horticulturae

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Oligosaccharins Used Together with Tebuconazole Enhances Resistance of Kiwifruit against Soft Rot Disease and Improves Its Yield and Quality

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Abstract: *Botryosphaeria dothidea* is one of the most frequent pathogens of soft rot disease in kiwifruit. The aim of this study was to investigate the role of oligosaccharins used together with tebuconazole to control soft rot and their influences on kiwifruit's disease resistance, growth and quality. The results show that tebuconazole displayed a toxicity against *B. dothidea* RF-1 with 0.87 mg kg^{-1} of EC_{50} value. Oligosaccharins used together with tebuconazole effectively managed soft rot with 84.83% of the field management effect by spraying tebuconazole + oligosaccharins (0.5:0.5, m/v) as a 5000-fold dilution liquid, which significantly ($p < 0.01$) exceeded the 72.05%, 52.59%, 62.17% and 33.52% effect of tebuconazole 2500-, oligosaccharins 2500-, tebuconazole 5000- and oligosaccharins 5000-fold liquids, respectively. Simultaneously, co-application of tebuconazole and oligosaccharins was more effective for enhancing the resistance, growth and quality of kiwifruit compared with tebuconazole or oligosaccharins alone. This work highlights that oligosaccharins used together with tebuconazole can be proposed as a practicable measure for managing kiwifruit soft rot and reducing the application of chemical synthetic fungicides.

Keywords: *Botryosphaeria dothidea*; kiwifruit; oligosaccharins; soft rot disease; tebuconazole

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Citation: Wang, Q.; Long, Y.; Ai, Q.; Su, Y.; Lei, Y. Oligosaccharins Used Together with Tebuconazole Enhances Resistance of Kiwifruit against Soft Rot Disease and Improves Its Yield and Quality. *Horticulturae* **2022**, *8*, 624. https://doi.org/10.3390/horticulturae8070624

Academic Editor: Esmaeil Fallahi

Received: 17 June 2022
Accepted: 9 July 2022
Published: 11 July 2022

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1. Introduction

Kiwifruit, a healthy, emerging, and typical third-generation fruit, has very high nutritious, medicinal and economical values [1,2]. The kiwifruit industry, a major industry for boosting agricultural modernization, alleviating poverty and vitalizing rural areas, has expanded rapidly in Guizhou Province, China, where it has more than 40,000 hm^2 of planting area [2,3]. Nonetheless, the soft rot disease of kiwifruit caused by *Botryosphaeria dothidea*, *Phomopsis* spp., *Botrytis cinerea* and *Cryptosporiopsis actinidiae*, etc., is the most serious postharvest disease [4–11]. Koh et al. [6] reported that *B. dothidea* caused soft rot in Korea with 83.3% of average isolation rate. Our previous study also found that *B. dothidea* and *Phomopsis* spp. were the most frequent pathogens of kiwifruit in Guizhou with 89.93% and 10.07% of average isolation rates, respectively [9]. Soft rot disease has had an annual incidence of about 30~50% in Guizhou Province since 2014, and seriously influences the yield and quality of kiwifruit, as well as frequently causes severe financial losses [9–11]. Accordingly, it is of realistic significance to excogitate various candidate measures against kiwifruit soft rot for the sustainable development of its industry.

Currently, chemical control are still the most typical, frequent and effective strategies for managing plant fungal disease. For example, Koh et al. [6] reported that some chemical synthetic fungicides including benomyl, thiophanate-methyl, tebuconazole, iprodione and flusilazole could be used as preventive fungicides against the postharvest rot diseases of kiwifruit. Considering the residual risks and pathogen resistance of chemical synthetic fungicides, however, reducing the application of chemical synthetic fungicides and their alternative or finding complementary approaches has been growing popular for plant disease management [12–14]. We previously reported that some natural products including tetramycin, chitosan, ferulic acid, matrine and their combinations could effectively control kiwifruit soft rot and enhance their resistance, growth and quality [9–11,15–17]. Nevertheless, natural products are often slower to take effects compared to chemical fungicides for controlling plant diseases. Meanwhile, considering the perniciousness of kiwifruit's soft rot, new candidate agronomic strategies need to be developed to help meet this challenge. Additionally, we also found that the co-application of chitosan and isopyrazam azoxystrobin effectively managed leaf spot disease in kiwifruit and reduced isopyrazam azoxystrobin application [12]. Similarly, whether natural products can be used together with chemical fungicides to effectively reduce the application of chemical fungicides and control kiwifruit's soft rot is extremely worth further exploring.

Tebuconazole, a broad-spectrum chiral triazole fungicide, is one of the most widespread sold pesticides in the world, and widely used for controlling many plant fungi diseases [18–20]. Its action mechanism has been demonstrated to inhibit the sterol biosynthesis of plant pathogenic fungi [21]. Ma et al. [22] reported that the 50% effective concentration's inhibition rate (EC_{50} value) for tebuconazole on 105 *B. dothidea* isolates from a commercial pistachio orchard in California was 0.019~0.159 μg mL^{-1}. Oligosaccharins, a part of the cell walls of plants and fungi, is capable of regulating plant growth and acting as regulators of the plant response when infected by plant pathogens [23,24]. When plant pathogens attack plants, oligosaccharins can activate the generation of oligosaccharins by means of the enzymatic lysis of the cell wall of the attacked plant [24,25]. Recently, oligosaccharins has received considerable attention due to its ability to enhance antipathogenic compound synthesis and trigger defense responses in plants [23,24,26–28]. For instance, Boyzo-Marín et al. [27] reported that glutathione-oligosaccharins application led to the lowest incidence (0.66~13%) of downy mildew on blackberry in Mexico compared to that of other fungicides or control. The foliar application of oligosaccharins could effectively promote the stem elongation, biomass production and yield formation of tomatoes [29,30]. However, to date, there is little documentation available about oligosaccharins for controlling kiwifruit soft rot disease. In that case, it is worth further exploring whether oligosaccharins can promote tebuconazole to control kiwifruit soft rot and decrease tebuconazole application.

In the present work, the toxicities of ten synthetic fungicides and oligosaccharins on *B. dothidea* were firstly determined. Then, the field control effects of tebuconazole + oligosaccharins, tebuconazole and oligosaccharins to manage soft rot disease in kiwifruit were investigated. Subsequently, the influences of tebuconazole + oligosaccharins, tebuconazole and oligosaccharins on the resistance, yield and quality of kiwifruit were evaluated. This finding excogitates a green, practicable and candidate agronomic measure for managing kiwifruit soft rot and decreasing chemical synthetic fungicide application.

2. Pathogen, Reagents and Methods

2.1. Experimental Materials

The tested isolate was *Botryosphaeria dothidea* RF-1 with a highly pathogenicity, which was isolated from the "Guichang" kiwifruit collected from Xifeng County, Guiyang city, Guizhou province, China. Strain RF-1 was identified based on a pathogenicity, morphology and gene phylogenetic analysis [9], and kept at −20 °C in the Institute of Crop Protection, Guizhou University, Guizhou Province, China. Potato dextrose agar was sterilized at 121 °C for 30 min [3]. The manufacturing information of the tested fungicides is shown in Table 1; they include eleven chemical fungicides and one natural fungicide.

Table 1. The manufacturing information of tested fungicides.

Fungicides	Dosage Forms	Manufactures	Manufacture Sites
80% Tebuconazole	WG	Meibang Pesticide Co., Ltd.	Shaanxi, China
20% Eugenol	EW	Jianpai Agrochemical Co., Ltd.	Jiangsu, China
125 g/L Epoxiconazole	SC	BASF Plant Protection (Jiangsu) Co., Ltd.	Jiangsu, China
500 g/L Fluazinam	SC	Hetian Chemical Co. Ltd.	Zhejiang, China
75% Trifloxystrobin tebuconazole	WG	Bayer Crop Science (China) Co., Ltd.	Zhejiang, China
33.5% Oxine-copper	SC	Xingnong Pharmaceutical (China) Co., Ltd.	Shanghai, China
10% Difenoconazole	WG	Ruidefeng Biotechnology Co., Ltd.	Guangdong, China
25% Flutriafol	SC	Yancheng Limin Agrochemical Co., Ltd.	Jiangsu, China
50% Azoxystrobin	WG	Guannong Agrochemical Co., Ltd.	Hebei, China
40% Pyrimethanil	SC	Bayer Crop Science (China) Co., Ltd.	Zhejiang, China
5% Oligosaccharins	AS	Kesheng Group Co., Ltd.	Jiangsu, China

WG: wettable granule, EW: emulsion in water, SC: suspension concentrate.

2.2. Field Site

The field management experiment of kiwifruit soft rot was carried out in an orchard with a 6-year-old "Guichang" cultivar in Zhongba village, Shidong Town, Xifeng country, Guizhou Province, China (27°04′, 106°55′), where there were 74 plants with 66 female plants per 666.7 m^2. Moreover, its sunshine duration, rainfall, temperature, frostless season and altitude were about 1139.2 h, 1293 mm, 13.2~15 °C, 266 days and 1300 m, respectively. The nutritional information of the planting soil is shown in Table 2. Moreover, its exchangeable calcium and pH value were 20.33 cmol kg^{-1} and 6.24, respectively.

Table 2. The nutritional information of kiwifruit's planting soils.

Nutrition Indices	Content (g kg^{-1})	Nutrition Indices	Content (mg kg^{-1})	Nutrition Indices	Content (mg kg^{-1})
Organic matter	36.68	Alkali-hydrolyzable nitrogen	101.35	Available zinc	0.97
Total nitrogen	1.51	Available phosphorus	8.96	Available iron	35.87
Total phosphorus	1.73	Available potassium	2.31	Available manganese	19.24
Total potassium	1.16	Exchangeable magnesium	320.05	Available boron	0.31

2.3. Fungicide Toxicity Tests In Vitro

The mycelium growth method was applied for testing the toxicities of different fungicides against *B. dothidea* RF-1 [3]. The tested fungicide solutions with five series concentrations were designed according to pre-experiment results and readied by sterilized water. First, 1 mL of tested fungicide solution and 9 mL of fresh PDA (45~55 °C) were uniformly mixed to ready a fungicide-containing PDA, and sterilized water was used as a control solution. Then, their mixed solution was fed in petri dishes (90 mm of diameter) and we waited until it solidified. Subsequently, a *B. dothidea* RF-1 disc (5 mm of diameter) was cut from a 7-day-old *B. dothidea* RF-1 PDA plate and placed on the center of the fungicide-containing PDA plate with the inoculum side down; we used three replicates. The mycelium diameter of *B. dothidea* RF-1 in the treated plates was recorded using the crisscrossing method [15] after incubation (12 h of light/12 h of darkness) under 28 °C until mycelium grew to almost overlay the control plates. The inhibition rates under different gradient concentrations of each fungicide against *B. dothidea* RF-1 were checked. Finally, the EC_{50} values and regression equation of different fungicides on *B. dothidea* RF-1 were calculated by SPSS 18.0 software (SPSS Inc., Chicago, IL, USA). The inhibition rate was calculated according to Equation (1):

$$\text{Inhibition rate (\%)} = 100 \times [(\text{Mycelium diameter of control plate} - \text{Mycelium diameter of fungicide plate})/(\text{Mycelium diameter of control plate} - 5)] \quad (1)$$

2.4. Field Control Experiment

The foliar spray method and a completely randomized experimental design were used for the field management experiment of kiwifruit soft rot. Based on the toxicity test results, six experimental treatments were designed for controlling soft rot disease. They were, respectively: (1) T + O 5000: 80% tebuconazole WG + 5% oligosaccharins AS (0.5:0.5, m/v) 5000-fold dilution liquid, (2) T 2500: 80% tebuconazole WG 2500-fold dilution liquid, (3) O 2500:5% oligosaccharins AS 2500-fold dilution liquid, (4) T 5000:80% tebuconazole WG 5000-fold dilution liquid, (5) O 5000:5% oligosaccharins AS 5000-fold dilution liquid, and (6) irrigation water as control. Each treatment was applied in three replicates and their eighteen plots were randomly arranged. Five trees on the diagonal in every plot were used for measuring. According to our previous study [11], the pathogen infection periods of "Guichang" kiwifruit soft rot was 20 May~13 June and 2 August~12 August. Thus, 1.5 L and 2.0 L of fungicide dilution liquid were sprayed on the fruits, leaves, buds and stems of each kiwifruit plant on 18 May and 30 July, respectively.

2.5. Determination of Control Effects

Five fruits were collected from the five directions of each treated trees on 26 September, and a total of 125 fruits were obtained from five trees on the diagonal in each plot and randomly divided into two groups. Ninety fruits were used for investigating the management effects on soft rot disease, the other 35 fruits were used for determining their resistance, growth and quality. The incidence rate and control effect of kiwifruit soft rot were counted as Equations (2) and (3), respectively:

$$\text{Incidence rate (\%)} = 100 \times (\text{Number of diseased fruits/Total number of fruits}) \quad (2)$$

$$\text{Control effect (\%)} = 100 \times ((\text{Incidence rate in control} - \text{Incidence rate in fungicide})/\text{Incidence rate of control}) \quad (3)$$

2.6. Determination of Disease Resistance, Growth and Quality

The disease resistance parameters of kiwifruit that reached edible state were analyzed. Phenolics and flavonoids were determined using the HCl-methyl alcohol method according to Wang et al. [16]. Soluble protein was measured using the Coomassie bright blue method, and malondialdehyde (MDA) was determined by the thiobarbituric acid method [9]. Superoxide dismutase (SOD), peroxidase (POD), polyphenoloxidase (PPO) and phenylalanine ammonia lyase (PAL) activities were measured as in our previous studies [10]. The longitudinal diameter, transverse diameter, lateral diameter, fruit shape index, single fruit volume of kiwifruit were measured and calculated using a digital caliper, and its weight was analyzed by an analytical balance. Simultaneously, the quality parameters of kiwifruit under edible state were also analyzed as in our previous studies [10]. Vitamin C was measured by a high-performance liquid chromatography system (1260, Agilent, Santa Clara, CA, USA), the amount of soluble solid was determined by a digital refractometer (Yishida Sunshine Tech Co. Ltd., Beijing, China), and the dry matter was measured by the oven-drying method. Soluble sugar was determined using the anthrone colorimetric method, and titratable acidity was measured via titrating with 0.01 mol L^{-1} NaOH; as the sugar/acid ratio was the ratio value of soluble sugar and titratable acidity.

2.7. Statistical Analysis

The mean value ± standard deviation (SD) of three replicate results is displayed. The variance (ANOVA) and normality (quantile–quantile plot test) were analyzed by a SPSS 18.0 software (SPSS Inc., Chicago, IL, USA). Charts were plotted by Origin 10.0 software (OriginLab, Northampton, MA, USA).

3. Results

3.1. Toxicity of Fungicides on B. dothidea RF-1

The toxicities of eleven fungicides on *B. dothidea* RF-1 soft rot pathogen, are exhibited in Table 3. Tebuconazole displayed a superior toxicity on *B. dothidea* RF-1 with 0.87 mg kg^{-1} of EC_{50} value, which was higher by 1.23, 1.37, 1.39, 3.03, 5.29, 6.39, 10.79, 22.11, 25.05 and 255.56 folds compared to flutriafol, epoxiconazole, trifloxystrobin tebuconazole, difenoconazole, fluazinam, oxine-copper, eugenol, azoxystrobin, pyrimethanil and oligosaccharins, respectively. The results indicate that tebuconazole could be used as an optimized field fungicide to control kiwifruit soft rot. Although oligosaccharins had a relatively inferior toxicity on *B. dothidea* RF-1 with 222.34 mg kg^{-1} of EC_{50} value, it is worth further studying whether oligosaccharins could be applied as a resistance inductor for inducing the management of kiwifruit soft rot.

Table 3. The toxicities of different fungicides on *B. dothidea* RF-1.

Fungicides	Regression Equation	Determination Coefficient (R^2)	EC_{50} (mg kg^{-1})
Tebuconazole	$y = 10.7335 + 1.8745\ x$	0.9846	0.87
Flutriafol	$y = 8.6168 + 1.2179\ x$	0.9680	1.07
Epoxiconazole	$y = 6.0359 + 0.3542\ x$	0.9898	1.19
Trifloxystrobin tebuconazole	$y = 11.4974 + 2.2281\ x$	0.9869	1.21
Difenoconazole	$y = 9.4045 + 1.7084\ x$	0.9911	2.64
Fluazinam	$y = 8.2184 + 0.9647\ x$	0.9686	4.60
Oxine-copper	$y = 7.6318 + 1.1672\ x$	0.9833	5.56
Eugenol	$y = 11.9786 + 1.9446\ x$	0.9863	9.39
Azoxystrobin	$y = 5.6272 + 0.8786\ x$	0.9802	19.24
Pyrimethanil	$y = 7.0833 + 1.2536\ x$	0.9951	21.79
Oligosaccharins	$y = 5.6260 + 0.9587\ x$	0.9917	222.34

x and y represent the fungicide concentration and inhibition rate, respectively.

3.2. Management Effects of Tebuconazole and Oligosaccharins on Soft Rot

The field management effects of tebuconazole + oligosaccharins, tebuconazole, and oligosaccharins on kiwifruit soft rot are shown in Table 4. The tebuconazole + oligosaccharins 5000-fold liquid, tebuconazole 2500-fold liquid, oligosaccharins 2500-fold liquid, tebuconazole 5000-fold liquid and oligosaccharins 5000-fold liquid could significantly ($p < 0.01$) decrease incidence rate, and tebuconazole + oligosaccharins had an optimal effect. The control effect of the tebuconazole + oligosaccharins 5000-fold liquid on soft rot was 84.83%, which significantly ($p < 0.01$) exceeded the 72.05% of the tebuconazole 2500-fold liquid, 52.59% of the oligosaccharins 2500-fold liquid, 62.17% of the tebuconazole 5000-fold liquid and the 33.52% of the oligosaccharins 5000-fold liquid. Moreover, the control effect of the tebuconazole 2500-fold liquid against soft rot disease was significantly ($p < 0.01$) exceeded by that of the tebuconazole 5000-fold liquid, and that of the oligosaccharins 2500-fold liquid was also significantly ($p < 0.01$) exceeded by that of the oligosaccharins 5000-fold liquid. These results demonstrate that oligosaccharins induced a management effect on soft rot, and when mixed together with tebuconazole, it could more effectively control the soft rot disease of kiwifruit compared to tebuconazole or oligosaccharins alone, as well as effectively reduce the application dosage of tebuconazole.

Table 4. The field control effects of tebuconazole and oligosaccharins on kiwifruit soft rot.

Treatments	Incidence Rate (%)	Control Effects (%)
T + O 5000	8.15 ± 1.70^{fE}	84.83 ± 3.61^{aA}
T 2500	15.19 ± 2.80^{eD}	72.05 ± 3.59^{bB}
O 2500	25.56 ± 1.11^{dCD}	52.59 ± 4.20^{dC}
T 5000	20.37 ± 1.70^{cC}	62.17 ± 4.63^{cC}
O 5000	35.93 ± 1.70^{bB}	33.52 ± 1.02^{eD}
Control	54.07 ± 3.39^{aA}	

The mean ± SD (n = 3) of three replicates is shown. The significant differences at the 5% ($p < 0.05$) and 1% ($p < 0.01$) levels are represented as different small and capital letters, respectively.

3.3. Effects of Tebuconazole and Oligosaccharins on Kiwifruit Resistance

The effects of tebuconazole + oligosaccharins, tebuconazole and oligosaccharins on the disease resistance substances of kiwifruit are shown in Figure 1. The total phenolics, total flavonoids and soluble protein contents of kiwifruit treated by oligosaccharins were significantly ($p < 0.05$) increased compared to control, and their MDA content was significant ($p < 0.05$) decreased. Compared to tebuconazole, oligosaccharins could more effectively improve the disease resistance substances of kiwifruit, and significantly ($p < 0.05$) reduced their MDA content. Tebuconazole + oligosaccharins significantly ($p < 0.05$) improved the disease resistance substances of kiwifruit compared to tebuconazole, oligosaccharins or control, and significantly ($p < 0.05$) reduced their MDA content. These results indicate that oligosaccharins had a notable potential for enhancing the disease resistance of kiwifruit, which, used together with tebuconazole, effectively promoted the improving or inhibiting effects of oligosaccharins or tebuconazole on the phenolics, flavonoids, soluble protein and MDA of kiwifruit.

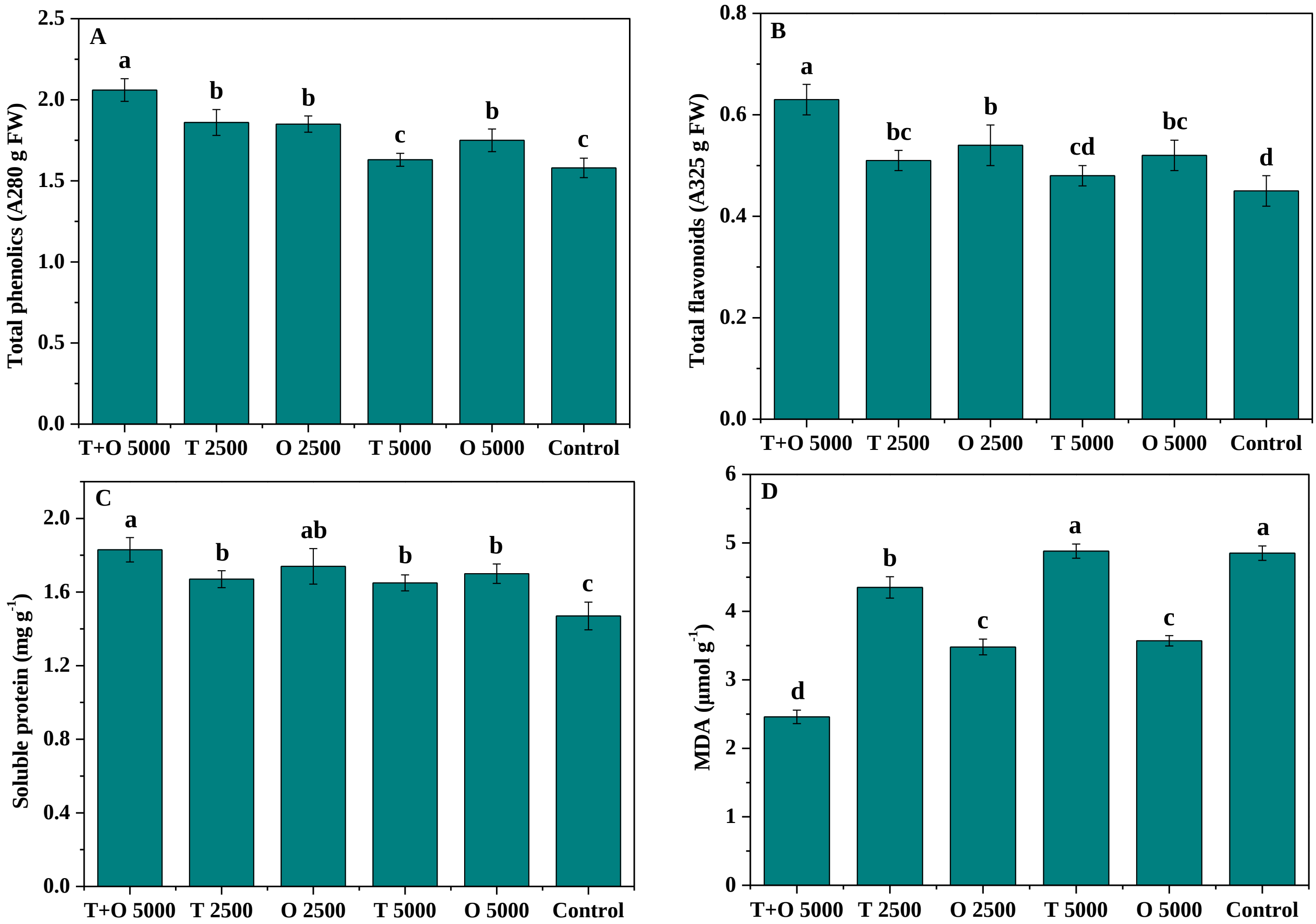

Figure 1. The effects of tebuconazole and oligosaccharins on phenolics (**A**) (unit: A280 g FW), flavonoids (**B**) (unit: A325 g FW), soluble protein (**C**) (unit: mg g^{-1}) and MDA (**D**) (unit: μmol g^{-1}) in kiwifruit. The mean ± SD (n = 3) of three replicates is shown. The significant differences at 5% ($p < 0.05$) are represented as different small letters.

The effects of tebuconazole + oligosaccharins, tebuconazole and oligosaccharins on the disease resistance enzyme activities of kiwifruit are shown in Figure 2. Tebuconazole + oligosaccharins significantly ($p < 0.05$) promoted the SOD, POD, PPO and PAL activities of fruits compared with tebuconazole, oligosaccharins or control. Compared to control, the oligosaccharins 2500- and 5000-fold liquids could significantly ($p < 0.05$) enhance SOD and PPO activities of fruits, and the oligosaccharins 2500-fold liquid could also significantly

($p < 0.05$) enhance their POD and PAL activities. Meanwhile, compared to control, the tebuconazole 2500-fold liquid could also significantly ($p < 0.05$) improve PPO and PAL activities of fruits. The findings further demonstrate that oligosaccharins used together with tebuconazole notably enhanced the SOD, POD, PPO and PAL activities of kiwifruit, thereby improving the resistance of kiwifruit against soft rot disease.

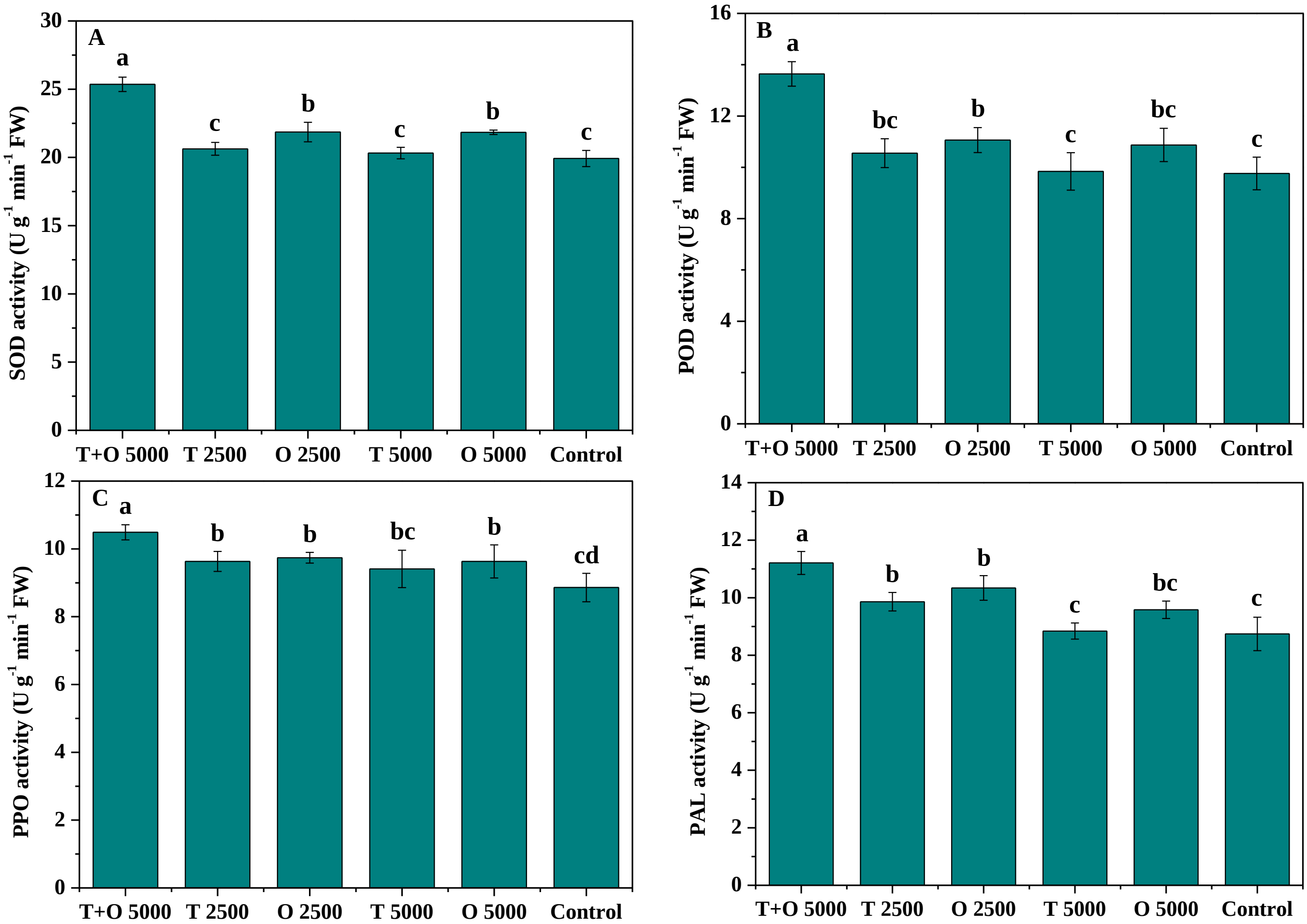

Figure 2. The effects of tebuconazole and oligosaccharins on SOD (**A**), POD (**B**), PPO (**C**) and PAL (**D**) activities in kiwifruit. Unit of all enzyme activities is U g^{-1} min^{-1} FW. The mean ± SD (n = 3) of three replicate is shown. The significant differences at 5% ($p < 0.05$) are represented as different small letters.

3.4. Effects of Tebuconazole and Oligosaccharins on Kiwifruit Growth

The effects of tebuconazole + oligosaccharins, tebuconazole and oligosaccharins on kiwifruit growth are shown in Table 5. Tebuconazole + oligosaccharins significantly ($p < 0.05$) enhanced the longitudinal diameter, transverse diameter, lateral diameter, volume and weight of kiwifruit. The fruit shape indexes of kiwifruit were not significantly ($p < 0.05$) different among the six treatments. Compared to control, the tebuconazole 2500-fold liquid, oligosaccharins 2500-fold liquid, and tebuconazole 5000-fold liquid could significant ($p < 0.05$) increase kiwifruit's volume, and the tebuconazole 2500-fold liquid could significant ($p < 0.05$) increase kiwifruit's weight. The volume and weight of kiwifruit treated by tebuconazole + oligosaccharins were 68.63 cm^3 and 97.22 g, which were significantly ($p < 0.05$) increased by 5.53%, 12.12%, 15.31%, 20.96% or 22.08%, and 4.68%, 7.77%, 8.00%, 9.71% or 10.17% compared to the tebuconazole 2500-fold liquid, oligosaccharins 2500-fold liquid, tebuconazole 5000-fold liquid, oligosaccharins 5000-fold liquid or control treatments, respectively. The results show that co-application of oligosaccharins and tebuconazole could effectively promote the fruit growth and yield of kiwifruit.

Table 5. The effects of tebuconazole and oligosaccharins on kiwifruit's development.

Treatments	Diameter (mm)			Fruit Shape Index	Fruit Volume (cm^3)	Fruit Weight (g)
	Longitudinal	Transverse	Lateral			
T + O 5000	79.81 ± 1.10 ^{a}	49.19 ± 1.22 ^{a}	41.60 ± 0.76 ^{a}	1.76 ± 0.03 ^{b}	68.36 ± 1.53 ^{a}	97.22 ± 2.19 ^{a}
T 2500	78.14 ± 1.72 ^{b}	49.08 ± 1.75 ^{a}	40.21 ± 1.20 ^{ab}	1.75 ± 0.07 ^{b}	64.58 ± 3.82 ^{b}	92.67 ± 1.42 ^{b}
O 2500	78.70 ± 2.42 ^{ab}	47.14 ± 1.37 ^{ab}	38.70 ± 0.41 ^{bc}	1.83 ± 0.09 ^{ab}	60.07 ± 0.82 ^{c}	89.66 ± 2.02 ^{bc}
T 5000	77.72 ± 1.62 ^{b}	46.53 ± 1.36 ^{ab}	38.25 ± 1.19 ^{cd}	1.83 ± 0.08 ^{ab}	57.90 ± 2.23 ^{c}	89.44 ± 1.23 ^{bc}
O 5000	73.88 ± 1.90 ^{c}	46.84 ± 1.66 ^{ab}	37.31 ± 0.64 ^{cd}	1.76 ± 0.05 ^{b}	54.03 ± 1.74 ^{d}	87.78 ± 1.72 ^{c}
Control	73.78 ± 1.62 ^{c}	46.25 ± 0.59 ^{b}	37.30 ± 1.26 ^{d}	1.77 ± 0.08 ^{b}	53.27 ± 1.29 ^{d}	87.33 ± 1.28 ^{c}

The mean ± SD (n = 3) of three replicate is shown. The significant differences at 5% ($p < 0.05$) are represented as different small letters.

3.5. Effects of Tebuconazole and Oligosaccharins on Kiwifruit's Quality

Table 6 displays the effects of tebuconazole + oligosaccharins, tebuconazole and oligosaccharins on kiwifruit's quality. Compared to control, the vitamin C, soluble sugar, soluble solid, dry matter and sugar/acid ratio of kiwifruit treated by tebuconazole + oligosaccharins significantly ($p < 0.05$) improved. Compared to tebuconazole, the vitamin C, soluble sugar, soluble solid and sugar/acid ratio of kiwifruit treated by tebuconazole + oligosaccharins was significantly ($p < 0.05$) increased. Meanwhile, compared to oligosaccharins, tebuconazole + oligosaccharins could significantly ($p < 0.05$) enhance the vitamin C, soluble sugar and soluble solid of kiwifruit. However, the improvement of tebuconazole or oligosaccharins alone on kiwifruit's quality was not obvious, and all quality parameters were not significantly ($p < 0.05$) different in tebuconazole alone, oligosaccharins alone and control treatments. These findings show that co-application of oligosaccharins and tebuconazole notably improved kiwifruit's quality, which should have a notably synergistic effect.

Table 6. The effects of tebuconazole and oligosaccharins on kiwifruit's quality.

Treatments	Vitamin C (g kg^{-1})	Soluble Solid (%)	Dry Matter (%)	Total Soluble Sugar (%)	Titratable Acidity (%)	Sugar/Acid Ratio
T + O 5000	2.11 ± 0.14 ^{a}	19.75 ± 0.17 ^{a}	12.91 ± 0.06 ^{a}	15.80 ± 0.18 ^{a}	1.07 ± 0.02 ^{ab}	12.06 ± 0.05 ^{a}
T 2500	1.91 ± 0.10 ^{b}	19.22 ± 0.23 ^{b}	12.53 ± 0.17 ^{ab}	15.07 ± 0.35 ^{b}	1.11 ± 0.04 ^{a}	11.19 ± 0.06 ^{b}
O 2500	1.89 ± 0.13 ^{b}	19.18 ± 0.18 ^{bc}	12.59 ± 0.24 ^{ab}	15.10 ± 0.14 ^{b}	1.09 ± 0.01 ^{a}	11.57 ± 0.11 ^{ab}
T 5000	1.87 ± 0.12 ^{b}	18.98 ± 0.18 ^{bc}	12.39 ± 0.15 ^{abc}	14.63 ± 0.13 ^{bc}	1.12 ± 0.05 ^{a}	11.07 ± 0.07 ^{b}
O 5000	1.86 ± 0.12 ^{b}	19.04 ± 0.39 ^{bc}	12.59 ± 0.28 ^{ab}	14.77 ± 0.16 ^{bc}	1.10 ± 0.03 ^{a}	11.44 ± 0.08 ^{ab}
Control	1.83 ± 0.05 ^{b}	18.82 ± 0.59 ^{bc}	12.06 ± 0.35 ^{b}	14.60 ± 0.29 ^{bc}	1.16 ± 0.03 ^{a}	10.41 ± 0.16 ^{bc}

The mean ± SD (n = 3) of three replicates is shown. The significant differences at 5% ($p < 0.05$) are represented as different small letters.

4. Discussion

Tebuconazole is one of the chiral triazole fungicides, and it has a broad-spectrum systemic activity on many plants' pathogenic fungi; it also has the three functions of protection, treatment and eradication [18–20]. Ma et al. [22] reported that the EC_{50} value of tebuconazole against 105 *B. dothidea* isolates from a pistachio orchard was 0.019~0.159 µg mL^{-1}. The results here showed that tebuconazole displayed a toxicity against *B. dothidea* RF-1 soft rot pathogen with 0.87 mg kg^{-1} of EC_{50} value, which was higher by 1.23, 1.37, 1.39, 3.03, 5.29, 6.39, 10.79, 22.11, 25.05 and 255.56 folds compared to flutriafol, epoxiconazole, trifloxystrobin tebuconazole, difenoconazole, fluazinam, oxine-copper, eugenol, azoxystrobin, pyrimethanil and oligosaccharins, respectively. This result is consistent with previous research. However, oligosaccharins had a relatively inferior toxicity on *B. dothidea* RF-1 with an EC_{50} value of 222.34 mg kg^{-1}.

Oligosaccharins can be used as a biostimulant for triggering the plant's defense responses and enhancing the plant's antipathogenic compound synthesis [23,24,26–28]. Boyzo-Marín et al. [27] found that glutathione-oligosaccharins application had the lowest incidence (0.66~13%) of downy mildew in blackberry compared to that of other fungicides.

In this study, tebuconazole + oligosaccharins, tebuconazole and oligosaccharins could significantly ($p < 0.01$) decrease fruit incidence rate, the field control effect of tebuconazole + oligosaccharins 5000-fold liquid on soft rot was 84.83%, which significantly ($p < 0.01$) exceeded the 72.05% of the tebuconazole 2500-fold liquid, 52.59% of the oligosaccharins 2500-fold liquid, 62.17% of the tebuconazole 5000-fold liquid, and 33.52% of the oligosaccharins 5000-fold liquid. Oligosaccharins exhibited an inducing control effect on soft rot disease, which, mixed together with tebuconazole, could more effectively control soft rot disease compared to tebuconazole or oligosaccharins alone, as well as effectively reduce tebuconazole application. The excellent controlling effect of tebuconazole + oligosaccharins on soft rot was possibly originated from the therapeutic, preventive and eradication characteristics of tebuconazole, and the inducing effect of oligosaccharins.

Phenolics and flavonoids themselves can not only be used as antagonists of pathogens, but also as precursors of lignin biosynthesis, resulting in host cell lignification and further producing disease resistance [31]. Soluble protein is the basis of material and energy metabolism, while MDA is an index to reflect membrane lipid peroxidation [11,31]. SOD is a key protective enzyme for scavenging free radicals in plants, and POD plays a role in catalyzing H_2O_2 decomposition in the final step of lignin biosynthesis [31,32]. PPO can catalyze the formation of lignin and other phenolic oxidation products to form a protective barrier against the invasion of pathogens, and can also play a direct role in disease resistance by forming quinones [31]. PAL is one of the enzymes for controlling the biosynthesis of phenolics and flavonoids [11,31]. In fact, oligosaccharins originates from the hydrolysis of chitosan [32]; while chitosan can induce protein increase, MDA reduces and triggers the defense enzyme activity [33–37]. In our previous studies, we reported that chitosan, tetramycin + chitosan and chitosan + ferulic acid could effectively improve the phenolics, flavonoids and soluble protein of kiwifruit, inhibit its MDA and enhance its SOD, POD, PPO and PAL activities [9–11,16]. The present results show that the co-application of oligosaccharins and tebuconazole notably enhanced the phenolics, flavonoids and soluble protein content of kiwifruit, reduced their MDA content and reliably promoted their SOD, POD, PPO and PAL activities. These results also emphasize that oligosaccharins had a notable potential for enhancing kiwifruit's disease resistance, and when used together with tebuconazole notably enhanced the improving effects of oligosaccharins or tebuconazole on the disease-resistant substances and resistant enzyme activities of kiwifruit, thereby providing more benefit for enhancing the disease resistance of fruits.

A good growth determines the fruit yield, quality and commodity of kiwifruit. Costales et al. [29] demonstrated that foliar application of oligosaccharins effectively promoted the biomass production and yield formation of tomatoes. Hernández et al. [24] indicated that oligosaccharins stimulated the photosynthesis of tomatoes and improved their fruit production. In grapes, oligosaccharides resulted in an increase of its color intensity and anthocyanin [38]. He et al. [39] demonstrated that chitosan oligosaccharides application improved the quality of strawberry fruits. Although not specifically oligosaccharins, we also found that its parent chitosan could effectively improve kiwifruit's yield and quality [33–37]. In this study, tebuconazole + oligosaccharins notably ($p < 0.05$) enhanced the growth and quality of kiwifruit. Meanwhile, this improving effects of tebuconazole + oligosaccharins were more obvious than those of oligosaccharins or tebuconazole alone. This probably derived from their labor division: tebuconazole protected kiwifruit from a pathogen infection, oligosaccharins induced its disease resistance and enhanced its growth, yield and quality.

Recently, a growing attention has been focused on reducing the application of chemical fungicides and natural products as the adjuvants or alternative approaches of chemical fungicides for plant disease management [12,40]. In China, the recommended concentration of tebuconazole on banana, apple, pear and other fruit trees is a 1250~7500-fold dilution liquid. To date, however, there are no registration and application of tebuconazole for controlling kiwifruit diseases in China. In the present study, oligosaccharins used together with tebuconazole more effectively controlled soft rot in kiwifruit and promoted its resistance, growth and quality compared with tebuconazole or oligosaccharins alone. Meanwhile, the tebuconazole + oligosaccharins 5000-fold dilution liquid was equivalent to tebuconazole 10,000 + oligosaccharins 10,000-fold dilution liquid, hence oligosaccharins used together with tebuconazole effectively decreased tebuconazole application compared with the tested concentration (2500- or 5000-fold) or the recommended concentration (1250~7500-fold) of tebuconazole. The field concentration of the tebuconazole 10,000-fold dilution liquid was relatively low. Moreover, oligosaccharins is a nontoxic natural product widely used in food, medicine, agriculture, cosmetics fields and so on [23,24]. Furthermore, the total time of safe interval and soft ripening periods of kiwifruit was more than 80 days. Accordingly, the potential safety risks of tebuconazole + oligosaccharins are very small and almost nonexistent. This study highlights that a 80% tebuconazole WG + 5% oligosaccharins AS 5000-fold dilution liquid can be proposed as an effective candidate practice for managing kiwifruit soft rot and reducing the application of chemical fungicides.

5. Conclusions

In conclusion, tebuconazole exhibited a good toxicity on *B. dothidea*, and oligosaccharins was an effective adjuvant of tebuconazole in managing soft rot disease of kiwifruit. Oligosaccharins used together with tebuconazole notably increased the resistance substances of kiwifruit, effectively reduced its MDA, and reliably promoted its resistance enzyme activity. Additionally, the co-application of tebuconazole and oligosaccharins effectively promoted kiwifruit's growth and quality. This work highlights that oligosaccharins used together with tebuconazole can be proposed as an effective candidate practice for managing kiwifruit soft rot.

Author Contributions: Y.L. (Youhua Long) conceived the project; Y.L. (Yang Lei), Y.S. and Q.W. designed the experiments; Q.W. and Q.A. performed the experiments; Q.W. and Y.S. analyzed the data; Q.W. wrote the paper; Y.L. (Youhua Long) revised the paper. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the China Agriculture Research System of MOF and MARA, the Science and Technology Innovation Talent Project of Guizhou Province (no. (2016)5672), the Support Plan Projects of Science and Technology Department of Guizhou Province (nos. (2021) YB237, (2020)1Y016, (2019)2703), the Support Plan Projects of Guiyang City (no. (2017)26-1).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The datasets analyzed in the current study are available from the corresponding author on reasonable request.

Conflicts of Interest: We declare that we do not have any commercial or associative interest that represents a conflict of interest in connection with the work submitted.

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