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Evaluation of Physiological and Quality Parameters of Green Asparagus Spears Subjected to Three Treatments against the Decline Syndrome

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Abstract: Green asparagus (*Asparagus officinalis* L.) is a widely grown and consumed crop which provides high-level nutritional interest. In recent years, the decline syndrome in asparagus plantations has been rapidly augmenting. This syndrome causes the early death of whole plants, also negatively affecting the new replanting. Decline causes notable economic losses in the sector. The objective of this work was to verify the effect of different treatments against asparagus decline syndrome on the physiological parameters and nutritional quality of the spears. To meet the objective, four different treatments were applied to asparagus plots strongly affected by decline syndrome: (T1) untreated control soil, (T2) biofumigation with *Brassica* pellets, (T3) biofumigation with chicken manure pellets, and (T4) disinfestation of the soil with Dazomet. The cumulative yield and physiological and quality parameters of green asparagus spears were studied. Thus, malondialdehyde (MDA), photosynthetic pigments, glutathione (GSH), ascorbate (AsA), total phenols, flavonoids, anthocyanin, antioxidant test, mineral nutrients, and the amino acid profile were measured on asparagus spears. The results showed that the *Brassica* pellets and Dazomet treatments were the most effective against the damage caused by the decline syndrome. However, it would be necessary to monitor the evolution in the following years.

Keywords: allelopathy; green asparagus; chicken manure pellets; *Brassica* manure pellets; Dazomet; decline syndrome

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1. Introduction

Asparagus is a very important crop, widely cultivated in the world, with a harvested area of 1,623,741 ha. Specifically, in Spain the harvested area is 14,084 ha, being the sixth largest producer in the world and the second in Europe after Germany [1]. Almost 56% of the cultivated area in Spain belongs to the province of Andalusia [2]. Asparagus is a high-quality food due to its antioxidant content, with the effect of preventing hypertension. It also has anti-tumor effects [3] due to its high content of compounds that intervene in the detoxification of reactive oxygen species (ROS). Some of the antioxidant compounds that are prominent in asparagus are phenolic compounds, ascorbate (AsA), carotenoids, and glutathione (GSH), among others [4].

The asparagus growing cycle traditionally lasts approximately 15 years in the field, but recently, this time has been severely shortened because of the asparagus plantation decline syndrome. Thus, this syndrome, which causes the premature death of whole asparagus plants, has increased in all asparagus-producing areas of the world [5]. When plantations affected by decline were replanted with new asparagus plants transplanted into the same soil severely affected by decline, they were also negatively affected [6,7]. This problem

leads to serious economic losses in the sector. Specifically, the Andalusia region is strongly affected by this syndrome and the harvested area has decreased by 21.5% from 2018 to the present, i.e., from 10,071 ha in 2018 to 7,555 ha in February 2021 [2].

A combination of biotic and abiotic stress is considered to be the main cause of asparagus decline and the problem in asparagus replanting. Asparagus infection by pathogens of *Fusarium* species was reported to be the main biotic stressor [8]. In fact, the decline syndrome is characterized by crown and root rot (CRR) that has been associated with several species of *Fusarium* [5,9,10]. However, the association of decline syndrome with *Fusarium* is not always evident, and among other possible causes, researchers point out the accumulation of autotoxins exuded by the roots of the asparagus plants themselves into the soil [9,11,12]. Although abiotic factors are mainly related to the release of allelochemical compounds, the nutrient imbalance, deterioration of soil physicochemical conditions, agronomic practices, and excessive harvest pressure could be important contributing factors to decline syndrome [8]. Furthermore, an interaction between allelopathic compounds and CRR could predispose plants to the development of decline syndrome [13]. However, although some studies related compounds such as saponins, tryptophan, and phenolics, the specific effect of these substances in asparagus cultivation has not been sufficiently studied and their possible interactions with soil microbes or the plant defenses are still unknown [8].

Regardless of the cause, the most obvious way to counteract the replanting problems is crop rotation or changing the cultivation locations; nevertheless, farmers do not always have this possibility [14]. A potential alternative is the fumigation of soils with chemicals such as Dazomet or metam sodium. Dazomet emerged as an alternative to methyl bromide, a fungicide that causes serious impacts on the environment by affecting the ozone layer, with various studies highlighting its lower toxicity and effectiveness in pest control [14]. For example, Gamiel et al. [15] showed that CRR in tomato plants, caused by *Fusarium oxysporum*, was significantly reduced when Dazomet was applied [16]. Dazomet reacts with water and chemical compounds present in the soil, leading to the production of methyl isothiocyanate (MITC), carbon sulfide (CS_2), and other volatile products. MITC is a toxic substance for nematodes, fungi, bacteria, insects, and weeds [17]. Furthermore, it is relatively soluble in water and diffuses slowly into the soil [18]. The soil, to be treated, is also usually covered with plastic sheets, thereby improving the effectiveness of Dazomet and reducing its application rate [16].

Biofumigation is an alternative option to Dazomet in order to minimize the use of chemicals in agriculture. This technique that uses biological material results in more sustainable soil disinfection and takes advantage of its toxic metabolites for disinfection while improving the soil characteristics. For instance, chicken manure pellets and *Brassica* pellets are two broadly employed treatments that could be useful against the problem of asparagus decline. Thus, the use of plant species from the Brassicaceae family has been studied widely, and its use is widespread, particularly due to the presence of metabolites such as glucosinolates (GSL) that release bioactive gases (i.e., isothiocyanates) after enzymatic hydrolysis [19]. Moreover, laboratory tests with three types of amendments (*Brassica* pellets, chicken manure pellets, and alperujo compost) demonstrated that chicken manure pellets were the most effective in mitigating the effects of *F. oxysporum* and *F. solani* [20], although these results have not been confirmed in the field. In addition to the important role that these organic amendments have as disinfectants in the control of soil pathogens, the application of biofertilizers with organic fertilizers increases their efficiency, the fertility of the soil, and the productivity of the crops. In addition, biofertilizers incorporate live microorganisms that are administered to the soil as inoculants to supply certain nutrients to the plants [21]. Likewise, biofertilizers provide other environmental and agronomic advantages, such as weed control, control of diurnal and seasonal fluctuations in soil temperature, protection against soil erosion, and greater water use efficiency. They can also reduce the toxicity of metals and increase P availability to crops [21].

In the present experiment, we study the effect of *Brassica* pellets, chicken manure pellets, and Dazomet on asparagus plots affected by decline syndrome. These treatments were chosen given all their advantages on field crops as previously described. We applied them to study their efficacy against decline syndrome and the replant problem in asparagus. In addition, some important parameters, indicative of its degree of stress due to decline and its nutritional quality for human consumption, were also analyzed. We think that these treatments could alleviate the symptoms of decline syndrome while improving the yield and quality of the asparagus. Therefore, the objective of this work was to verify the effect of different treatments against asparagus decline syndrome on the physiological parameters and nutritional quality of the asparagus spears.

2. Materials and Methods

2.1. Plant Material and Growth Conditions

As plant material, we used *Asparagus Officinalis* L., var. Grande, from the seed trading company (INTERSEMILLAS S.A., Valencia, Spain), whose seeds were sown in a seed nursery on 7 March 2018 in forestry trays. The transplantation to the trial plots affected by decline syndrome was carried out on 11 June 2018. The planting frame used was 1.3 m between the lines and 0.33 m between the plants. The traditional agronomic practices, as carried out by the local farmers, were performed for crop management. Irrigation was done, by flooding, with the surface water. The crop fertilization was achieved through the application of the fertilizer units of macro and micronutrients to cover the nutritional needs of plants. The commercial fertilizers were: ammonium nitrosulphate (FERTIBERIA S.A., Madrid, Spain), Multi-K® (Haifa Iberia, Madrid, Spain).

2.2. Experimental Design, Treatments, and Plant Sampling

All plots had an area of 12 × 4.2 m^2. The experimental design comprised a randomized complete block with 4 treatments, 4 repetitions per treatment, thus a total of 16 plots. Each elementary plot was totally isolated, surrounded on its perimeter by an irrigation dike built in soil 50 cm high and 50 cm wide, to avoid contamination between the different plots by irrigation water. They were also separated at a distance of 2 m from each other. Trial was carried out within the asparagus-growing area in the province of Granada, specifically in the town of Loja (37°10′23.4″ N 4°10′49.1″ W).

The treatments carried out were: (T1) untreated control; (T2) biofumigation with *Brassica* pellets of Brassica *carinata* seeds (5000 kg/ha), Biofence® (Triumph Italia of Cerealtoscana Group, Livorno, Italy), composed of 6% N, 7% P_2O_5, 2.6% K_2O, 4.4% SO_3, 0.9% MgO, 84.2% MO; (T3) biofumigation with chicken manure pellets (5000 kg/ha), Riger® (Ferm o Feed, Helmond, The Netherlands), composed of 4% N, 3,4% P_2O_5, 3,2% K_2O, 0,9% MgO, 65% MO, 8% Ca; and (T4) disinfection with Dazomet (600 kg/ha), Basamid® (Certiseurope, Elche, Spain), composed of Dazomet 98%.

All the plots where the tests were carried out showed strong symptoms of the decline syndrome, where almost all of the pre-trial crop was dead due to the decline syndrome. On 4 May 2018, the *Brassica* pellet and chicken manure pellet treatments were applied manually and directly to the soil in a homogeneous way. For Dazomet application, a duly calibrated manual fertilizer spreader was used. Subsequently, all the treatments were incorporated into the soil using rotavator plowing. Finally, a light irrigation was applied to increase the moisture of the soil. Once the irrigation was finished, the elementary plots were covered with a transparent Totally Impermeable Film (TIF) of 120 gages for 25 days. After this period, the film was removed and the asparagus plants were transplanted.

The sampling was carried out coinciding with the periods of maximum production of spears, specifically the last week of harvest. All the sampled spears from the 4 plots of a treatment were mixed to obtain a homogeneous sample. A part of this sample was frozen at −40 °C (LTF-420, Dairei, Denmark) and was used later for biochemical analysis. The other part of the sample, after drying in a forced-air oven (DAF-635, RAYPA, Terrassa, Spain), was used to determine the concentration of mineral elements.

2.3. Yield

Harvesting was undertaken between 26 February and 19 March, to avoid exhaustion as these were small first-year plants.

To obtain the accumulated average yield value of asparagus, a harvest area was delimited in each elementary plot where one harvest was carried out per week. To obtain this value, only the fresh weight of the commercial spears, cut to 24 cm in length, was taken into account as green asparagus spears are marketed. The yield of each elemental plot was summed up and then the average for each treatment was calculated, followed by the accumulated average production of green asparagus.

2.4. Determination of Malondialdehyde (MDA) Concentration

For the extraction of MDA, 0.1 g of frozen plant material was macerated in 1 mL of 50 mM buffer (0.07% NaH_2PO_4 2 H_2O and 1.6% Na_2HPO_4 12 H_2O), and the extract was centrifuged at 20,000× *g* for 25 min. Subsequently, an aliquot of the supernatant was mixed in test tubes with 4 mL of 20% trichloroacetic acid containing 0.5% thiobarbituric acid. The resulting mixture was heated at 95 °C for 30 min and then rapidly cooled in an ice bath. The samples were then centrifuged (Digicen 20-R, Ortoalresa, Spain) at 10,000× *g* for 10 min and the absorbance of the supernatant was measured at 532 nm using a spectrophotometer (Infinite 200 Nanoquant, Tecan, Switzerland). The value for non-specific absorption at 600 nm was subtracted from the reading obtained at 532 nm as reported previously [22]. The centrifuge and the spectrophotometer were also used in the following analytical determinations.

2.5. Determination of Photosynthetic Pigment Concentration

The concentration of photosynthetic pigments was analyzed following the method of Wellburn [23] with minor modifications. A total of 0.1 g of frozen asparagus spears was macerated in 1 mL of methanol. Subsequently, it was centrifuged for 5 min at 2200× *g*. The absorbance was measured at 3 different wavelengths: 653 nm, 666 nm, and 470 nm. The calculation was carried out as follows:

$$\text{Chlorophyll a (Chl } a\text{)} = 15.65 \times \text{A666} - 7.34 \times \text{A653}$$

$$\text{Chlorophyll b (Chl } b\text{)} = 27.05 \times \text{A653} - 11.21 \times \text{A666}$$

$$\text{Carotenes} = (1000 \times \text{A470} - 2.86 \times \text{Chl } a - 129.2 \times \text{Chl } b)/221$$

In the results, the units of these bioindicators are expressed in milligram per gram of fresh weight (mg g^{-1} FW).

2.6. Determination of Reduced Glutathione (GSH) and Total Ascorbate (AsA) Concentrations

For the extraction and quantification of AsA, the method of Law et al. [24] was followed. This method is based on the reduction of Fe^{3+} to Fe^{2+} by AsA in an acidic solution. A total of 0.1 g of turion was homogenized in liquid N_2 with 5 mL of 5% (*w*/*v*) metaphosphoric acid and centrifuged at 4 °C for 15 min. Then, 0.2 mL of supernatant was added to a test tube along with 0.5 mL of 150 mM sodium phosphate buffer (pH 7.5) and 0.1 mL of 10 mM dithiothreitol (DTT). The mixture was shaken and incubated at room temperature in the dark for 10 min. Then, 0.1 mL of 0.5% (*w*/*v*) N-ethylmaleimide was added together with 0.4 mL of 44% (*v*/*v*) orthophosphoric acid, 0.4 mL of 2.20–4% (*w*/*v*) bipyridyl in 70% ethanol and 0.2 mL of 3% (*w*/*v*) $FeCl_3$. The resulting reaction mixture was shaken and incubated at 40 °C in the dark for 40 min. Finally, the absorbance was measured at 525 nm against a standard AsA curve, prepared following the same procedure.

For the reduced GSH concentration, 0.1 g of turion was macerated in 1 mL of 0.2 M HCl, centrifuged at 16,000× *g* for 10 min. An aliquot of 500 µL was taken followed by the addition of 500 µL of sodium phosphate buffer (pH 7.5). Afterward, an aliquot of 25 µL was taken and was poured in with 90 µL of sodium phosphate buffer, 10 µL of 10 mM

EDTA, 10 µL of 10 mM NADPH, 10 µL 6 mM DTNB, 35 µL of distilled H_2O, and 10 µL of GR 10 UD per mL. The reduced GSH concentration was measured at 412 nm [25].

2.7. Determination of Total Phenol Concentration

The phenols of the plant tissue were extracted according to Rivero et al. [26] with minor modifications. The content was quantified at an absorbance of 725 nm using the Folin–Ciocalteu reagent. A sample of 0.1 g was ground and mixed with 500 µL of methanol, 500 µL of chloroform, and 250 µL of 1% NaCl. It was then centrifuged for 10 min at 5000 rpm.

2.8. Determination of Flavonoid Concentration

The total flavonoid concentration was determined by the colorimetric method described by Kim et al. [27], with minor modifications. The plant sample was subjected to the same extraction process as for the determination of total phenols. Subsequently, the methanolic phase was mixed with distilled water and 5% $NaNO_2$. After 5 min, a 10% $AlCl_3$ solution was added, the mixture was allowed to stand for another 5 min and then 1 M NaOH was added. The reaction solution was mixed well and held for 15 min, and the absorbance was measured at 415 nm. The total flavonoid concentration was calculated using a rutin curve and expressed as mg of rutin g^{-1} fresh weight (FW).

2.9. Determination of Anthocyanin Concentration

The anthocyanin concentration was determined according to the differential pH method described by Giusti and Wrolstad [28]. Two buffer systems were used: potassium chloride (0.025 M), pH 1.0, and sodium acetate (0.4 M) with a pH of 4.5. Each of the buffers was added to plant material separately. The resulting solutions were then incubated at room temperature for 20 min. Finally, the absorption of both solutions was measured at 640 and 710 nm. The anthocyanin concentration was obtained by subtracting the absorbance values as follows: [(A640-A710) pH1.0]-[(A640-A710) pH4.5] while considering a molar extinction coefficient of cyanidin-3-glucoside 26,900 with a molecular weight of 449.2. The results were expressed as mg of cyanidin-3-glucoside per g FW.

2.10. Determination of Antioxidant Tests: FRAP and TEAC

The assay was performed using FRAP reagent and absorbance was measured at 593 mm [29]. The TEAC (Trolox Equivalent Antioxidant Activity) test was performed using a modified version of the method by Cai et al. [30] based on the reaction with 2,2′-azinobis-(3-ethylbenzothiazolin-6-sulfonic acid) (ABTS) and measuring absorbance at 734 nm.

2.11. Amino Acid Profile Determination

The method described by Bieleski and Turner [31] was used with some modifications for amino acid extraction. A total of 0.1 g of spears was homogenized in 1 mL of methanol: chloroform: water (12:5:1). Then, 50 µL of L-2 aminobutyric acid was added as an internal standard. The mixture was centrifuged at 2300× *g* for 10 min. The resulting supernatant was added to 700 µL of Milli-Q water and 1.2 mL of chloroform and samples were kept for 24 h at 4 °C. Then, the aqueous phase was obtained and was lyophilized. The resulting extract was diluted with 0.1 M HCl. The analysis of soluble amino acids was carried out using the precolumn AccQ Tag Ultra Derivatization Kit (Waters, Milford, MA, USA). LC fluorescence analysis was performed on the Waters Acquity® UPLC System equipped with the Acquity fluorescence detector. UPLC separation was performed on the AccQ Tag Ultra column (2.1 × 100 mm, 1.7 µm) from Waters. The flow rate was 0.7 mL min^{-1}, and the column temperature was kept at 55 °C. The injection volume was 1 µL, and the detection was set at a 266-nm excitation wavelength and a 473-nm emission wavelength. The solvent system consisted of two eluents: 1:20 Dilution of AccQ Tag Ultra eluent A concentrate and AccQ Tag Ultra eluent B.

2.12. Determination of Nutrient Concentration

The samples were mineralized by wet digestion method as described by Wolf [32]. For this, 0.1 g of lyophilized spears was ground and mineralized with 98% H_2SO_4 and 30% H_2O_2 at 300 °C. The mineral nutrients were analyzed by ICP-OES and expressed as mg or µg g^{-1} dry weight (DW). For N analysis, a sample of 0.1 g DW was digested with H_2SO_4 and H_2O_2. After dilution with deionized water, a 1-mL aliquot of the digest was added to a reaction medium containing sodium silicate/sodium nitroprusside and sodium hydroxide, and sodium dichloroisocyanurate. Samples were incubated at room temperature for 45 min, and N concentration was determined using a spectrophotometer according to Krom [33].

2.13. Statistical Analysis

The experiment was carried out using a randomized complete block with 4 treatments, 4 repetitions per treatment, thus a total of 16 plots. A statistical analysis was carried out comparing the 4 elementary plots of the same treatment. No significant differences were shown between the plots. Then, to assess the differences between treatments, a one-way analysis of variance (ANOVA) with 95% confidence was performed. The mean and standard error of each treatment was calculated from the 9 individual data of each parameter analyzed. Means were compared using Fisher's least significant differences ($LSD_{0.05}$). The significance levels were indicated as * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, or NS (not significant). All statistical analyses were carried out using the Statgraphics Centurion 16.1.03 software.

3. Results

3.1. Asparagus Yield

Plots treated with *Brassica* pellets (T2) and Dazomet (T4) showed a higher cumulative yield of spears in comparison to the other plots. Thus, the yield in plots treated with *Brassica* pellets (T2) and Dazomet (T4) reached cumulative yield values of 734 kg/ha (T2) and 731 kg/ha (T4), compared to 413 kg/ha in plots supplied with chicken manure (T3) and 485 kg/ha in untreated plots (T1) (Figure 1).

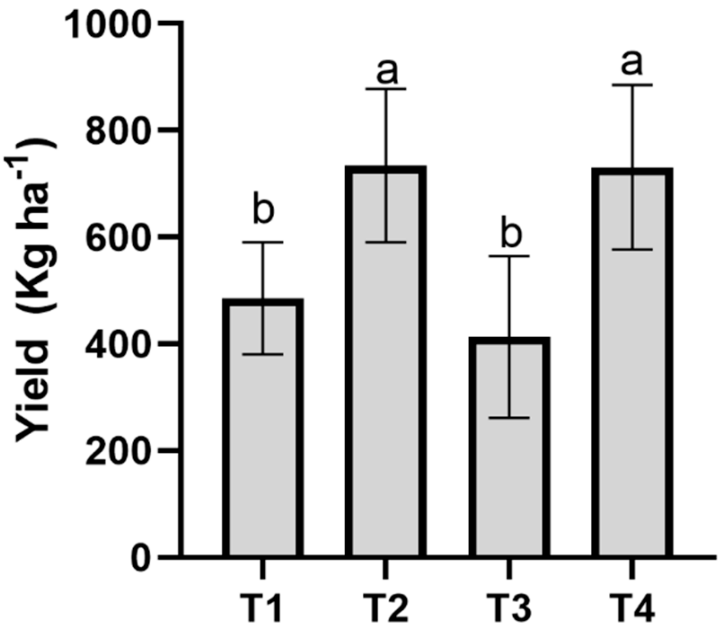

Figure 1. Cumulative yield in spears from the four different plots: untreated control (T1), *Brassica* pellets (T2), chicken manure pellets (T3), and Dazomet (T4). Values are expressed as means ± standard error ($n = 4$). Bars marked with different letters indicate significant differences among genotypes based on the $LSD_{0.05}$ test ($p < 0.05$).

3.2. Oxidative Stress Indicator (MDA)

Significant differences for MDA concentration were observed between spears from the plot treated with chicken manure pellets (T3) and spears from the untreated control plot (T1) and the Dazomet treated plot (T4), whereas no significant differences were observed between the other treatments (Figure 2).

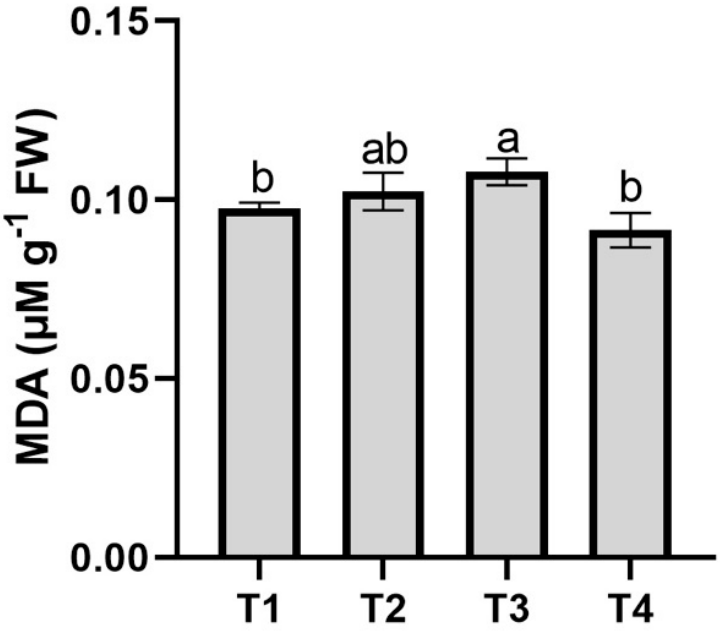

Figure 2. MDA concentration in spears from the four different plots: untreated control (T1), *Brassica* pellets (T2), chicken manure pellets (T3), and Dazomet (T4). Values are expressed as means ± standard error (n = 9). Bars marked with different letters indicate significant differences among genotypes based on the $LSD_{0.05}$ test ($p < 0.05$).

3.3. Photosynthetic Pigment Concentration

Treatment with Dazomet (T2) did not cause significant differences compared to the other treatments in spears regarding Chl *a*, Chl *b*, and carotenoid concentrations. The chicken manure pellet treatment (T3) showed significantly lower values in comparison to the other treatments for all the pigments, except for Chl *b* in comparison to spears treated with Dazomet (T4), where no significant differences were obtained (Table 1).

Table 1. Photosynthetic pigment concentrations in spears from the four different plots: untreated control (T1), *Brassica* pellets (T2), chicken manure pellets (T3), and Dazomet (T4).

	Chl a (mg g^{-1} FW)	**Chl b (mg g^{-1} FW)**	**Carotenoids (mg g^{-1} FW)**
T1	0.053 ± 0.006 a	0.041 ± 0.006 a	0.034 ± 0.005 a
T2	0.048 ± 0.007 ab	0.040 ± 0.006 ab	0.032 ± 0.005 ab
T3	0.035 ± 0.002 c	0.032 ± 0.001 c	0.023 ± 0.001 c
T4	0.045 ± 0.005 b	0.036 ± 0.002 bc	0.029 ± 0.003 b
p-value	***	**	***
$LSD_{0.05}$	0.005	0.004	0.003

Values are means ± standard deviation (n = 9). Values with different letters indicate significant differences among treatments. The levels of significance were represented as $p < 0.01$ (**), and $p < 0.001$ (***).

3.4. GSH and AsA Concentrations

Spears from plots treated with *Brassica* pellets (T2) showed significantly higher GSH concentrations compared to the spears from the chicken manure pellet (T3) and Dazomet (T4) treated plots. Considering AsA, spears from the plots treated with Dazomet (T4) showed the highest values in comparison to the other treatments (Table 2).

Table 2. GSH and AsA concentrations in spears from the four different plots: untreated control (T1), *Brassica* pellets (T2), chicken manure pellets (T3), and Dazomet (T4).

	GSH (μg g^{-1} FW)	**AsA (μg g^{-1} FW)**
T1	0.53 ± 0.06 ab	0.28 ± 0.02 c
T2	0.54 ± 0.09 a	0.28 ± 0.02 c
T3	0.46 ± 0.05 bc	0.33 ± 0.01 b
T4	0.40 ± 0.09 c	0.36 ± 0.05 a
p-value	**	***
$LSD_{0.05}$	0.07	0.03

Values are means ± standard deviation (n = 9). Values with different letters indicate significant differences among treatments. The levels of significance were represented as $p < 0.01$ (**), and $p < 0.001$ (***).

3.5. Total Phenol, Flavonoid, and Anthocyanin Concentration

Spears from plots supplied with *Brassica* pellet (T2) treatment presented significantly higher values of flavonoids and anthocyanins compared to spears from the other treatments. Moreover, T2-treated spears showed significantly higher total phenol values compared to spears treated with the chicken manure pellet (T3) and Dazomet (T4) treatments (Table 3).

Table 3. Total phenol, flavonoid, and anthocyanin concentrations in spears from the four different plots: untreated control (T1), *Brassica* pellets (T2), chicken manure pellets (T3), and Dazomet (T4).

	Total Phenols (mg g^{-1} FW)	**Flavonoids (mg g^{-1} FW)**	**Anthocyanins (mg g^{-1} FW)**
T1	4.32 ± 0.53 a	4.57 ± 0.55 b	8.95 ± 0.42 b
T2	4.59 ± 0.78 a	5.22 ± 0.61 a	10.73 ± 0.97 a
T3	2.95 ± 0.73 b	2.89 ± 0.28 c	7.67 ± 0.35 c
T4	3.27 ± 0.48 b	4.25 ± 0.36 b	5.88 ± 2.56 d
p-value	***	***	***
$LSD_{0.05}$	0.61	0.45	0.50

Values are means ± standard deviation (n = 9). Values with different letters indicate significant differences among treatments. The level of significance was represented as $p < 0.001$ (***).

3.6. Antioxidant Test: FRAP and TEAC

The application of *Brassica* pellet treatment (T2) enhanced the FRAP and TEAC values of asparagus spears in comparison to spears that received the other treatments and from the untreated control plots. In addition, spears supplied with chicken manure showed the lowest FRAP levels and spears from untreated plots presented the lowest TEAC levels (Figure 3).

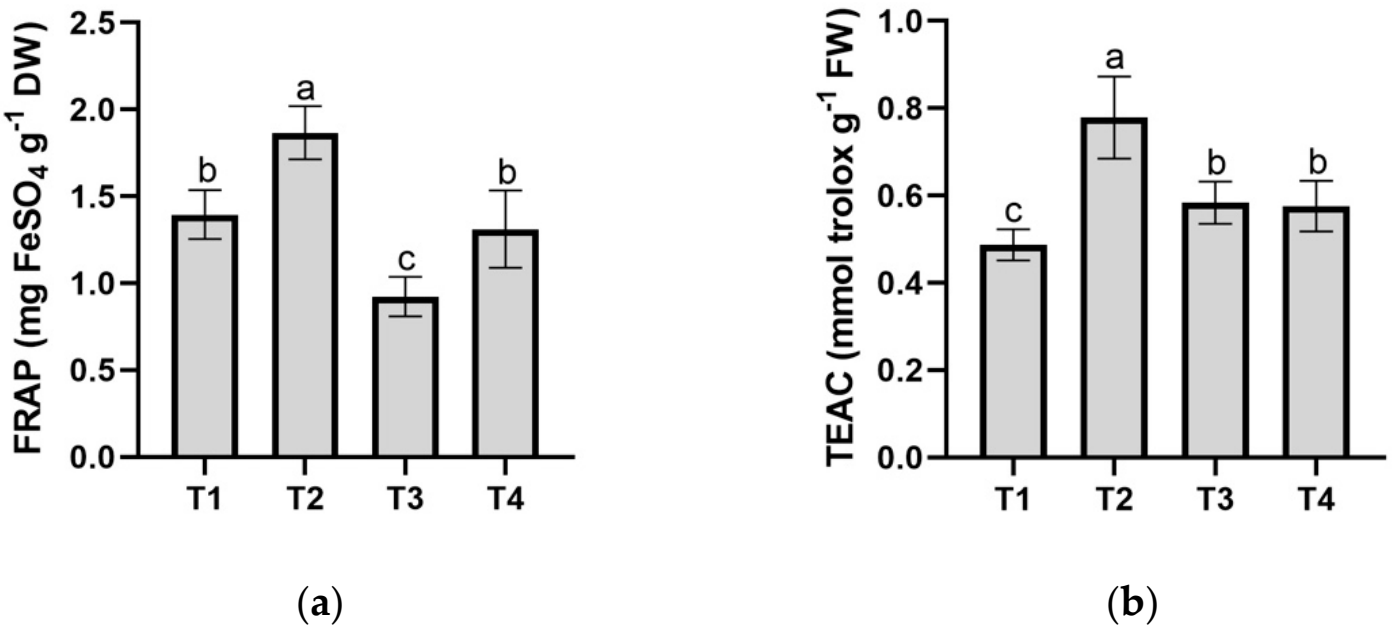

Figure 3. FRAP (**a**) and TEAC (**b**) values in spears from the four different plots: untreated control (T1), *Brassica* pellets (T2), chicken manure pellets (T3), and Dazomet (T4). Values are expressed as means ± standard error (n = 9). Bars marked with different letters indicate significant differences among genotypes based on the $LSD_{0.05}$ test ($p < 0.01$).

3.7. Amino Acids Profile

The results of the amino acids profile showed that *Brassica* pellet (T2) application increased the concentrations of Arg, His, Ser, Thr, Ala, Tyr, Val, Ile, and Met in spears in comparison to spears from control untreated plots (T1). Indeed, spears supplied with T2 treatment registered the highest Ala and Met levels in comparison to the spears from the other treatments. In contrast, spears from the plots supplied with chicken manure pellets (T3) presented a lower concentration of most amino acids in comparison to T1 spears. Furthermore, spears from plots treated with Dazomet (T4) registered higher concentrations of all amino acids except His, Glu, Asp, and Met in comparison to spears from untreated plots (T1) and reached the highest Ser, Thr, Tyr, Val, Ile, Leu, and Phe concentrations (Table 4).

3.8. Nutrient Concentration

Spears from plots supplied with *Brassica* pellets (T2) presented a higher macronutrient concentration, except for N and P, which did not differ in comparison to spears grown in untreated plots (T1). Moreover, spears from the plots supplied with chicken manure pellets (T3) showed the greatest increments in macronutrients except for N concentration, which was lower compared to spears from untreated plots (T1). Finally, Dazomet application (T4) caused a reduction in N, P, and Ca levels but increased S concentration. In addition, these spears from T4 plots registered the highest Ca levels in comparison to the other treatments (Table 5).

Table 4. Amino acid concentrations (μmol g^{-1} FW) in spears from the four different plots: untreated control (T1), *Brassica* pellets (T2), chicken manure pellets (T3), and Dazomet (T4).

	Arg	His	Glu	Asp	Pro	Ser	Gly	Thr	Ala	Tyr	Val	Ile	Leu	Phe	Met
T1	20.56 ± 0.82 b	15.57 ± 0.19 b	3.43 ± 0.14 a	1.91 ± 0.08 a	16.99 ± 0.68 b	4.67 ± 0.19 c	2.08 ± 0.08 b	1.27 ± 0.05 c	5.20 ± 0.21 c	9.31 ± 0.37 c	1.35 ± 0.05 c	0.07 ± 0.00 d	0.26 ± 0.01 c	0.22 ± 0.01 b	nd
T2	24.91 ± 1.00 a	17.10 ± 0.21 a	3.52 ± 0.14 a	1.32 ± 0.05 b	10.10 ± 0.40 c	5.35 ± 0.21 b	2.16 ± 0.09 b	1.21 ± 0.05 b	6.60 ± 0.26 a	11.89 ± 0.48 b	1.41 ± 0.06 b	0.08 ± 0.00 c	0.28 ± 0.01 c	0.15 ± 0.01 c	0.26 ± 0.01 a
T3	14.31 ± 0.57 c	11.94 ± 0.16 c	1.97 ± 0.08 b	1.16 ± 0.05 c	5.72 ± 0.23 d	4.02 ± 0.16 d	2.21 ± 0.09 b	1.06 ± 0.04 b	4.09 ± 0.16 d	2.81 ± 0.11 d	1.10 ± 0.04 b	0.13 ± 0.01 b	0.33 ± 0.01 b	nd	0.15 ± 0.01 b
T4	26.18 ± 1.05 a	10.89 ± 0.25 c	2.94 ± 0.12 c	1.96 ± 0.08 a	25.45 ± 1.02 a	6.28 ± 0.25 a	2.75 ± 0.11 a	1.95 ± 0.08 a	6.08 ± 0.24 b	16.21 ± 0.65 a	2.44 ± 0.10 a	0.22 ± 0.01 a	0.74 ± 0.03 a	0.57 ± 0.02 a	nd
p-value	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***
$LSD_{0.05}$	1.66	1.06	0.23	0.12	1.23	0.39	0.17	0.11	0.42	0.84	0.12	0.01	0.03	0.02	0.01

Values are means ± standard deviation (n = 9). Values with different letters indicate significant differences among treatments. The level of significance was represented as $p < 0.001$ (***).

Table 5. Macronutrient concentrations (mg g^{-1} DW) in spears from the four different plots: untreated control (T1), *Brassica* pellets (T2), chicken manure pellets (T3), and Dazomet (T4).

	N	K	P	S	Ca	Mg
T1	84.54 ± 7.97 a	18.00 ± 0.36 c	4.69 ± 0.09 b	2.83 ± 0.06 d	2.22 ± 0.04 c	0.74 ± 0.01 c
T2	76.31 ± 13.15 ab	19.52 ± 0.39 b	4.85 ± 0.10 b	4.26 ± 0.09 b	3.50 ± 0.07 b	0.88 ± 0.02 b
T3	67.03 ± 13.48 bc	22.91 ± 0.46 a	6.19 ± 0.12 a	5.00 ± 0.10 a	3.39 ± 0.07 b	1.12 ±0.02 a
T4	59.72 ± 6.42 c	17.65 ± 0.35 c	4.25 ± 0.09 c	3.41 ± 0.07 c	3.92 ± 0.08 a	0.79 ± 0.02 d
p-value	***	***	***	***	***	***
$LSD_{0.05}$	10.29	0.74	0.19	0.15	0.12	0.03

Values are means ± standard deviation ($n = 9$). Values with different letters indicate significant differences among treatments. The level of significance was represented as $p < 0.001$ (***).

Considering the micronutrient results, spears from treated plots presented lower B concentrations in comparison to spears from untreated plots (T1). In contrast, the spears from treated plots showed higher concentrations of the rest of the micronutrients. Specifically, spears from plots treated with Dazomet (T4) registered the highest Fe levels, and spears from plots treated with chicken manure presented the highest Mn, Zn, and Cu concentrations (Table 6).

Table 6. Micronutrient concentrations (μg g^{-1} DW) in spears from the four different plots: untreated control (T1), *Brassica* pellets (T2), chicken manure pellets (T3), and Dazomet (T4).

	B	Fe	Mn	Zn	Cu
T1	15.86 ± 0.32 a	135.47 ± 2.71 d	16.19 ± 0.32 d	31.66 ± 0.63 d	5.33 ± 0.11 d
T2	10.29 ± 0.21 b	232.35 ± 4.65 b	19.42 ± 0.39 b	40.01 ± 0.80 b	7.07 ± 0.14 b
T3	9.13 ± 0.18 c	177.82 ± 3.56 c	21.70 ± 0.43 a	56.36 ± 1.13 a	7.11 ± 0.14 a
T4	9.10 ± 0.18 c	255.43 ± 5.11 a	18.25 ± 0.36 c	33.96 ± 0.68 c	6.83 ± 0.14 c
p-value	***	***	***	***	***
$LSD_{0.05}$	0.20	7.74	0.72	1.57	0.25

Values are means ± standard deviation ($n = 9$). Values with different letters indicate significant differences among treatments. The level of significance was represented as $p < 0.001$ (***).

4. Discussion

Adequate crop yields are considered crucial to the crop economic viability, and such is the case with asparagus cultivation. Reduced asparagus yields are the most obvious effect of the decline syndrome [2]. Indeed, the plots used in this experiment showed a considerable reduction in yield in the years preceding the study. However, the application of *Brassica* pellets and Dazomet was effective to alleviate decline syndrome because these treatments enhanced the average yields. This enhancement could mean large differences in economic performance, thereby anticipating the application of these treatments to be beneficial for obtaining higher yields. On the other hand, chicken manure was not effective in improving asparagus yield in spite of the fact that it is a widely used product by farmers as an organic amendment and for biofumigation.

Decline syndrome is a stressful condition for plants that may explain the reduction in asparagus yield [8]. Many plants are continually subjected to stressful situations such as salinity, drought, high temperature, herbicides, pathogens, etc., which induce the generation of ROS [34]. These ROS are highly reactive, among other harmful effects, and can provoke lipid peroxidation, during which secondary products, i.e., (MDA), are generated. In this way, MDA has been used as an indicator of lipid peroxidation and, therefore, oxidative stress [35]. The MDA results suggest that spears from the plot treated with chicken manure presented higher levels of oxidative stress, which could explain the lower average yield observed in these plots.

Furthermore, photosynthetic pigments such as Chls and carotenoids are indicators of stress in plants. Thus, a lower concentration of these pigments is usually indicative of a higher incidence of stress, i.e., oxidative stress [36]. The photosynthetic pigment results

also suggest that plants from plots supplied with chicken manure present higher stress levels. On the other hand, spears from plots treated with *Brassica* pellets maintained similar pigment and MDA levels as the untreated plots, indicating that this treatment did not cause stress to the asparagus plants. This result is also positive given that Chls and carotenes are crucial for maintaining the nutritional and sensory quality of asparagus [37].

Plants have non-enzymatic antioxidant systems such as GSH and AsA to defend and detoxify the ROS produced under stress conditions [38]. GSH forms complexes with the MITC in plant cells to avoid the toxicity of these compounds, leading to a depletion in GSH free levels [39]. This fact could explain the decrease in GSH levels in spears treated with Dazomet. Thus, the MITC generated by Dazomet application would have been detoxified by GSH in spears, leading to the depletion of free GSH levels. This lack of free GSH for ROS detoxification might be compensated by the higher AsA level, which also has an important antioxidant capacity [38]. In addition, chicken manure treatment was positive for AsA accumulation. The higher concentrations of AsA might be because of the decline syndrome that was invoking stress in these plants, thereby leading to increased concentrations of MDA and ROS. As a result, AsA, being an antioxidant agent, would have played its role by limiting the ROS massive accumulation, as described previously.

Other antioxidant compounds in plants are phenols or phenolic compounds that are secondary metabolites. In addition, they contribute to the flavor, texture, and color of foods [40]. Phenols are not essential for humans, but their benefits are mainly attributed to their antioxidant activity [41]. In our study, Dazomet and chicken manure treatments had a negative impact on the accumulation of phenolic compounds such as flavonoids and anthocyanins. Nevertheless, the application of *Brassica* pellet treatment enhanced their accumulation. Thus, phenolic compounds could provide asparagus plants an enhanced tolerance against decline syndrome, i.e., enriching their antioxidant defense system along with the nutritional quality.

There are several tests used to measure the antioxidant activity of vegetables, such as FRAP and TEAC. These tests show the capacity of plants to detoxify ROS through antioxidant compounds such as GSH, AsA, and phenolics. Likewise, antioxidant tests indicate the antioxidant potential of food crops for human consumption [42]. In our experiment, we found a clear correlation between the concentration of antioxidant compounds, FRAP, and TEAC. Thus, the application of *Brassica* pellets was effective to enhance antioxidant compound accumulation and, consequently, higher values were observed for these antioxidant tests. These results are supported by Farooq et al.'s study, which observed enhanced antioxidant defenses in plants supplied with *Brassica* plant extracts [43]. On the other hand, spears from plots treated with chicken manure pellets presented a minor accumulation of antioxidants and also the lowest levels of FRAP.

Considering amino acids, the results showed that the application of chicken manure (T3) caused a negative impact on the accumulation of most amino acids. Therefore, asparagus plants neither effectively assimilated the amino acids present in chicken manure nor was their catabolism enhanced. In contrast, the other treatments (T2 and T4) increased the accumulation of most amino acids in asparagus plants. Thus, asparagus supplied with *Brassica* pellets (T2) showed a higher accumulation of amino acids related to the nitrogen metabolism such as Arg, His, and Ala. The accumulation of these amino acids could be regarded as a response to decline because they are crucial for N storage in plants growing under stress conditions [44,45]. Furthermore, the application of Dazomet treatment (T4) enhanced the accumulation of other amino acids such as Thr, Tyr, and Phe. These amino acids are the precursors for secondary metabolites that protect against stress conditions [45,46]. Interestingly, the treated plots produced asparagus with higher concentrations of essential amino acids such as His, Thr, Val, Ile, Leu, Phe, and Met, which also hold a prominent place and are essential [47]. Specifically, asparagus from plots treated with *Brassica* pellets (T2) accumulated higher His and Met levels, whereas the most effective treatment for essential amino acid accumulation was Dazomet (T4), as spears from this plot showed greater Thr, Val, Ile, and Phe concentrations.

The other important elements that affect plant performance and quality are macro and micronutrients [48]. Thus, an adequate supply of these mineral elements is crucial for plant growth and higher yield. In addition, nutrients such as Ca, Mg, Fe, and Zn are essential in the human diet and crops should supply an adequate content of them [49]. In general, in the present experiment, the treatments' effect was positive for mineral nutrient accumulation. Therefore, the application of the treatments did not hinder the accumulation of nutrients, and can even promote the production of asparagus with higher nutritional quality. The results for Zn accumulation are noteworthy, since all the treatments applied improved its accumulation. Zn is an essential micronutrient for both plants and animals that fulfills important roles such as being a cofactor of antioxidant enzymes [50]. Specifically, the positive effect in nutrient accumulation was more remarkable in plots treated with chicken manure pellets (T3). A possible explanation could be a concentration effect because these plots reached lower plant biomass, and consequently spears could have a higher mineral nutrient concentration. Besides, chicken manure provides a supply of K, P and Mg, which are a considerable percentage of its composition. However, we did not observe this effect for N concentration. The accumulation of this nutrient is indicative of higher biomass production [48]. Consequently, the lower N concentration is related to the lower biomass production, and in spears treated with *Brassica* pellets (T2), no reduction in N concentration was observed and these spears showed higher average yield.

The action of the different treatments on the reduction in the inoculum density of different species of *Fusarium* was verified. This analysis was carried out before and after the application of the treatments. The results showed that all the treatments were effective in reducing the presence of *Fusarium* in the soil. However, the application of the *Brassica* pellet (T2) and Dazomet (T4) treatments obtained 20% more reduction in the *Fusarium* inoculums in comparison to chicken manure (T3) treatment (data not shown). These results could explain the higher harvest yield, the lower stress and the higher quality of the asparagus spears from the plots that received the *Brassica* (T2) and Dazomet (T4) treatments.

5. Conclusions

The present study shows that plots treated with *Brassica* pellets and Dazomet showed an enhancement in most analyzed parameters, thereby establishing the basis, in all cases, for the acquired accumulated production. On the other hand, in this study we verify that chicken manure, in spite of being widely used by farmers, is not effective against asparagus decline and that other types of organic matter such as *Brassica* pellets are more useful against decline and to obtain better harvest yields than chicken manure. Thus, *Brassica* pellets and also Dazomet increased yield, and spears from plots treated with *Brassica* pellets showed lower stress symptoms and higher antioxidant potential. These results indicate that *Brassica* pellet and Dazomet treatments can be a promising option to take into account for alleviating the damages and yield losses caused by the decline syndrome, and to increase asparagus quality. Further studies can effectively address these challenges, and it would also be worthy to monitor the crop evolution in the following years.

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References

1. FAOSTAT. Available online: http://fenix.fao.org/faostat/internal/es/#data/QC (accessed on 23 March 2021).
2. Boletín Mensual de Estadística. Available online: https://www.mapa.gob.es/en/estadistica/temas/publicaciones/boletin-mensual/default.aspx (accessed on 23 March 2021).
3. Wang, J.; Liu, Y.; Zhao, J.; Zhang, W.; Pang, X. Saponins Extracted from By-Product of *Asparagus Officinalis* L. Suppress Tumour Cell Migration and Invasion through Targeting Rho GTPase Signalling Pathway. *J. Sci. Food Agric.* **2013**, *93*, 1492–1498. [CrossRef]
4. Noperi-Mosqueda, L.C.; López-Moreno, F.J.; Navarro-León, E.; Sánchez, E.; Blasco, B.; Moreno, D.A.; Soriano, T.; Ruiz, J.M. Effects of Asparagus Decline on Nutrients and Phenolic Compounds, Spear Quality, and Allelopathy. *Sci. Hortic.* **2020**, *261*, 109029. [CrossRef]
5. Summerell, B.A.; Leslie, J.F.; Backhouse, D.; Bryden, W.L.; Burgess, L.W. Fusarium: Paul E. Nelson Memorial Symposium. In *Fusarium: Paul E. Nelson Memorial Symposium*; APS Press: St. Paul, MN, USA, 2001.
6. Motoki, S.; Ozawa, T.; Komatsu, K.; Tsukada, M.; Hattori, T.; Komura, T.; Oka, J. Allelopathy in Asparagus 1: Reduction of the Allelopathic Effect on Asparagus by the Flowable Agent in Activated Carbon. In X International Asparagus Symposium. International Society for Horticultural Science. *Acta Hortic.* **2002**, *589*, 381–386. [CrossRef]
7. Elmer, W.H. Management of Fusarium Crown and Root Rot of Asparagus. *Crop Prot.* **2015**, *73*, 2–6. [CrossRef]
8. Liu, J.; Matsubara, Y.-I. Interaction between Allelochemicals and *Fusarium* Root Rot in Asparagus Seedlings Cultured In Vitro. *Am. J. Plant Sci.* **2018**, *9*, 543–551. [CrossRef]
9. Blok, W.J.; Bollen, G.J. The Role of Autotoxins from Root Residues of the Previous Crop in the Replant Disease of Asparagus. *Neth. J. Plant Pathol.* **1993**, *99*, 29–40. [CrossRef]
10. Schreuder, W.; Lamprecht, S.C.; Marasas, W.F.O.; Calitz, F.J. Pathogenicity of Three Fusarium Species Associated with Asparagus Decline in South Africa. *Plant Dis.* **1995**, *79*, 177–181. [CrossRef]
11. Schofield, P.E. Asparagus Decline and Replant Problem in New Zealand. *N. Z. J. Crop Hortic. Sci.* **1991**, *19*, 213–220. [CrossRef]
12. Lake, R.J.; Falloon, P.G.; Cook, D.W.M. Replant Problem and Chemical Components of Asparagus Roots. *N. Z. J. Crop Hortic. Sci.* **1993**, *21*, 53–58. [CrossRef]
13. Hartung, A.C.; Stephens, C.T. Effects of Allelopathic Substances Produced by Asparagus on Incidence and Severity of Asparagus Decline Due to *Fusarium* Crown Rot. *J. Chem. Ecol.* **1983**, *9*, 1163–1174. [CrossRef] [PubMed]
14. Dos Santos, C.A.; de Abboud, A.C.S.; do Carmo, M.G.F. Biofumigation with Species of the Brassicaceae Family: A Review. *Cienc. Rural* **2021**, *51*, 1–17.
15. Gamliela, A.; Siti, M.; Arbel, A.; Katan, J. Soil Solarization as a Component of the Integrated Management of Fusarium Crown and Root Rot in Tomato. *Acta Hortic.* **2009**, *808*, 321–326. [CrossRef]
16. Hwang, S.F.; Ahmed, H.U.; Strelkov, S.E.; Zhou, Q.; Gossen, B.D.; McDonald, M.R.; Peng, G.; Turnbull, G.D. Suppression of Clubroot by Dazomet Fumigant. *Can. J. Plant Sci.* **2017**, *98*, 172–182. [CrossRef]
17. Fritsch, H.J.; Huber, R. Basamid granular—A halogen-free soil disinfestant. *Acta Hortic.* **1995**, 76–85. [CrossRef]
18. Prider, J.; Williams, A. Using Dazomet to Reduce Broomrape Seed Banks in Soils with Low Moisture Content. *Crop Prot.* **2014**, *59*, 43–50. [CrossRef]
19. Kantikowati, E.; Yusdian, Y.; Suryani, C. Chicken Manure and Biofertilizer for Increasing Growth and Yield of Potato (*Solanum Tuberosum* L.) of Granola Varieties. *IOP Conf. Ser. Earth Environ. Sci.* **2019**, *393*, 012017.
20. Borrego-Benjumea, A.; Basallote-Ureba, M.J.; Melero-Vara, J.M. Eficacia de Enmiendas Orgánicas, Temperatura de Suelo y Cultivares En El Control de La Podredumbre de Raíces y Cuello de Espárrago. In Proceedings of the Resúmenes del XV Congreso de la Sociedad Española de Fitopatología, Vitoria-Gasteiz, Spain, 27 September–1 October 2010; pp. 383–undefined.
21. Vida, C.; Vicente, A.; Cazorla, F.M. The Role of Organic Amendments to Soil for Crop Protection: Induction of Suppression of Soilborne Pathogens. *Ann. Appl. Biol.* **2020**, *176*, 1–15. [CrossRef]
22. Heath, R.L.; Packer, L. Photoperoxidation in Isolated Chloroplasts. I. Kinetics and Stoichiometry of Fatty Acid Peroxidation. *Arch. Biochem. Biophys.* **1968**, *125*, 189–198. [CrossRef]
23. Wellburn, A.R. The Spectral Determination of Chlorophylls a and b, as Well as Total Carotenoids, Using Various Solvents with Spectrophotometers of Different Resolution. *J. Plant Physiol.* **1994**, *144*, 307–313. [CrossRef]
24. Law, M.Y.; Charles, S.A.; Halliwell, B. Glutathione and Ascorbic Acid in Spinach (*Spinacia Oleracea*) Chloroplasts. The Effect of Hydrogen Peroxide and of Paraquat. *Biochem. J.* **1983**, *210*, 899–903. [CrossRef]

25. Gronwald, J.W.; Fuerst, E.P.; Eberlein, C.V.; Egli, M.A. Effect of Herbicide Antidotes on Glutathione Content and Glutathione S-Transferase Activity of Sorghum Shoots. *Pestic. Biochem. Physiol.* **1987**, *29*, 66–76. [CrossRef]
26. Rivero, R.M.; Ruiz, J.M.; García, P.C.; López-Lefebre, L.R.; Sánchez, E.; Romero, L. Resistance to Cold and Heat Stress: Accumulation of Phenolic Compounds in Tomato and Watermelon Plants. *Plant Sci.* **2001**, *160*, 315–321. [CrossRef]
27. Kim, D.O.; Jeong, S.W.; Lee, C.Y. Antioxidant Capacity of Phenolic Phytochemicals from Various Cultivars of Plums. *Food Chem.* **2003**, *81*, 321–326. [CrossRef]
28. Giusti, M.M.; Wrolstad, R.E. Characterization and Measurement of Anthocyanins by UV-Visible Spectroscopy. *Curr. Protoc. Food Anal. Chem.* **2001**, *1*, F1.2.1–F1.2.13. [CrossRef]
29. Benzie, I.F.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] [PubMed]
30. Cai, Y.; Luo, Q.; Sun, M.; Corke, H. Antioxidant Activity and Phenolic Compounds of 112 Traditional Chinese Medicinal Plants Associated with Anticancer. *Life Sci.* **2004**, *74*, 2157–2184. [CrossRef]
31. Bieleski, R.L.; Turner, N.A. Separation and Estimation of Amino Acids in Crude Plant Extracts by Thin-Layer Electrophoresis and Chromatography. *Anal. Biochem.* **1966**, *17*, 278–293. [CrossRef]
32. Wolf, B. A Comprehensive System of Leaf Analyses and Its Use for Diagnosing Crop Nutrient Status. *Commun. Soil Sci. Plant Anal.* **1982**, *13*, 1035–1059. [CrossRef]
33. Krom, M.D. Spectrophotometric Determination of Ammonia: A Study of a Modified Berthelot Reaction Using Salicylate and Dichloroisocyanurate. *Anal.* **1980**, *105*, 305–316. [CrossRef]
34. Mittler, R. Oxidative Stress, Antioxidants and Stress Tolerance. *Trends Plant Sci.* **2002**, *7*, 405–410. [CrossRef]
35. Marnett, L.J. Lipid Peroxidation—DNA Damage by Malondialdehyde. *Mutat. Res. - Fundam. Mol. Mech. Mutagenesis* **1999**, *424*, 83–95. [CrossRef]
36. Ashraf, M.; Harris, P.J.C. Photosynthesis under Stressful Environments: An Overview. *Photosynthetica* **2013**, *51*, 163–190. [CrossRef]
37. Tenorio, M.; Villanueva, M.; Sagardoy, M. Changes in Carotenoids and Chlorophylls in Fresh Green Asparagus (*Asparagus Officinalis* L) Stored under Modified Atmosphere Packaging. *J. Sci. Food Agric.* **2004**, *84*, 357–365. [CrossRef]
38. Noctor, G.; Foyer, C.H. Ascorbate and Glutathione: Keeping Active Oxygen under Control. *Annu. Rev. Plant Biol.* **1998**, *49*, 249–279. [CrossRef] [PubMed]
39. Li, Y.; Liu, Y.; Zhang, Z.; Cao, Y.; Li, J.; Luo, L. Allyl Isothiocyanate (AITC) Triggered Toxicity and FsYvc1 (a STRPC Family Member) Responded Sense in *Fusarium Solani*. *Front. Microbiol.* **2020**, *11*, 870. [CrossRef] [PubMed]
40. Papoulias, E.; Siomos, A.; Koukounaras, A.; Gerasopoulos, D.; Kazakis, E. Effects of Genetic, Pre- and Post-Harvest Factors on Phenolic Content and Antioxidant Capacity of White Asparagus Spears. *Int. J. Mol. Sci.* **2009**, *10*, 5370–5380. [CrossRef] [PubMed]
41. Rickman, J.C.; Barrett, D.M.; Bruhn, C.M. Nutritional Comparison of Fresh, Frozen and Canned Fruits and Vegetables. Part 1. Vitamins C and B and Phenolic Compounds. *J. Sci. Food Agric.* **2007**, *87*, 930–944. [CrossRef]
42. Rosales, M.A.; Cervilla, L.M.; Sánchez-Rodríguez, E.; Rubio-Wilhelmi, M.d.M.; Blasco, B.; Ríos, J.J.; Soriano, T.; Castilla, N.; Romero, L.; Ruiz, J.M. The Effect of Environmental Conditions on Nutritional Quality of Cherry Tomato Fruits: Evaluation of Two Experimental Mediterranean Greenhouses. *J. Sci. Food Agric.* **2011**, *91*, 152–162. [CrossRef]
43. Farooq, M.; Rizwan, M.; Nawaz, A.; Rehman, A.; Ahmad, R. Application of Natural Plant Extracts Improves the Tolerance against Combined Terminal Heat and Drought Stresses in Bread Wheat. *J. Agron. Crop Sci.* **2017**, *203*, 528–538. [CrossRef]
44. Galili, G.; Amir, R.; Fernie, A.R. The Regulation of Essential Amino Acid Synthesis and Accumulation in Plants. *Annu. Rev. Plant Biol.* **2016**, *67*, 153–178. [CrossRef]
45. Batista-Silva, W.; Heinemann, B.; Rugen, N.; Nunes-Nesi, A.; Araújo, W.L.; Braun, H.P.; Hildebrandt, T.M. The Role of Amino Acid Metabolism during Abiotic Stress Release. *Plant Cell Environ.* **2019**, *42*, 1630–1644. [CrossRef]
46. Holländer-Czytko, H.; Grabowski, J.; Sandorf, I.; Weckermann, K.; Weiler, E.W. Tocopherol Content and Activities of Tyrosine Aminotransferase and Cystine Lyase in Arabidopsis under Stress Conditions. *J. Plant Physiol.* **2005**, *162*, 767–770.
47. Hou, Y.; Wu, G. Nutritionally Essential Amino Acids. *Adv. Nutr.* **2018**, *9*, 849–851. [CrossRef] [PubMed]
48. Marschner, H. *Marschner's Mineral Nutrition of Higher Plants*; Academic Press: Cambridge, MA, USA, 2011.
49. White, P.J.; Broadley, M.R. Biofortification of Crops with Seven Mineral Elements Often Lacking in Human Diets—Iron, Zinc, Copper, Calcium, Magnesium, Selenium and Iodine. *New Phytol.* **2009**, *182*, 49–84. [CrossRef] [PubMed]
50. Tsonev, T.; Lidon, F.J.C. Zinc in Plants—An Overview. *Emir. J. Food Agric.* **2012**, *24*, 322–334.