agronomy

MDPI

Article

Adding Sulfur to Soil Improved Cucumber Plants' Resistance to Powdery Mildew

Hongwei Jia [1,†], Zifan Wang [1,†], Xinna Kang [1], Jing Wang [1], Yahong Wu [1], Zeyang Yao [1], Yanwei Zhou [1], Yuke Li [2], Yu Fu [2], Yuan Huang [1], Jianhua Shi [1,*] and Zhonglin Shang [2,*]

[1] Key Laboratory for Agricultural Information Perception and Intelligent Control of Shijiazhuang, Shijiazhuang Academy of Agricultural and Forestry Sciences, Shijiazhuang 050041, China; jiahongwei_dxx@163.com (H.J.); princefans@126.com (Z.W.); nkykang@163.com (X.K.); nkykjfw@163.com (J.W.); wyhydszhd@163.com (Y.W.); sjznkyrsk@163.com (Z.Y.); zhouyanweinky@163.com (Y.Z.); nkyhuangyuan@sina.com (Y.H.)

[2] Ministry of Education Key Laboratory of Molecular and Cellular Biology, Hebei Collaboration Innovation Center for Cell Signaling and Environmental Adaptation, Hebei Research Center of the Basic Discipline of Cell Biology, Hebei Key Laboratory of Molecular and Cellular Biology, College of Life Sciences, Hebei Normal University, Shijiazhuang 050024, China; liyuke@stu.hebtu.edu.cn (Y.L.); hbsdfuyu@stu.hebtu.edu.cn (Y.F.)

* Correspondence: nkyshijianhua@163.com (J.S.); shangzhonglin@hebtu.edu.cn (Z.S.)

† These authors contributed equally to this work.

Abstract: Chemical fungicides can effectively prevent and control powdery mildew, but they can also leave pesticide residues in the environment and on cucumbers. In this study, we added sulfur powder to the soil where cucumbers were grown to see how it affected the occurrence of powdery mildew. The results showed that adding sulfur increased sulfur absorption by the cucumbers, improved plant immunity, and reduced the incidence of powdery mildew. Furthermore, adding sulfur to the soil increased soluble protein content in cucumber leaves, enhanced photosynthesis, and significantly increased fruit yield. Additionally, sulfur addition decreased soil dehydrogenase activity and increased sucrase activity, potentially impacting soil microbial activity. In conclusion, this study found that adding sulfur had a positive inhibitory effect on the occurrence of cucumber powdery mildew while not significantly impacting the soil environment. These findings provide valuable insights for developing new control methods that are easy to implement, cost-effective, reliable, and environmentally safe.

Keywords: cucumber; powdery mildew; sulfur; soil; reactive oxygen species

Citation: Jia, H.; Wang, Z.; Kang, X.; Wang, J.; Wu, Y.; Yao, Z.; Zhou, Y.; Li, Y.; Fu, Y.; Huang, Y.; et al. Adding Sulfur to Soil Improved Cucumber Plants' Resistance to Powdery Mildew. *Agronomy* **2024**, *14*, 1799. https://doi.org/10.3390/agronomy14081799

Academic Editor: Loukas Kanetis

Received: 11 July 2024
Revised: 2 August 2024
Accepted: 14 August 2024
Published: 15 August 2024

1. Introduction

Powdery mildew is a common fungal disease that affects approximately 10,000 types of flowering plants, including many important fruit and vegetable crops [1,2]. When cucumbers and melons are infected with powdery mildew in a controlled environment, such as a greenhouse, large white colonies form on the surface of leaves, petioles, and stems. These colonies hinder photosynthesis and cell viability, severely impacting plant growth and fruit yield [3]. The pathogens responsible for powdery mildew are generally *Sphaerotheca cucurbitae* and *Erysiphe cichoracearum* [4]. The occurrence of powdery mildew is closely linked to environmental conditions, such as high temperatures and humidity in greenhouses during summer and autumn. Under these conditions, the conidia reproduce rapidly and spread through the air, invading leaf tissues within 24 h and rapidly spreading within 7 days upon contact with cucumber plants [5].

Currently, various measures such as chemical, agricultural, and biological controls are being used to prevent and manage powdery mildew. Chemicals commonly used for controlling cucumber powdery mildew include chlorofluoroconazole, azoxystrobin, tebuconazole, hexaconazole, etc. However, prolonged use of these chemicals can lead to pesticide residues in agricultural products and soil, posing risks to food safety and

environmental safety [6]. Reports indicate that powdery mildew pathogens have resisted certain chemical agents, for example, difenoxystrobin, hexaconazole, and organophosphorus fungicides [7,8]. Breeding disease-resistant cucumber varieties has been an effective agricultural control method. Many countries have developed powdery mildew-resistant cucumber varieties, such as Jinzao 3, Jinyan 2, Zhongnong 8, GLE, Green Magic, etc. [9] However, disease resistance in these varieties is usually controlled by a single dominant gene, making them susceptible to losing resistance after widespread cultivation. Other agricultural methods, such as intercropping, grafting, and growing in high-temperature environments, can also contribute to powdery mildew prevention, but their effectiveness is limited [10]. Biological control methods involve using biocontrol microorganisms and plant extracts to combat powdery mildew. Studies have shown that extracts of *Euphorbia humifusa* and *Robinia pseudoacacia* [11–13], and emodin from rhubarb root [14], have preventive and therapeutic effects on cucumber powdery mildew. Additionally, biocontrol fungi (e.g., *Fusarium solani*, *Trichoderma harzianum*) and biocontrol extracts (e.g., ninamycin, aminopyrimidine) have shown effectiveness against powdery mildew [15,16]. However, it is important to note that prolonged use of biocides and their sources can become resistant to pathogens.

The addition of mineral elements can improve plant resistance. For instance, incorporating 20 ppm of phosphorus in a hydroponic cucumber system enhanced the plants' resistance to powdery mildew. Applying phosphate, potassium, and silica salts to the leaves can effectively control powdery mildew [17–22]. Nitrate can strengthen cucumber immunity to *Fusarium wilt* by controlling citrate exudation and inducing resistance to *Fusarium oxysporum* in cucumbers [23,24]. Foliar spraying of $CaCl_2$, $MgCl_2$, KCl, and Ka_2SO_4 solutions or using high concentrations of N and Mg in irrigation water can reduce powdery mildew disease in cucumbers. However, medium P and high K concentrations aggravated powdery mildew [25]. Additionally, Mn–amino acid complex foliar sprays strengthen cell wall structure, reduce water loss from infected leaves, and control the extent of cucumber powdery mildew [26–28]. Furthermore, a high-carbon–silicon solution concentration can notably alleviate the damage of powdery mildew to cucumbers [29].

Powdery mildew is highly susceptible to sulfur, and sulfur fumigation has been proven as effective in controlling powdery mildew during production [30]. A combination of *Trichoderma afroharzianum* and sulfur in foliar sprays has been reported to be effective in controlling powdery mildew in vineyards [31]. A spray treatment using 3 $g \cdot L^{-1}$ of sulfur mixed with vegetable oil effectively reduced the severity of powdery mildew on grape bunches [32]. However, it remains unverified whether adding sulfur to the soil can control the powdery mildew of cucumber. In this study, we conducted a pot experiment to investigate the impact of sulfur on the occurrence and severity of powdery mildew in cucumbers by introducing sulfur powder to the soil where the cucumbers were cultivated. This study aimed to preliminarily assess the preventive effect of soil sulfur application on the development of powdery mildew in cucumbers.

2. Materials and Methods

2.1. Plant Materials

The experiments were implemented in the solar greenhouse of Zhaoxian Experimental Station, located at 114.83′13.18″ E, 37.83′28.12″ N, part of the Shijiazhuang Academy of Agriculture and Forestry Sciences. Pots with a bottom diameter of 15 cm, top diameter of 20 cm, and height of 17.5 cm were used for potting. The cucumber variety used was Jin You 301, obtained from Tianjin Kerun Agricultural Science and Technology Co., Tianjin, China.

2.2. Cultivation Conditions

The experiment took place between March and October 2023. There were five groups, each with 40 pots containing 2.6 kg of soil. The nutrient content of the tested soil was total nitrogen at 2.52 $g \cdot kg^{-1}$, total phosphorus at 2.31 $g \cdot kg^{-1}$, and total potassium at 1.42 $g \cdot kg^{-1}$. The sulfur powder (Qingdao Luchuan Chemical Co., Ltd., Qingdao, China) used

had a purity of 99.99%. In the control group, no sulfur was added. In the four treatment groups, sulfur was added in different amounts (in $g \cdot kg^{-1}$): 0.2 (T1), 0.4 (T2), 0.6 (T3), and 0.8 (T4), respectively. The dosage was determined according to the method outlined by Jiang et al. [33]. The experiment was conducted twice in 2023. The first round started on 5th March, with powdery mildew inoculation on 7th April, and ended on 5th May. The second round started on 17th August, with powdery mildew inoculation on 18th September, and ended on 9th November. The inoculation was conducted 33 days after cucumber planting. Measurements and statistics of powdery mildew and photosynthetic parameters were taken every 10 days after inoculation. The fruit quality parameters, antioxidant indexes, and leaf active oxygen indexes were sampled 20 days after inoculation. At the end of the experiment, measurements were taken for cucumber leaves, soil sulfur content, and soil enzyme activity.

The pathogenic fungal spores causing cucumber powdery mildew were collected from the diseased leaves of fresh cucumbers in the greenhouse at Zhaoxian Experimental Station. The pathogen was identified as *Sphaerotheca fuligenea*. The leaves were washed with distilled water and filtered through 2–4 layers of gauze to obtain a spore suspension. The pathogenic fungal spores in the culture medium were counted using a hemocytometer. The concentration of the spore suspension was adjusted to 1×10^7 spores per mL, and the suspension was evenly sprayed on the front and back of cucumber leaves using a nebulizer.

2.3. Detection of Morbidity

After the plants were inoculated, 10 plants per treatment were randomly selected at 10-day intervals, following the reported method [6]. The 4th or 5th leaf of the cucumber plant was then photographed, and the images were processed using Photoshop 2023 software, based on the approach outlined by Cui et al. (2017), to measure the area of lesions on the leaves [34]. The disease level on the entire plant was assessed by examining all the leaves. Based on the extent of leaf damage, the grading criteria were as follows: grade 0 for no spots, grade 1 for spots covering less than 5% of the leaf area, and so on up to grade 11 for spots covering more than 75% of the leaf area. The disease index was calculated using the following formula: disease index = Σ (number of diseased leaves at each level × relative level value)/(total number of leaves surveyed × 11) × 100. The control effect was calculated as control effect (%) = (control disease index − treatment disease index)/control disease index × 100.

2.4. Detection of Photosynthetic Indicators

Photosynthetic measurements were conducted using an instrument (1102G, Yaxin Liyi Technology, Beijing, China). The instrument was used to measure the top new leaves of the cucumber, starting from the 4th or 5th leaf, to determine the net photosynthetic rate of the leaves, transpiration rate, intercellular CO_2 concentration, and stomatal conductivity.

2.5. Detection of Yield Indicators

Ten plants were labeled for each treatment, and the average yield per plant was recorded after each harvest. The total yield was calculated after the final harvest and converted into yield per hectare.

2.6. Detection of Fruit Quality Indicators

Detection of soluble solids: After grinding the cucumber into a homogenate, it was tested using a hand-held digital Brix meter refractometer (PAL-1, ATAGO CHINA Guangzhou Co., Ltd., Guangzhou, China) to measure the soluble solid content.

Detection of soluble protein: 0.2 g cucumber fruit tissue was ground into a homogenate. Then, distilled water was added to the homogenate to make a total volume of 10 mL. After centrifugation at 5000 rpm for 10 min, a 0.1 mL supernatant was mixed with 0.9 mL of a Coomassie brilliant blue G-250 solution ($0.1\ g \cdot L^{-1}$). The absorbance of the mixture at

595 nm was measured using a spectrometer (Evolution One Plus, ThermoFisher Scientific, Waltham, MA, USA).

Detection of vitamin C: A mixture was made by adding 4 g of cucumber fruit tissue to 5 mL of 2% oxalic acid. The mixture was then ground into a homogenate, filtered, and adjusted to 50 mL. After that, 10 mL of the filtrate was taken and titrated with a 2,6-dichloroindophenol solution until the solution turned pink, and the volume of 2,6-dichloroindophenol used was recorded as V1. Subsequently, 10 mL of a 2% oxalic acid solution was titrated with 2,6-dichloroindophenol until the endpoint, and the amount of 2,6-dichloroindophenol used (V2) was recorded. Then, V1 and V2 were used to calculate the vitamin C content.

Detection of soluble sugar: 0.5 g of cucumber fruit tissue was mixed with 5 mL of 80% ethanol and ground into a homogenate. The homogenate was placed in a water bath at 80 °C for 30 min and centrifuged at 3500 rpm for 10 min. The collected supernatant was mixed with an anthrone–H_2SO_4 reagent, heated at 100 °C for 10 min, and then cooled to room temperature. The absorbance of the solution at 620 nm was measured using a spectrometer.

Detection of titratable acid: 5 g of cucumber fruit tissue was ground into a homogenate. Then, distilled water was added to the homogenate to make a total volume of 50 mL. The homogenate was placed in a water bath at 80 °C for 30 min and then cooled to room temperature. The mixture was filtered using a filter paper, and 20 mL of the filtrate was titrated using a NaOH solution. The amount of NaOH used was recorded to calculate the total acid content.

2.7. Detection of Reactive Oxygen Species in Leaf Cells

The DAB (3,3-diaminobenzidine) staining method was utilized to visualize ROS. Cucumber leaves were soaked in a DAB solution with a pH of 3.8. Following a 24 h incubation period in the dark, the leaves were immersed in warm ethanol until fully decolorized. Subsequently, the leaves were photographed using a stereo microscope (SZX16, Olympus Corporation, Tokyo, Japan).

2.8. Detection of Antioxidant Enzymes' Activity

Detection of peroxidase (POD) activity: 0.5 g of cucumber leave tissue was ground into a homogenate in an ice bath, and distilled water was added to make a total volume of 50 mL. The homogenate was centrifuged at 5000 rpm for 10 min at 4 °C. In total, 1 mL of the supernatant was mixed with 1 mL of a guaiacol solution, 6.9 mL of water, and 1 mL of H_2O_2. The mixture was incubated at 25 °C for 10 min. Then, the reaction was terminated by adding 0.2 mL of a 5% metaphosphoric acid solution. The absorbance of the solution at 470 nm was measured.

Detection of superoxide dismutase (SOD) activity: 0.5 g of cucumber leaf tissue was ground into a homogenate in an ice bath, and a phosphate buffer was added to make a total volume of 10 mL. The homogenate was centrifuged at 10,000 rpm for 15 min at 4 °C. A 0.1 mL supernatant was mixed with 0.5 mL of distilled water, 1.5 mL of a phosphate buffer, 0.3 mL of a methionine solution, 0.3 mL of an NBT (nitrogen chloride blue tetrazolium) solution, 0.3 mL of a riboflavin solution, and 0.3 mL of an EDTA-Na_2 solution. A fluorescent lamp illuminated the mixture for 20 min. The absorbance of the solution at 560 nm was measured.

Detection of catalase (CAT) activity: 1.0 g of cucumber leaf tissue was ground into a homogenate in an ice bath, and a phosphate buffer was added to make a total volume of 10 mL. The homogenate was centrifuged at 10,000 rpm for 15 min at 4 °C. A 0.1 mL supernatant was mixed with 1 mL Tris-HCl buffer and 1.7 mL distilled water. The mixture was incubated at 25 °C for 3 min. After that, 0.2 mL of H_2O_2 was added, and the absorbance of the solution at 240 nm was measured every 30 s for 3 min. The data obtained were used to calculate the enzyme activity.

2.9. Detection of Sulfur Content in Cucumber Leaves

In total, 0.3 g of dried cucumber leaf tissue was soaked in 3 mL concentrated HNO_3 for 12 h and then boiled at 150 °C for 1 h. After adding 2 mL of 70% $HClO_4$, the solution was boiled at 235 °C for 2 h. Then, 1 mL of concentrated HCl was added, and the solution was boiled at 150 °C for 20 min. After cooling, 35 mL of water and 10 mL of a buffer (1 L of buffer containing 40 g $MgCl_2 \cdot 6H_2O$, 4.1 g NaOAc, 0.8 g KNO_3, and 28 mL ethanol) were added to make a final volume of 50 mL. Finally, 0.3 g of $BaCl_2 \cdot 2H_2O$ was added, and the absorbance of the solution at 440 nm was measured.

2.10. Detection of Soil Sulfur Content

In total, 0.5 g soil was placed in a high-temperature tube electric furnace with a silicon carbon rod for complete combustion. The gas produced was then passed through a 5% copper sulfate solution, 5% potassium permanganate solution, and concentrated sulfuric acid. Finally, it was introduced into a hydrochloric acid–sweet potato starch absorption solution. The absorption solution was titrated with 0.001 $mol \cdot L^{-1}$ potassium iodate (KIO_3). The amount of sulfur present was calculated based on the consumption of KIO_3.

2.11. Detection of Soil Enzymes' Activity

Detection of sucrase activity: 5 g soil was mixed with 0.25 mL methylbenzene, 5 mL 8% sucrose solution, and 5 mL phosphate buffer (pH 5.5). The mixture was incubated at 37 °C for 24 h and then centrifuged at 2000 rpm for 5 min. A 1 mL supernatant was mixed with 3 mL 0.5% 3,5-dinitrosalicylic acid solution and then boiled in a 100 °C water bath for 5 min. The absorbance of the solution at 508 nm was measured.

Detection of dehydrogenase activity: 5 g soil was mixed with 5 mL of a reaction buffer (containing 0.5% TTC (2,3,5-triphenyl tetrazolium chloride) and 0.5% glucose). The mixture was incubated at 37 °C for 24 h. After adding 5 mL methanol, the mixture was centrifuged at 2000 rpm for 5 min. The absorbance of the supernatant at 485 nm was measured.

Detection of urease activity: 5 g soil was mixed with 1 mL methylbenzene, 10 mL 10% urea solution, and 20 mL citrate buffer (pH 6.8). The mixture was incubated at 37 °C for 24 h and then centrifuged at 2000 rpm for 5 min. A 1 mL supernatant was mixed with 4 mL 1.35 $mol \cdot L^{-1}$ sodium phenolate and 3 mL 1% sodium hypochlorite. After 20 min, the absorbance of the supernatant at 485 nm was measured.

Detection of phosphatase activity: 5 g soil was mixed with 0.25 mL methylbenzene and 20 mL 0.5% disodium phosphate phenyl solution. The mixture was incubated at 37 °C for 24 h and then centrifuged at 2000 rpm for 5 min. A 5 mL supernatant was mixed with 0.5 mL 2% 4-aminoantiflavine solution and 0.5 mL 8% potassium ferrocyanide solution. After 15 min, the absorbance of the solution at 510 nm was measured.

Detection of protease activity: 5 g soil was mixed with 25 mL Tris-HCl buffer (50 mM, pH 6.8) and 25 mL 2% casein solution. After being incubated at 40 °C for 2 h, the mixture was mixed with 25 mL 15% trichloroacetic acid solution and centrifuged at 2000 rpm for 5 min. A 5 mL supernatant was mixed with 7.5 mL solution (5% NaOH-Na_2CO_3, 0.01% $CuSO_4$, 0.02% potassium tartrate, and 5 mL of 33% Folin reagent). After 1 h, the absorbance of the solution at 700 nm was measured.

2.12. Data Processing and Analysis

The data were statistically analyzed using Microsoft Office 2021 (Microsoft Corporation, Redmond, WA, USA) and SPSS22.0 (SPSS Inc., Chicago, IL, USA) and graphed using GraphPad Prism 9 (GraphPad Software, San Diego, CA, USA) software.

3. Results

3.1. Adding Sulfur to the Soil Promoted Photosynthesis and Fruit Yield of Cucumber

Several photosynthetic parameters were measured to assess the impact of sulfur addition on photosynthesis. In groups T1 and T2, there were no significant differences in stomatal conductance compared to the control. However, the T1 and T2 treatments showed

higher intercellular CO_2 levels, lower transpiration rates, and higher net photosynthetic rates than the control. In groups T3 and T4, stomatal conductance was significantly higher than in the control, while intercellular CO_2 levels showed no significant difference. The transpiration and net photosynthetic rates were significantly higher than the control group (Figure 1A–D). These results indicate that adding sulfur promotes the absorption and assimilation of CO_2.

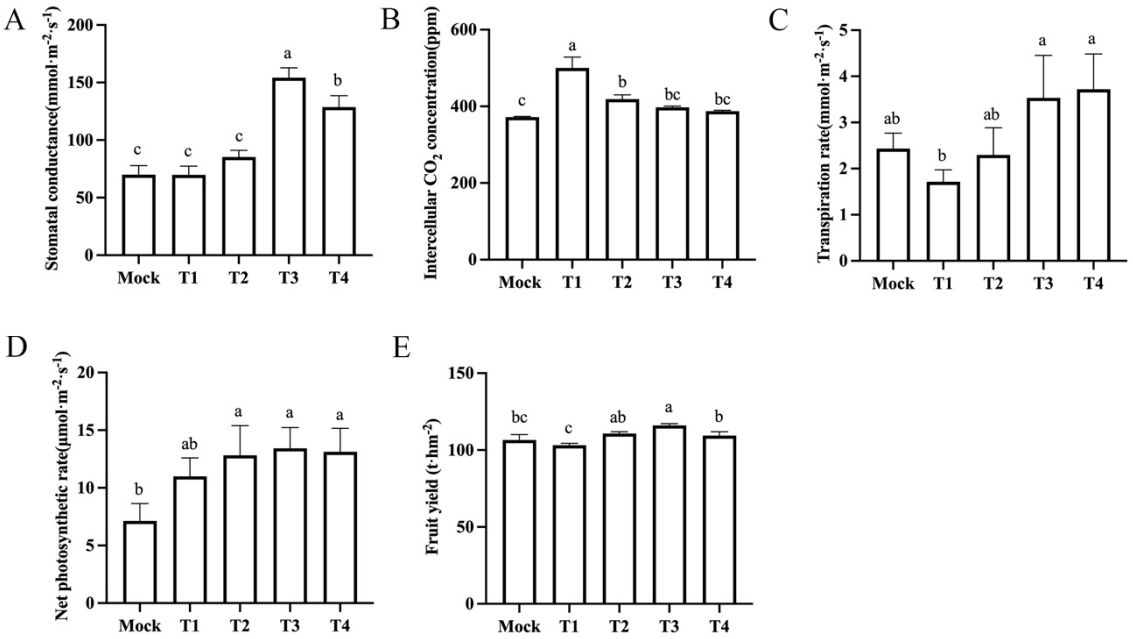

Figure 1. Adding sulfur to the soil enhanced the process of photosynthesis and the yield of cucumber plants. During the mid-growth period of the cucumber, the photosynthesis-related parameters, includingstomatal conductance (**A**), intercellular CO_2 concentration (**B**), transpiration rate (**C**), and net photosynthetic rate (**D**), of the 4th or 5th leaf (counting from the top) were measured using a portable photosynthesis system. (**E**) The total fruit yield after the final harvest. In (**A**–**D**), 5 plants were detected. In (**E**), 10 plants were detected. Results from three replicates were calculated to obtain the mean ± SE and statistically analyzed using one-way ANOVA. The different lowercase letters indicate that differences between treatments were significant at the $p < 0.05$ level.

The differences in fruit yield between the T1, T2, and T4 groups and the control group were insignificant. However, the fruit yield of the T3 group increased by 8.86% ($p < 0.05$) compared with the control group (Figure 1E). This indicates that adding sulfur promotes the fruit-productive ability of cucumber plants to a certain extent.

3.2. Adding Sulfur to the Soil Increased the Nutrient Content in Cucumber Fruit

The impact of sulfur addition on the quality of cucumber fruits was investigated by analyzing the nutrient content. In comparison to the control group, the T1, T2, T3, and T4 groups showed the following changes: Titratable acid content increased by 14.58 ± 0.85%, 15.67 ± 0.80%, 15.50 ± 0.85%, and 17.17 ± 0.80% (Figure 2A). Soluble protein content increased by 45.2 ± 0.58%, 66.38 ± 3.50%, 56.9 ± 6.50%, and 23.84 ± 5.05% (Figure 2B). Vitamin C content decreased by 11.86 ± 2.84%, 20.34 ± 3.57%, 35.59 ± 2.41%, and 32.2 ± 8.42% (Figure 2E). Soluble solids' content increased by 9.54 ± 2.02% and 12.37 ± 1.75% in the T1 and T2 groups (Figure 2C). Soluble sugar content decreased by 24.26 ± 4.76% and 36.24 ± 5.76% in the T3 and T4 groups (Figure 2D).

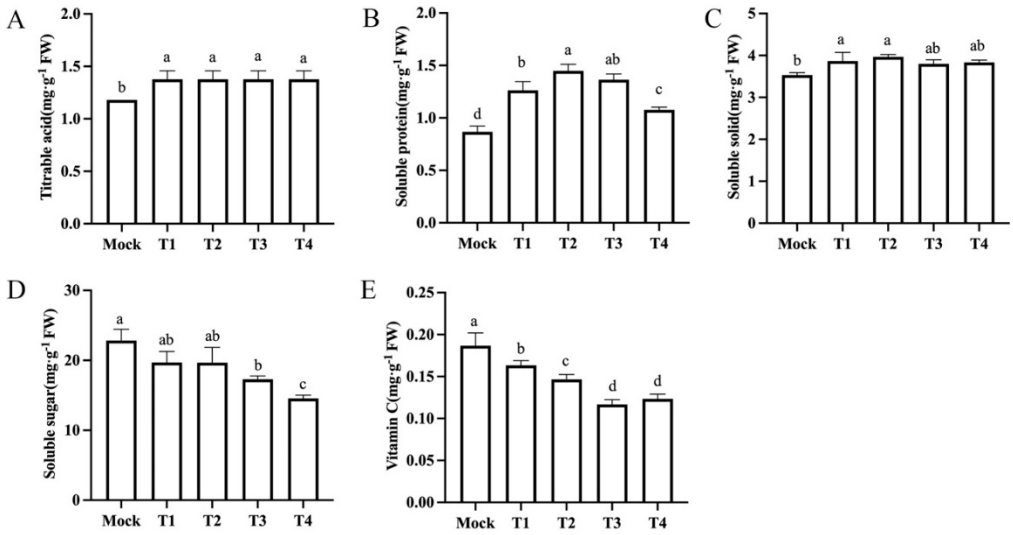

Figure 2. Adding sulfur to the soil altered the content of several nutrients in cucumber fruits. Fruits with commerciality were taken as material. The content of five nutrients, including titratable acid (**A**), soluble protein (**B**), soluble solid (**C**), soluble sugar (**D**), and vitamin C (**E**), were measured. In each experiment, 5 fruits were detected. Results from three replicates were calculated to obtain the mean ± SE and statistically analyzed using one-way ANOVA. The different lowercase letters indicate that differences between treatments were significant at the $p < 0.05$ level.

3.3. Adding Sulfur into Soil Reduced the Incidence of Powdery Mildew in Cucumber

Ten, twenty, and thirty days after the inoculation of powdery mildew, disease onset was detected on the leaves. The control group exhibited severe symptoms, with many large spots. The powdery mildew condition in the T1 and T2 groups was significantly reduced, showing a decrease in the number and size of spots on the leaves compared to the control group. The T3 and T4 groups displayed milder symptoms, significantly decreasing the number and size of spots (Figure 3).

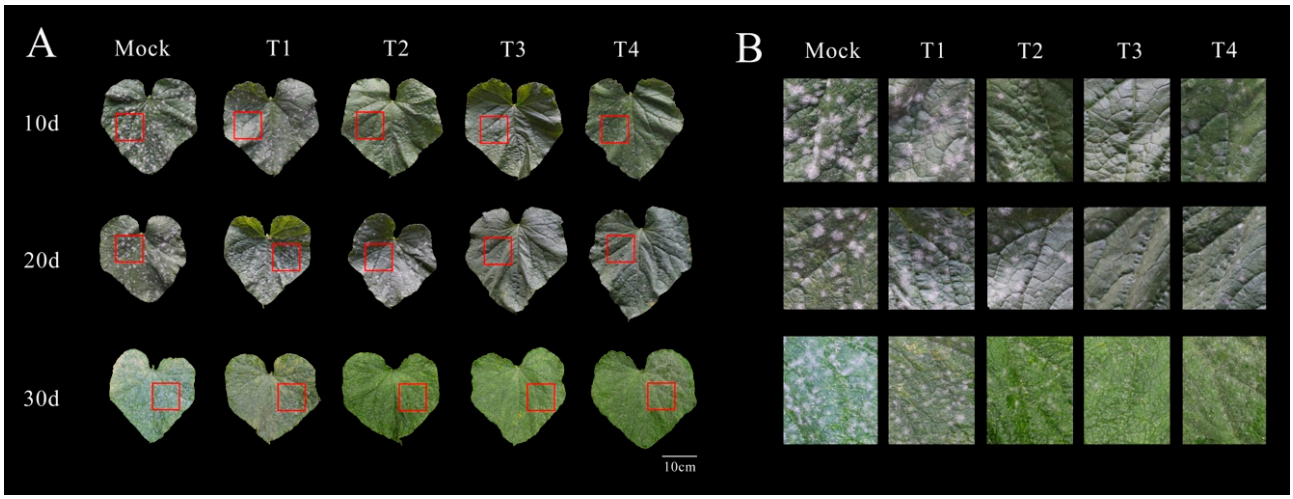

Figure 3. Adding sulfur to the soil significantly reduced powdery mildew damage to cucumber leaves. Photographs were taken of the 4th or 5th leaf (counted downwards from the top of the plants) ten, twenty, and thirty days after the powdery mildew fungus was introduced. (**A**) shows a complete leaf, and (**B**) shows an enlarged view of the portion framed in red in (**A**).

To compare the degree of incidence more accurately, 10 plants were randomly selected from each group of mid-epidemic plants, and the degree of incidence grading and disease index calculation were carried out. The disease index for the control group was 89, while the disease index for the T1–T4 groups was significantly lower than that of the control group (Table 1). The lowest disease index was observed in the T3 group (17.27), suggesting that the optimal sulfur addition level may be 0.6 g·Kg^{-1}.

Table 1. The powdery mildew incidence of leaves in different treatments.

Treatment	Level Classification							Disease Index	Control Efficiency (%)
	0	1	3	5	7	9	11		
Mock	0	0	0	0	1	4	5	89.09 ± 0.05 a	0.00 ± 0.00 e
T1	0	0	1	1	2	2	4	76.36 ± 1.13 b	14.28 ± 1.27 d
T2	2	2	2	2	1	1	0	30.91 ± 0.39 d	65.31 ± 0.44 b
T3	3	2	4	1	0	0	0	17.27 ± 0.95 e	80.61 ± 1.06 a
T4	1	1	2	3	2	1	0	40.91 ± 0.42 c	54.08 ± 0.47 c

Note: Ten plants were randomly selected from each group 30 days after inoculation with powdery mildew fungus. The data in the table show the number of plants at different disease levels. The disease index and control efficiency were also counted. Data from 3 replicates were calculated to obtain the mean ± SE and statistically analyzed using one-way ANOVA. The different lowercase letters indicate that differences between treatments were significant at the $p < 0.05$ level.

3.4. Adding Sulfur to the Soil Increased the Antioxidant Capacity of Cucumber Plants

To verify the effect of sulfur addition on the antioxidant capacity of cucumber cells, the 5th leaf (counted from the top) was used to detect the activity of SOD, POD, and CAT. Compared with the control group, SOD activity was significantly enhanced in the T1, T2, T3, and T4 groups. Additionally, POD activity was significantly enhanced in T1 and T2 groups. Furthermore, CAT activity increased gradually with the increase in sulfur dosage (Figure 4).

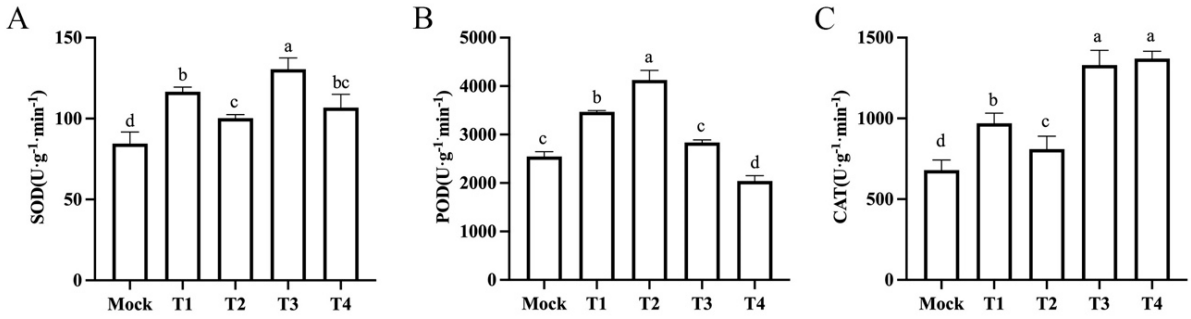

Figure 4. Adding sulfur to the soil increased the activity of antioxidant enzymes in cucumber leaves. The activities of SOD (**A**), POD (**B**), and CAT (**C**) were measured. Five leaves were tested in each experiment. Data from three replicates were calculated to obtain the mean ± SE and statistically analyzed using one-way ANOVA. The different lowercase letters indicate that differences between treatments were significant at the $p < 0.05$ level.

Leaves were stained with DAB to observe the accumulation of ROS. Compared to the control leaves, which exhibited numerous brown spots, leaves in the T2 and T3 groups showed a reduced number of brown spots (Figure 5).

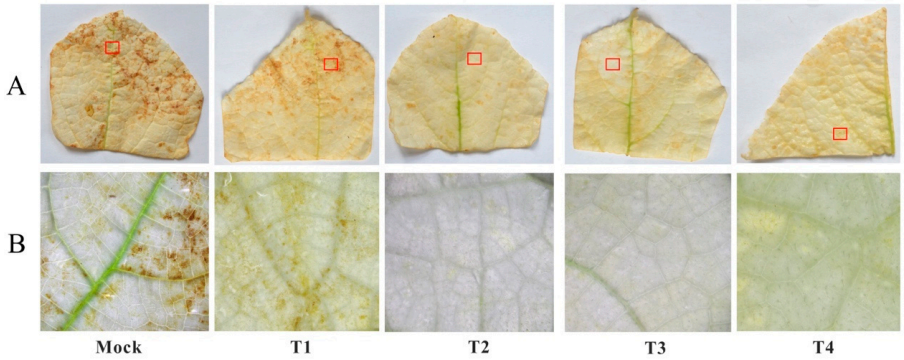

Figure 5. Adding sulfur to the soil reduced ROS accumulation in cucumber leaves. The 4th or 5th leaf (counted downwards from the top) was taken 10 days after the inoculation of powdery mildew fungus. ROS content was visualized using DAB staining. (**A**) shows a photograph of a leaf, and (**B**) shows an enlarged view of the portion framed in red in (**A**).

3.5. Cucumber Plants Absorbed Added Sulfur

To analyze the sulfur absorption of cucumber plants, the sulfur content in both the soil and leaves was measured. The soil sulfur content in each treatment group decreased significantly after cucumber harvesting compared to the pre-planting soil. The reductions were as follows: 58.02 ± 2.61% in the T1 group, 56.98 ± 1.62% in the T2 group, 53.81 ± 2.42% in the T3 group, and 41.08 ± 7.26% in the T4 group. Additionally, there was a slight increase in leaf sulfur content. The T2 and T3 groups showed slightly higher leaf sulfur content than the control group, while the T4 group exhibited a significant increase (Figure 6).

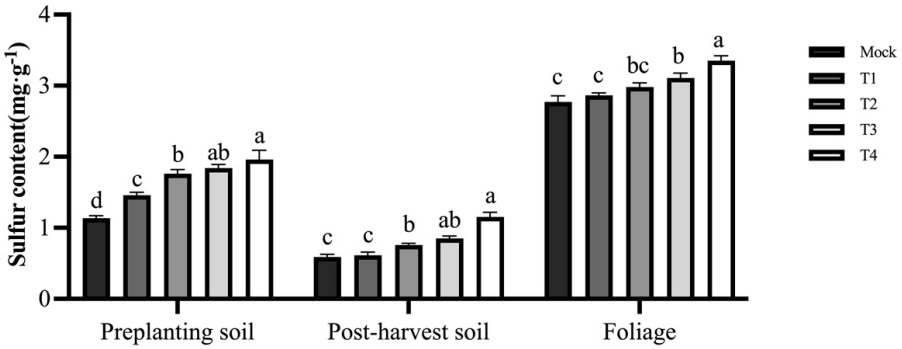

Figure 6. The sulfur content in soil and cucumber leaves. Five leaves or soil samples were detected in each experiment. Data from 3 replicates were calculated to obtain the mean ± SE and statistically analyzed using one-way ANOVA. The different lowercase letters indicate that differences between treatments were significant at the $p < 0.05$ level.

3.6. The Effect of Sulfur Addition on Soil Vitality

The effects of sulfur addition on soil vitality were studied by measuring the activity of several enzymes in the soil after different treatments. The results indicated that protease and urease activity remained unchanged after sulfur addition. However, dehydrogenase activity decreased significantly (13.66 ± 3.63%, 41.97 ± 4.50%, 35.36 ± 2.68%, and 25.58 ± 4.26% in T1-T4 groups, respectively), while sucrase activity increased significantly (41.46 ± 4.03%, 49.69 ± 8.80%, 69.54 ± 2.26%, and 84.85 ± 3.54% in T1–T4 groups, respectively). Additionally, alkaline phosphatase activity was increased dramatically in the T3 group (Figure 7).

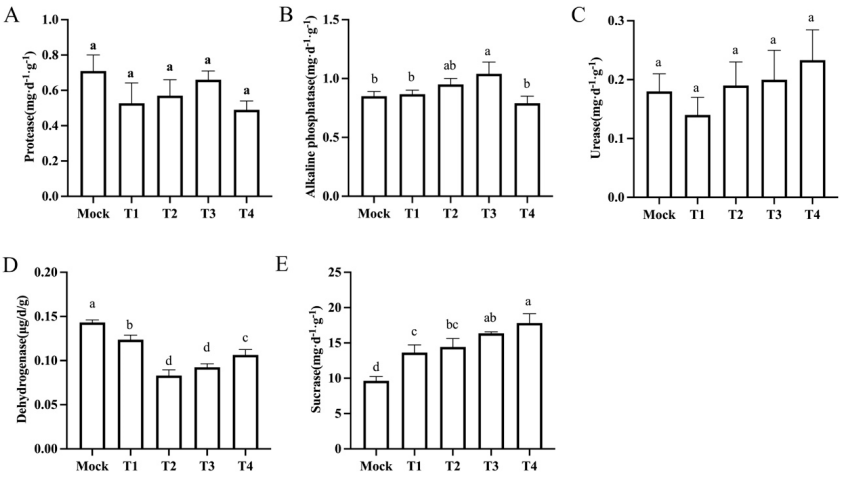

Figure 7. The effect of sulfur addition on soil enzymes' activity. The activities of protease (**A**), alkaline phosphatase (**B**), urease (**C**), dehydrogenase (**D**), and sucrase (**E**) were measured. Five soil samples were detected in each experiment. Data from 3 replicates were calculated to obtain the mean ± SE and statistically analyzed using one-way ANOVA. The different lowercase letters indicate that differences between treatments were significant at the $p < 0.05$ level.

4. Discussion

Various methods have been used to control cucumber powdery mildew, but the control has been unsatisfactory due to the frequent and widespread infestation of powdery mildew organisms [35]. Additionally, the frequent use of chemicals increases the risk to food and environmental safety [36]. Applying specific mineral elements to plants or cultivated soil can improve crop resistance to powdery mildew. For example, foliar sprays of soluble silicon enhanced resistance to powdery mildew in cucumber and wheat [29,37]. Foliar sprays of KH_2PO_4, K_2HPO_4, and $MgSO_4$ reduced the incidence of powdery mildew in sunflowers [38]. Treatments such as lithium chloride on cucumber leaves infected with powdery mildew reduced the spore production and infectivity of the powdery mildew fungus [39]. Foliar spraying of Mn–amino acid complexes (Mn–methionine (Mn-Met) and Mn–lysine (Mn-Lys)) strengthened plant defenses and helped prevent powdery mildew development [26]. Using 0.02 $g \cdot day^{-1} \cdot m^{-3}$ sulfur fumigation for 2 h daily has eliminated powdery mildew fungi. However, this fumigation method also damaged cucumber leaves, elevated levels of proline and triglycerides, and involved changes in the carbon and nitrogen metabolism within the leaves [40]. The application of sulfur to the soil was found to be effective in controlling powdery mildew. The powdery mildew in lentils decreased when sulfur and zinc were added to the soil [41]. Adding bio-sulfur to the soil enhanced the tomato's immunity to powdery mildew [42]. The results here showed that adding sulfur to the soil significantly increased cucumber's resistance to powdery mildew, indicating that sulfur application to the soil may effectively enhance multiple plants' immunity to powdery mildew.

High concentrations of sulfur in the soil may have negative effects by increasing soil acidification. However, the proper dose benefits soil enzyme activities, positively affecting the abiotic environment [43]. Sulfur addition can increase the activities of urease, neutral phosphatase, and polyphenol oxidase by enhancing microbial activity in the soil [44]. In this work, adding sulfur to the soil reduced sulfur residues in the plant. This addition had no significant effect on soil protease and urease, but it did reduce dehydrogenase activity and increase sucrase activity. Soil dehydrogenase activity reflects the abundance of active microorganisms in the soil and their ability to degrade organic matter. Sucrase, secreted by microorganisms, is mainly used to break down sucrose in the soil. Adding sulfur monomers to soil may affect the soil's microbial degradation properties and potentially impact plant health in the long term. However, it can also assist plants in absorbing sucrose

and promote their growth and development to some extent. Therefore, further study is needed to understand the overall impact of sulfur monomers on the soil environment.

When plants are under stress from pathogens, they produce and accumulate ROS. ROS can help destroy pathogenic microorganisms and activate internal disease-resistant mechanisms. However, an excessive buildup of free radicals can also harm the plant cells. Plant cells have an antioxidant system to prevent excessive ROS accumulation and maintain normal cellular metabolism. Antioxidant enzymes within the cells play a crucial role in preventing the excessive accumulation of ROS. Research has demonstrated that the antioxidant system becomes active in cucumbers infected with powdery mildew. This results in a decrease in chlorophyll content in the leaves, while the activities of peroxidase (POD) and superoxide dismutase (SOD), along with malondialdehyde (MDA) content, increase [45]. When D-pinitol was sprayed on cucumber leaves infected by powdery mildew, the levels of secondary metabolites, e.g., total phenols, GAD, and flavonoids, increased in the plant. This treatment also boosted the activity of antioxidant enzymes like PPO and SOD and activated defense-related genes, ultimately enhancing the plant's resistance to the disease [46]. Similarly, balsam pear (*Momordica charantia*) exhibited a significant increase in the activity of POD, CAT, and SOD following powdery mildew infection. Additionally, there was a notable rise in the accumulation of ROS, leading to an enhanced rate of lignin accumulation in the cell wall. Spraying of *Bacillus cereus* significantly inhibited the germination of powdery mildew spores and induced JA pathway-dependent disease resistance in balsam pear [47]. In this experiment, adding sulfur to the soil significantly increased SOD and CAT activities in cucumber leaves dealing with powdery mildew infection. Preliminary results from ROS staining suggest that sulfur addition influenced the accumulation of ROS in leaf cells. The results indicate that sulfur enhances the antioxidant capacity of cucumber leaf cells, possibly contributing to the plant's improved immunity against powdery mildew fungus.

Sulfur is an essential element that synthesizes proteins, chlorophyll, and vitamins [48]. Research has shown that adding sulfur-containing fertilizers to soil can activate sulfur-related metabolic processes in plants and boost their resistance to pathogens. Sulfur is also involved in the synthesis of GSH (glutathione), which helps to scavenge ROS [49], and in the synthesis of Glucosinolates, which are important for triggering defensive responses [50]. Moreover, certain sulfur-rich proteins have essential roles in defending against pathogens [51]. Adding sulfur to the soil at a rate of 0.5 $g \cdot kg^{-1}$ can effectively alleviate sulfur deficiency in maize. The sulfur content of maize leaves, stems, and roots progressively increased with increasing sulfur application [52]. Additionally, sulfur application in the soil significantly increased the sulfur content in the straw and kernels of the wheat–soybean cropping sequence [53]. Furthermore, the application of sulfur fertilizer in the soil, along with fungicide spraying, significantly increased crop yield [54]. In this experiment, it was revealed that the sulfur content of the soil decreased significantly during cucumber growth while the sulfur content in the plant increased significantly. This suggests that the plant absorbed the added sulfur, stimulating metabolic reactions and enhancing resistance to powdery mildew.

Sulfur contributes to improved crop yield and quality. Research has shown that sulfur fertilizer increases wheat seeds' weight and protein content [55]. Additionally, applying sulfur to the soil led to higher levels of soluble solids and vitamin C in cucumber fruits [56], and increased yield and protein content in pepper [57]. Here, it was also found that adding sulfur to the soil significantly increased the levels of soluble protein, titratable acid, and soluble solids in cucumber fruits while also enhancing disease resistance. Vitamin C can be used as a reducing agent [58]. The addition of sulfur led to a decrease in the vitamin C content of cucumber fruits, so further research may be required to determine its impact on resistance to powdery mildew.

Compared to traditional methods such as breeding resistant varieties, developing microbial fungicides, various chemicals, and soil high-temperature abatement [59–61], adding sulfur to soil can enhance the resistance of cucumber to powdery mildew. It has

no significant negative impact on soil activity, cucumber yield, and quality. Furthermore, the cost is also lower. In the future, research on how plants absorb soil sulfur and how it is transported and transformed in plants will help reveal the mechanism of sulfur's action further. This will provide more valuable clues for developing new measures for powdery mildew control. The impact of adding sulfur to soil on microbial communities must be studied more. The research will provide insights into how sulfur affects the structure of soil microbial communities, their metabolic pathways, and soil pH and will help create a theoretical framework for properly utilizing sulfur's nutritional and ecological functions in vegetable cultivation.

5. Conclusions

Adding 0.2–0.8 g/kg of sulfur to the soil can effectively increase the sulfur content of cucumber plants. This increase in sulfur content significantly promotes photosynthesis, leading to the synthesis and accumulation of organic matter and a significant increase in fruit yield. It also increases titratable acid and soluble protein content in cucumber fruits, improving their nutritional value. Furthermore, adding sulfur to the soil improves the resistance of cucumber leaves to powdery mildew, significantly reducing the incidence rate of powdery mildew and the area of disease spots. Additionally, the activities of SOD and CAT in cucumber leaves increase with the addition of sulfur, improving the antioxidant capacity of leaf cells and counteracting the adverse effects of excessive reactive oxygen species accumulation. The application of sulfur has minimal impact on the soil environment, with a decrease in dehydrogenase activity and an increase in sucrase activity, which may affect the degradation performance of soil microorganisms but can help plants absorb sucrose. These results suggest that adding sulfur to the soil can be used as an effective measure for controlling cucumber powdery mildew.

Author Contributions: Z.S., J.S., and Z.W. conceived and designed the research and finished writing the article; H.J., Z.W., X.K., Y.Z., and J.W. conducted the experiments; Y.W., Z.Y., Y.L., Y.F., and Y.H. contributed to the research data analysis. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the Shijiazhuang Science and Technology Leader Project (248790066A), the Modern Agricultural Science and Technology Innovation of Shijiazhuang Research Institute of Agricultural and Forestry Sciences (2023-5), and the Heibei Open-field Vegetable Innovation Team of Modern Agro-industry Technology Research System (HBCT2021200101).

Data Availability Statement: The manuscript contains all data that were created or examined during the research.

Acknowledgments: We thank all the colleagues who helped with the development of different parts of this manuscript.

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

References

1. Sarhan, E.A.D.; Abd-Elsyed, M.H.F.; Ebrahiem, A.M.Y. Biological control of cucumber powdery mildew (*Podosphaera xanthii*) (Castagne) under greenhouse conditions. *Egypt. J. Biol. Pest Control.* **2020**, *30*, 7. [CrossRef]
2. Asalf, B.; Trandem, N.; Stensvand, A.; Wekesa, V.W.; De Moraes, G.J.; Klingen, I. Influence of sulfur, powdery mildew, and the predatory mite *Phytoseiulus persimilis* on two-spotted spider mite in strawberry. *Biol. Control.* **2012**, *61*, 121–127. [CrossRef]
3. Nuñez-Palenius, H.; Hopkins, D.; Cantliffe, D. Powdery mildew of cucurbits in florida. *EDIS* **2006**, *20*, 1–9. [CrossRef]
4. Shen, H.; You, C.; Zhang, Y.Y.; Cheng, F.M.; Guo, S.R.; Sun, J. Advances in integrated control of cucumber powdery mildew. *Heilongjiang Agric. Sci.* **2017**, 133–137.
5. Kuzuya, M.; Yashiro, K.; Tomita, K.; Ezura, H. Powdery mildew (*Podosphaera xanthii*) resistance in melon is categorized into two types based on inhibition of the infection processes. *J. Exp. Bot.* **2006**, *57*, 2093–2100. [CrossRef] [PubMed]
6. Hai, F.; Yuan, S.X.; Liu, S.M. Control effcacy and safety evaluation of the combination of azoxystrobin and tebuconazole oncucumber powdery mildew (*Sphaerotheca fuligenea*). *China Cucurbit. Veget.* **2022**, *35*, 86–91.
7. Wang, M.; Han, P.; Lu, X.H.; Liu, X.L. Determination of sensitivity baseline of Sphaerotheca fuligine to ZJ0712 and assessment of Its laboratory resistant risk. *Acta Phytopathol. Sin.* **2007**, *37*, 660–665.

8. Yang, L.L.; Mu, W.; Liu, F.; Chen, Z.L.; Zhai, R.H. Induction of resistance to hexaconazole and biological properties of hexaconazole-resistant strains of sphaerotheca fuliginea. *Chin. J. Pestic. Sci.* **2007**, *9*, 29–33.
9. Dehestani, A.; Babaeizad, V.; Moradi, N.; Rahimian, H. Comparative study of selected cucumber cultivars resistant to powdery mildew caused by *Sphaerotheca fuliginea*. *Plant Cell Biotechnol. Mol. Biol.* **2017**, *18*, 30–38.
10. Sakata, Y.; Sugiyama, M.; Ohara, T.; Morishita, M. Influence of rootstocks on the resistance of grafted cucumber (*Cucumis sativus* L.) scions to powdery mildew (*Podosphaera xanthii* U. Braun & N. Shishkoff). *J. Jpn. Soc. Hortic. Sci.* **2006**, *75*, 135–140.
11. Liu, F.; Zhuge, Y.Y.; Yang, C.Y.; Jin, S.X.; Chen, J.; Li, H.; Dai, G. Control effects of some plant extracts against cucumber powdery mildew (*Sphaerotheca fuliginea*) and their stability study. *Eur. J. Hortic. Sci.* **2010**, *75*, 147–152.
12. Chen, J.; Dai, G.H. Effect of D-pinitol isolated and identified from *Robinia pseudoacacia* against cucumber powdery mildew. *Sci. Hortic.* **2014**, *176*, 38–44. [CrossRef]
13. Zhang, Z.Y.; Dai, G.H.; Zhuge, Y.Y.; Li, Y.B. Protective effect of *Robinia pseudoacacia* Linn1 extracts against cucumber powdery mildew fungus, *Sphaerotheca fuliginea*. *Crop Prot.* **2008**, *27*, 920–925. [CrossRef]
14. Yang, X.; Ma, X.; Yang, L.; Yu, D.; Qian, Y.; Ni, H. Efficacy of *Rheum officinale* liquid formulation on cucumber powdery mildew. *Crop Prot.* **2009**, *28*, 1031–1035. [CrossRef]
15. Lu, X.Y.; Li, S.Z.; Ma, P.; Gao, S.G.; Zhao, Y.; Sun, Y.S. Creening, identifcation and action mechanism of bacteria as biocontrol agent acainst cucumber powdery mildew. *Chin. J. Bio. Control.* **2006**, *S1*, 54–58.
16. Lu, F.; Wang, W.Q. Vegetable fungicides and their application in China. *China Veget.* **2017**, 6–13.
17. Reuveni, M.; Agapov, V.; Reuveni, R. Controlling powdery mildew caused by *Sphaerotheca fuliginea* in cucumber by foliar sprays of phosphate and potassium salts. *Crop Prot.* **1996**, *15*, 49–53. [CrossRef]
18. Wei, G.Q.; Zhu, Z.J.; Qian, Q.Q.; Li, J. Effect of silicon on resistance of cucumber powdery mildew and its physiological mechanism. *Plant Nutr. Fert. Sci.* **2004**, *10*, 202–205.
19. Menzies, J.; Bowen, P.; Ehret, D.; Glass, A. Foliar applications of potassium silicate reduce severity of powdery mildew on cucumber, muskmelon, and zucchini squash. *J. Am. Soc. Hortic. Sci.* **1992**, *117*, 902–905. [CrossRef]
20. Reuveni, M.; Agapov, V.; Reuveni, R. Induced systemic protection to powdery mildew in cucumber by phosphate and potassium fertilizers: Effects of inoculum concentration and post-inoculation treatment. *Can. J. Plant Pathol.* **1995**, *17*, 247–251. [CrossRef]
21. Reuveni, M.; Agapov, V.; Reuveni, R. Suppression of cucumber powdery mildew (*Sphaerotheca fuliginea*) by foliar sprays of pbospbate and potassium salts. *Plant Pathol.* **2007**, *44*, 31–39. [CrossRef]
22. Reuveni, R.; Dor, G.; Raviv, M.; Reuveni, M.; Tuzun, S. Systemic resistance against *Sphaerotheca fuliginea* in cucumber plants exposed to phosphate in hydroponics system, and its control by foliar spray of mono-potassium phosphate. *Crop Prot.* **2000**, *19*, 355–361. [CrossRef]
23. Zhou, J.; Wang, M.; Sun, Y.; Gu, Z.; Wang, R.; Saydin, A.; Shen, Q.; Guo, S. Nitrate increased cucumber tolerance to *Fusarium wilt* by regulating fungal toxin production and distribution. *Toxins* **2017**, *9*, 100. [CrossRef] [PubMed]
24. Wang, M.; Sun, Y.; Gu, Z.; Wang, R.; Sun, G.; Zhu, C.; Guo, S.; Shen, Q. Nitrate protects cucumber plants against *Fusarium oxysporum* by regulating citrate exudation. *Plant Cell Physiol.* **2016**, *57*, 2001–2012. [CrossRef]
25. Barnea, D.; Yermiyahu, U.; Rav-David, D.; Elad, Y. Effect of mineral nutrition and salt spray on cucumber downy mildew (*Pseudoperonospora cubensis*). *Plants.* **2022**, *11*, 1007. [CrossRef]
26. Eskandari, S.; Khoshgoftarmanesh, A.H.; Sharifnabi, B. The effect of foliar-applied manganese in mineral and complex forms with amino acids on certain defense mechanisms of cucumber (*Cucumis sativus* L.) against powdery mildew. *J. Plant Growth Regul.* **2018**, *37*, 481–490. [CrossRef]
27. Eskandari, S.; Höfte, H.; Zhang, T. Foliar manganese spray induces the resistance of cucumber to *Colletotrichum lagenarium*. *J. Plant Physiol.* **2020**, *246–247*, 153129. [CrossRef]
28. Eskandari, S.; Sharifnabi, B. The modifications of cell wall composition and water status of cucumber leaves induced by powdery mildew and manganese nutrition. *Plant Physiol. Biochem.* **2019**, *145*, 132–141. [CrossRef] [PubMed]
29. Wolff, S.; Karoliussen, I.; Rohloff, J.; Strimbeck, R. Foliar application of silicone against powdery mildew infection in cucumber greenhouse production. *J. Food, Agric. Environ.* **2012**, *10*, 355–359.
30. Khan, M.R.; Khan, M.W.; Pasha, M.J. Effects of sulfur dioxide on the development of powdery mildew of cucumber. *Environ. Exp. Bot.* **1998**, *40*, 265–273. [CrossRef]
31. Sawant, I.S.; Wadkar, P.N.; Ghule, S.B.; Rajguru, Y.R.; Salunkhe, V.P.; Sawant, S.D. Enhanced biological control of powdery mildew in vineyards by integrating a strain of *Trichoderma afroharzianum* with sulphur. *Biol. Control* **2017**, *114*, 133–143. [CrossRef]
32. Savocchia, S.; Mandel, R.; Crisp, P.; Scott, E. Evaluation of 'alternative' materials to sulfur and synthetic fungicides for control of grapevine powdery mildew in a warm climate region of Australia. *Australas. Plant Path.* **2010**, *40*, 20–27. [CrossRef]
33. Jiang, W.; Li, Q.; Wang, H.; Chen, R.; Xiang, L.; Liu, E.; Chen, X.; Shen, X.; Yin, C.; Mao, Z. Effect of sulfur on apple replanted soil environment and growth of *Malus hupehensis* seedlings. *Acta Hortic. Sin.* **2020**, *47*, 1225–1236.
34. Cui, S.G.; Qin, J.H. Study on the determination of leaf area of rape by image processing. *Hubei Agric. Sci.* **2017**, *56*, 2756–2757.
35. Abo-Elyousr, K.A.; Seleim, M.; Almasoudi, N.; Bagy, H.M. Evaluation of native bacterial isolates for control of cucumber powdery mildew under greenhouse conditions. *Horticulturae* **2022**, *8*, 1143. [CrossRef]
36. Madbouly, A.K.; Abo Elyousr, K.A.M.; Ismail, I.M. Biocontrol of *Monilinia fructigena*, causal agent of brown rot of apple fruit, by using endophytic yeasts. *Biol. Control* **2020**, *144*, 104239. [CrossRef]

37. Rémus-Borel, W.; Menzies, J.G.; Bélanger, R.R. Silicon induces antifungal compounds in powdery mildew-infected wheat. *Physiol. Mol. Plant Pathol.* **2005**, *66*, 108–115. [CrossRef]
38. Abo-Elyousr, K.; Ahmed, H.A.M.; Hassan, M.A.E.; El-Fatah, B.E.S.A. Influence of foliar application of some salts, phyto-extracts and essential oils for contolling powdery mildew disease of *Helianthus annuus*. *J. Plant Pathol.* **2022**, *104*, 735–747. [CrossRef]
39. Al-Janabi, J.; Lösel, D.; Ayres, P. Changes in abundance and infectivity of powdery mildew conidia from cucumber plants treated systemically with lithium chloride. *Plant Pathol.* **1992**, *41*, 255–261.
40. Liu, J.; Gao, Y.; Gong, F.; Hou, F.; Zhang, Z.; Cheng, X.; Du, W.; Zhang, L.; Wang, J.; Xu, J.; et al. The transcriptome and metabolome reveal stress responses in sulfur-fumigated cucumber (*Cucumis sativus* L.). *Front. Plant Sci.* **2021**, *12*, 778956. [CrossRef]
41. Singh, A.K.; Singh, K.M.; Bharati, R.C.; Chandra, N.; Bhatt, B.P.; Pedapati, A. Potential of residual sulfur and zinc nutrition in improving powdery mildew (*Erysiphe trifolii*) disease tolerance of lentil (*Lens culunaris* L). *Commun. Soil Sci. Plan.* **2014**, *45*, 2807–2818. [CrossRef]
42. Llorens, E.; Agustí-Brisach, C.; González-Hernández, A.I.; Troncho, P.; Vicedo, B.; Yuste, T.; Orero, M.; Ledó, C.; García-Agustín, P.; Lapeña, L. Bioassimilable sulphur provides effective control of *Oidium neolycopersici* in tomato, enhancing the plant immune system. *Pest Manag. Sci.* **2017**, *73*, 1017–1023. [CrossRef]
43. Tabak, M.; Lisowska, A.; Filipek-Mazur, B. Bioavailability of sulfur from waste obtained during biogas desulfurization and the effect of sulfur on soil acidity and biological activity. *Processes* **2020**, *8*, 863. [CrossRef]
44. Zhao, Y.; Xiao, X.; Bi, D.; Hu, F. Effects of sulfur fertilization on soybean root and leaf traits, and soil microbial activity. *J. Plant Nutr.* **2008**, *31*, 473–483. [CrossRef]
45. Zhang, Q.; Zhou, M.; Wang, J. Increasing the activities of protective enzymes is an important strategy to improve resistance in cucumber to powdery mildew disease and melon aphid under different infection/infestation patterns. *Front. Plant Sci.* **2022**, *13*, 950538. [CrossRef]
46. Chen, J.; Fernandez, D.; Wang, D.D.; Chen, Y.J.; Dai, G.H. Biological control mechanisms of D-pinitol against powdery mildew in cucumber. *Physiol. Mol. Plant Pathol.* **2014**, *88*, 52–60. [CrossRef]
47. Xiang, J.Y.; Shang, S.; Tian, L.B. Study on the control mechanism of *Bacillus velezensis* N46 against powdery mildew of bitter melon. *Chin. J. Trop. Corps.* **2023**, 1–17, *ahead of print*.
48. Scherer, H.; Pacyna, S.; Spoth, K.; Schulz, M. Low levels of ferredoxin, ATP and leghemoglobin contribute to limited N_2 fixation of peas (*Pisum sativum* L.) and alfalfa (*Medicago sativa* L.) under S deficiency conditions. *Biol. Fert. Soils.* **2008**, *44*, 909–916. [CrossRef]
49. Zhou, J.; Hao, M.; Liu, Y.; Huang, G.; Fu, Q.; Zhu, J.; Hu, H. Effects of exogenous sulfur on growth and Cd uptake in Chinese cabbage (*Brassica campestris* spp. pekinensis) in Cd-contaminated soil. *Environ. Sci. Pollut. Res. Int.* **2018**, *25*, 15823–15829. [CrossRef]
50. Sotelo, T.; Lema, M.; Soengas, P.; Cartea, M.E.; Velasco, P. In vitro activity of glucosinolates and their degradation products against brassica-pathogenic bacteria and fungi. *Appl. Environ. Microbiol.* **2015**, *81*, 432–440. [CrossRef]
51. Rausch, T.; Wachter, A. Sulfur metabolism: A versatile platform for launching defence operations. *Trends Plant Sci.* **2005**, *10*, 503–509. [CrossRef]
52. Karimizarchi, M.; Soltangheisi, A.; Husin, A.; Yusop, M.K.; Othman, R. Sulfur uptake and translocation in maize (*Zea mays*) grown in a high pH soil treated with elemental sulfur. *J. Plant Nutr.* **2018**, *41*, 1798–1806. [CrossRef]
53. Singh, S.; Singh, R.; Singh, M.; Singh, V. Impact of sulfur fertilization on different forms and balance of soil sulfur and the nutrition of wheat in wheat-soybean cropping sequence in tarai soil. *J. Plant Nutr.* **2014**, *37*, 618–632. [CrossRef]
54. Sutherland, K.G.; Booth, E.J.; Walker, K.C. Effects of added sulphur on fungicide control of light leaf spot. In *HGCA Project Report*; Home Grown Cereals Authority: London, UK, 2004; pp. 1–69.
55. Hřivna, L.; Kotková, B.; Buresova, I. Effect of sulphur fertilization on yield and quality of wheat grain. *Cereal Res. Commun.* **2015**, *1*, 344–352. [CrossRef]
56. Motior, R.; Abdou, S.; Darwish, F.; El-Tarabily, K.; Awad, M.; Faruq, G.; Azirun, M. Influence of elemental sulfur on nutrient uptake, yield and quality of cucumber grown in sandy calcareous soil. *Aust. J. Crop Sci.* **2011**, *5*, 1610–1615.
57. Smatanova, M.; Richter, R.; Hlušek, J. Spinach and pepper response to nitrogen and sulphur fertilization. *Plant Soil Environ.* **2004**, *50*, 303–308. [CrossRef]
58. Siddiqui, Z.S. Effects of double stress on antioxidant enzyme activity in *Vigna radiata* (L.) Wilczek. *Acta Bot. Croat.* **2013**, *72*, 145–156. [CrossRef]
59. Cerkauskas, R.F.; Brown, J. Aspects of the epidemiology and control of powdery mildew (*Oidium neolycopersici*) on tomato in Ontario, Canada. *Can. J. Plant Pathol.* **2015**, *37*, 448–464. [CrossRef]
60. Hamza, A.; Mohamed, A.; Derbalah, A. Chemical inducers for resistance induction against powdery mildew of cucumber under greenhouse conditions. *Acta Phytopathol. Entomol. Hung.* **2017**, *52*, 49–60. [CrossRef]
61. Elagamey, E.; Abdellatef, M.A.E.; Haridy, M.S.A.; Abd El-aziz, E.-S.A.E. Evaluation of natural products and chemical compounds to improve the control strategy against cucumber powdery mildew. *Eur. J. Plant Pathol.* **2023**, *165*, 385–400. [CrossRef]