Cristoffanini, C.; Alesami, N.; Djébali, N.; Gracia-Romero, A.; Serret, M.D.; López-Carbonell, M.; Araus, J.L.; Hamada, W. A novel aspect of essential oils: Coating seeds with thyme essential oil induces drought resistance in wheat. *Plants* **2019**, *8*, 371. [CrossRef]
18. Moumni, M.; Allagui, M.B.; Mezrioui, K.; Ben Amara, H.; Romanazzi, G. Evaluation of seven essential oils as seed treatments against seedborne fungal pathogens of *Cucurbita maxima*. *Molecules* **2021**, *26*, 2354. [CrossRef]
19. Holc, M.; Primc, G.; Iskra, J.; Titan, P.; Kovač, J.; Mozetič, M.; Junkar, I. Effect of oxygen plasma on sprout and root growth, surface morphology and yield of garlic. *Plants* **2019**, *8*, 462. [CrossRef]
20. Liu, H.J.; Huang, C.P.; Tong, P.J.; Yang, X.; Cui, M.M.; Cheng, Z.H. Response of axillary bud development in garlic (*Allium sativum* L.) to seed cloves soaked in gibberellic acid (GA3) solution. *J. Integr. Agric.* **2020**, *19*, 1044–1054. [CrossRef]
21. Holc, M.; Junkar, I.; Primc, G.; Iskra, J.; Titan, P.; Grobelnik Mlakar, S.; Mozetic, M. Improved Sprout Emergence of Garlic Cloves by Plasma Treatment. *Plasma Med.* **2016**, *6*, 325–338. [CrossRef]
22. Geraldine, R.M.; Soares, N.d.F.F.; Botrel, D.A.; de Almeida Gonçalves, L. Characterization and effect of edible coatings on minimally processed garlic quality. *Carbohydr. Polym.* **2008**, *72*, 403–409. [CrossRef]
23. Srivastava, A.; Rao, D.P. Enhancement of seed germination and plant growth of wheat, maize, peanut and garlic using multiwalled carbon nanotubes. *Eur. Chem. Bull.* **2014**, *3*, 502–504. [CrossRef]
24. Wu, C.; Wang, M.; Dong, Y.; Cheng, Z.; Meng, H. Growth, bolting and yield of garlic (*Allium sativum* L.) in response to clove chilling treatment. *Sci. Hortic.* **2015**, *194*, 43–52. [CrossRef]
25. Youssef, N.S. Growth and bulbing of garlic as influenced by low temperature and storage period treatments. *World Rural Obs.* **2013**, *5*, 47–57.
26. Cantwell, M.I.; Kang, J.; Hong, G. Heat treatments control sprouting and rooting of garlic cloves. *Postharvest Biol. Technol.* **2003**, *30*, 57–65. [CrossRef]
27. De Santis, D.; Garzoli, S.; Vettraino, A.M. Effect of gaseous ozone treatment on the aroma and clove rot by *Fusarium proliferatum* during garlic postharvest storage. *Heliyon* **2021**, *7*, e06634. [CrossRef]
28. Park, M.H.; Kim, J.G. Low-dose UV-C irradiation reduces the microbial population and preserves antioxidant levels in peeled garlic (*Allium sativum* L.) during storage. *Postharvest Biol. Technol.* **2015**, *100*, 109–112. [CrossRef]

29. Andresen, M.; Cedergreen, N. Plant growth is stimulated by tea-seed extract: A new natural growth regulator? *HortScience* **2010**, *45*, 1848–1853. [CrossRef]
30. Khan, S.; Basra, S.M.A.; Nawaz, M.; Hussain, I.; Foidl, N. Combined application of moringa leaf extract and chemical growth-promoters enhances the plant growth and productivity of wheat crop (*Triticum aestivum* L.). *S. Afr. J. Bot.* **2020**, *129*, 74–81. [CrossRef]
31. Sharma, A.; Rajendran, S.; Srivastava, A.; Sharma, S.; Kundu, B. Antifungal activities of selected essential oils against *Fusarium oxysporum* f. sp. lycopersici 1322, with emphasis on *Syzygium aromaticum* essential oil. *J. Biosci. Bioeng.* **2017**, *123*, 308–313. [CrossRef] [PubMed]
32. Wei, Y.; Shao, X.; Wei, Y.; Xu, F.; Wang, H. Effect of preharvest application of tea tree oil on strawberry fruit quality parameters and possible disease resistance mechanisms. *Sci. Hortic.* **2018**, *241*, 18–28. [CrossRef]
33. Mulugeta, T.; Muhinyuza, J.B.; Gouws-Meyer, R.; Matsaunyane, L.; Andreasson, E.; Alexandersson, E. Botanicals and plant strengtheners for potato and tomato cultivation in Africa. *J. Integr. Agric.* **2020**, *19*, 406–427. [CrossRef]
34. Ramírez, P.G.; Ramírez, D.G.; Mejía, E.Z.; Ocampo, S.A.; Díaz, C.N.; Rojas Martínez, R.I. Extracts of *Stevia rebaudiana* against *Fusarium oxysporum* associated with tomato cultivation. *Sci. Hortic.* **2020**, *259*, 108683. [CrossRef]
35. Šernaitė, L.; Rasiukevičiutė, N.; Valiuškaitė, A. The extracts of cinnamon and clove as potential biofungicides against strawberry grey mould. *Plants* **2020**, *9*, 613. [CrossRef] [PubMed]
36. Achimón, F.; Brito, V.D.; Pizzolitto, R.P.; Ramirez Sanchez, A.; Gómez, E.A.; Zygadlo, J.A. Chemical composition and antifungal properties of commercial essential oils against the maize phytopathogenic fungus *Fusarium verticillioides*. *Rev. Argent. Microbiol.* **2021**, *53*, 292–303. [CrossRef]
37. Šernaitė, L.; Rasiukevičiūtė, N.; Dambrauskienė, E.; Viškelis, P.; Valiuškaitė, A. Biocontrol of strawberry pathogen *Botrytis cinerea* using plant extracts and essential oils. *Zemdirb.-Agric.* **2020**, *107*, 147–152. [CrossRef]
38. Rolli, E.; Marieschi, M.; Maietti, S.; Sacchetti, G.; Bruni, R. Comparative phytotoxicity of 25 essential oils on pre- and post-emergence development of *Solanum lycopersicum* L.: A multivariate approach. *Ind. Crops Prod.* **2014**, *60*, 280–290. [CrossRef]
39. Morkeliūnė, A.; Rasiukevičiūtė, N.; Šernaitė, L.; Valiuškaitė, A. The use of essential oils from thyme, sage and peppermint against colletotrichum acutatum. *Plants* **2021**, *10*, 114. [CrossRef]
40. Chrapačienė, S.; Rasiukevičiūtė, N.; Valiuškaitė, A. Control of Seed-Borne Fungi by Selected Essential Oils. *Horticulturae* **2022**, *8*, 220. [CrossRef]
41. Dėnė, L.; Laužikė, K.; Rasiukevičiūtė, N.; Chrapačienė, S.; Brazaityte, A.; Viršilė, A.; Vaštakaitė-Kairienė, V.; Miliauskienė, J.; Sutulienė, R.; Samuolienė, G.; et al. Defense response of strawberry plants against *Botrytis cinerea* influenced by coriander extract and essential oil. *Front. Plant Sci.* **2023**, *13*, 1098048. [CrossRef] [PubMed]
42. Maksoud, S.A.; Gad, K.I.; Hamed, E.Y.M. The potentiality of biostimulant (*Lawsonia inermis* L.) on some morpho-physiological, biochemical traits, productivity and grain quality of *Triticum aestivum* L. *BMC Plant Biol.* **2023**, *23*, 95. [CrossRef] [PubMed]
43. Findura, P.; Kocira, S.; Hara, P.; Pawłowska, A.; Szparaga, A.; Kangalov, P. Extracts from Artemisia vulgaris L. in Potato Cultivation—Preliminary Research on Biostimulating Effect. *Agriculture* **2020**, *10*, 356. [CrossRef]
44. Khaliq, G.; Ramzan, M.; Baloch, A.H. Effect of *Aloe vera* gel coating enriched with *Fagonia indica* plant extract on physicochemical and antioxidant activity of sapodilla fruit during postharvest storage. *Food Chem.* **2019**, *286*, 346–353. [CrossRef] [PubMed]
45. Re, R.; Pellegrini, N.; Proteggente, A.; Pannala, A.; Yang, M.; Rice-Evans, C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radic. Biol. Med.* **1999**, *26*, 1231–1237. [CrossRef] [PubMed]
46. Sharma, O.P.; Bhat, T.K. DPPH antioxidant assay revisited. *Food Chem.* **2009**, *113*, 1202–1205. [CrossRef]
47. Benzie, I.F.; Strain, J.J. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": The FRAP assay. *Anal. Biochem.* **1996**, *239*, 70–76. [CrossRef]
48. Ainsworth, E.; Gillespie, K. Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin–Ciocalteu reagent. *Nat. Protoc.* **2007**, *2*, 875–877. [CrossRef]
49. Engida, A.M.; Kasim, N.S.; Tsigie, Y.A.; Ismadji, S.; Huynh, L.H.; Ju, Y.H. Extraction, identification and quantitative HPLC analysis of flavonoids from sarang semut (*Myrmecodia pendan*). *Ind. Crops Prod.* **2013**, *41*, 392–396. [CrossRef]
50. Oliveira, M.S.; Costa, W.A.; Pereira, D.S.; Botelho, J.R.S.; Menezes, T.O.A.; Andrade, E.H.A.; Silva, S.H.M.; Filho, A.P.S.S.; Carvalho Junior, R.N. Chemical composition and phytotoxic activity of clove (*Syzygium aromaticum*) essential oil obtained with supercritical CO_2. *J. Supercrit. Fluids* **2016**, *118*, 185–193. [CrossRef]
51. Škrovánková, S.; Mlček, J.; Snopek, L.; Planetová, T. Polyphenols and antioxidant capacity in different types of garlic. *Potravin. Slovak J. Food Sci.* **2018**, *12*, 267–272. [CrossRef] [PubMed]
52. Dinu, M.; Soare, R.; Babeanu, C.; Botu, M. Evaluation of Productivity Components and Antioxidant Activity of Different Types of Garlic Depending on the Morphological Organs. *Horticulturae* **2023**, *9*, 1039. [CrossRef]
53. Chen, S.; Shen, X.; Cheng, S.; Li, P.; Du, J.; Chang, Y.; Meng, H. Evaluation of Garlic Cultivars for Polyphenolic Content and Antioxidant Properties. *PLoS ONE* **2013**, *8*, e79730. [CrossRef] [PubMed]