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A Chitosan Composite Film Sprayed before Pathogen Infection Effectively Controls Postharvest Soft Rot in Kiwifruit

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Abstract: Soft rot caused by *Botryosphaeria dothidea* and *Phomopsis* sp. is a critical disease in kiwifruit. In order to efficiently control soft rot, a 28.6% chitosan composite film (CCF) containing chitosan, dextrin, ferulic acid, calcium, and auxiliaries was successfully developed. The results showed that CCF had a strong inhibitory effect on mycelia growth of *B. dothidea* and *Phomopsis* sp., with mycelial EC_{50} values of 68.11 and 50.34 mg L^{-1}, respectively. The concentration of 0.71–1.42 g L^{-1} CCF had noticeably preventive and curative effects against soft rot. The spray of CCF before pathogen infection effectively reduced the incidence of soft rot, remarkably increased the content of resistance compounds, and activated the activity of defense enzymes. Moreover, it notably enhanced the yield and quality and prolonged the shelf life of kiwifruit. Therefore, the excellent control effects of CCF against soft rot might be associated with its film-forming property and antifungal activity, which prevent infection and induce plant defense mechanisms. The concentration of 0.71–1.42 g L^{-1} CCF was optimal for the field application before the onset of disease symptoms in plants with *B. dothidea* and *Phomopsis* sp.

Keywords: chitosan composite film; kiwifruit; pathogen infection; soft rot; disease resistance; storage quality

1. Introduction

Kiwifruit (*Actinidia deliciosa*) is very popular among consumers due to its high nutritional value. The harvested area and yield of kiwifruit in the world has continuously increased in the 21st century [1,2]. However, postharvest diseases caused by pathogens are a serious problem for the storage of kiwifruit and have resulted in decay and a decrease in quality, shelf life, and consumer acceptability [3,4]. Soft rot is a key disease of postharvest kiwifruit, which is mainly caused by *Botryosphaeria dothidea*, *Phomopsis* sp., *Cryptosporiopsis actinidiae*, *Botrytis cinerea*, *Cylindrocarpon* sp., and *Phoma exigua* [5–9]. According to our previous results, *B. dothidea* and *Phomopsis* sp. are the key fungal pathogens causing decay in the kiwifruit cultivar Guichang [10]. They can enter kiwifruit tissues at early growth stages and remain latent there until the fruit ripens; then, the fungal pathogens begin to recover their infectious capacity, eventually causing fruit rot symptoms during storage [9,11]. Therefore, the safe and effective control of soft rot in kiwifruit is a challenge.

The kiwifruit cultivar Guichang has the highest yield, good quality and storage performance, and planting areas in Southwest China of 30,000 hm^2 [10]. According to our previous results, soft rot occurs in Guichang kiwifruit and has resulted in yield losses of 30–50% [10]. Generally, the control of postharvest decay of kiwifruit mainly depends on chemical fungicides [7,12]. Although synthetic fungicides can effectively control fruit diseases, the potentially harmful impacts of their residues on human health and the environment have resulted in increasing concerns. Recently, alternative strategies of chemical control have been developed. For example, hot water, quercetin, oxalic acid, and harpin have been used to stimulate defense responses in kiwifruit [2,10,13,14]. However, the induction of disease resistance in kiwifruit during the preharvest period has received little attention.

Chitosan (CTS) is a natural compound with antibacterial, film-forming, nontoxic, antioxidant, renewable, and biocompatible properties [15–17]. Previous studies showed that it has good antifungal activity against some phytopathogenic fungi which cause postharvest diseases [18–21]. Moreover, it can trigger plant defense responses by inducing a variety of defense-related reactions [9], and it has also been used as nutrient to enhance the growth and yield of maize and rice [18,22]. However, chitosan alone has shown low inhibitory activity against *B. dothidea* and *Phomopsis* sp. (for detailed results, see Supplementary Table S1–S3). Dextrin is an edible film-forming material that is widely used in the storage and preservation of various fruits [23]. Ferulic acid is an abundant cinnamic acid derivative found in plants with good antibacterial performance [24]. Calcium plays a decisive role in the development, quality, and storability of kiwifruit [25]. To date, no studies are available on the use of a composite film of chitosan, dextrin, ferulic acid, and calcium against postharvest soft rot of kiwifruit.

The aims of this study were (i) to evaluate the effects of a chitosan composite film (CCF) against soft rot sprayed before pathogen infection, and (ii) to study the mechanisms involved in the resistance to kiwifruit soft rot. The findings provide an economically efficient and environmentally friendly approach for the control of kiwifruit soft rot.

2. Materials and Methods

2.1. Materials

Botryosphaeria dothidea and *Phomopsis* sp. isolates were selected from the culture collection obtained from the kiwifruit orchard at Xiuwen county, Guizhou province, China. The following compounds were used in the experiments: CTS (deacetylation ≥ 90.00%), Huarun Bioengineering Co. Ltd., Zhenzhou, China; dextrin (≥99.00%) and sodium benzoate (≥99.00%), Kermel Chemical Reagent Co. Ltd., Tianjin, China; ferulic acid (≥ 99.00%, FA), Aladdin Industrial Co. Ltd., Shanghai, China; calcium nitrate (≥99.50%, Ca-N) and glycerol (≥99.50%), Jinshan Chemical Reagent Co. Ltd., Chongqin, China; organosilicon (100%), Zhengan Agricultural Sci & Tech Co. Ltd., Shijiazhuang, China; 80% thiophanate-methyl wettable powder (Thiopsin-M WP), Meibang Pesticide Co. Ltd., Xian, China; and potato dextrose agar (PDA), Xiya Reagent Co. Ltd., Chengdu, China. The CCF contained CTS (8.00%), dextrin (10.00%), FA (1.00%), Ca-N (5.00%), sodium benzoate (0.60%), glycerol (3.00%), organosilicon (1.00%), and sterile water (72.40%) (for detailed results, see Supplementary Table S1–S13). The following steps were performed: step 1, dextrin was stirred and dissolved in sterile water under an 85 °C water bath for 20 min; step 2, other components were mixed with the dextrin solution and stirred using an electric stirrer (JJ-1, Fuhua Instrument Co. Ltd., Jintan, China) for 12 h; finally, the CCF solution was subjected to ultrasonication (scientz-08, Xinzhi Bio & Tech Co. Ltd., Ningbo, China) for 30 min.

2.2. In Vitro Toxicity Tests and Artificial Inoculation of Fruit

To evaluate the fungi toxicity of CCF to the selected fungal isolates, 9 mL of potato dextrose agar (PDA) was emptied into glass petri dishes at a temperature of 40–45 °C and 1 mL of the tested solution of CCF was evenly coated on the PDA plate after it solidified. Then, a 5 mm in diameter cake originating from a 7-day-old colony of *B. dothidea* and *Phomopsis* sp. was inoculated in the center of the plate with the inoculum side down, respectively. The diameters of the fungal growth in the treated and

control plates were independently measured after being incubated at 28 °C for 2, 3, 4, 5, 6, and 7 days. The growth inhibition of fungi was calculated according to Equation (1):

$$\text{Inhibition rate (\%)} = [(D_c - D_t)/(D_c - 5)] \times 100 \quad (1)$$

where D_t and D_c represent the mycelial growth diameter in the treated and control conditions, respectively. The inhibition rates of *B. dothidea* and *Phomopsis* sp. at different concentrations of 0.34, 0.17, 0.085, 0.0425, and 0.02125 g L^{-1} CCF were independently tested.

To ensure the sterility of the test fruit, bagged fruits were used for the artificial inoculation. The fruit bagging began after petal fall (14 May). The bagged fruit was sterilized in 1% sodium hypochlorite for 30 min and then ultraviolet sterilized and dried on an ultraclean worktable. The preventive effect evaluation (inoculation after CCF application) was as follows: The film solutions with different concentrations were sprayed on the fruit surface. Then, the same size of pathogenic fungus block (5 mm diameter) was inoculated on the equator of each fruit surface at 1, 3, and 7 days. The curative effect evaluation (CCF application after inoculation) was as follows: The pathogenic fungus block was inoculated on the equator of each fruit surface, and then the film solutions were sprayed at 1, 3, and 7 days. Control fruits were sprayed with sterilized water. After spray treatment, all fruits were stored at 28 °C and 80–90% relative humidity for 21 days. The lesion diameter was measured and the preventive and curative effects were evaluated.

2.3. Field Experiments

2.3.1. Orchard Site

Experiments were carried out in 2017 and 2018 in an orchard of the kiwifruit cultivar Guichang, which was planted in 2002 in Xiuwen county, Guizhou province, China (26°49′02″ N, 106°28′23″ E). A concrete "T"-type frame held up the kiwifruit plants with a spacing of 3.00 × 3.00 m. Female kiwifruit plants accounted for 1/9 of the total. The soil (0–60 cm deep) had total organic matter of 29.56 g kg^{-1}, alkali-hydrolyzable nitrogen of 98.47 mg kg^{-1}, available phosphorus of 4.40 mg kg^{-1}, available iron of 48.31 mg kg^{-1}, total zinc of 50.66 mg kg^{-1}, available manganese of 19.17 mg kg^{-1}, exchangeable calcium of 17.84 cmol kg^{-1}, and a pH value of 5.86.

2.3.2. Infection Period

Bagging protected the fruit from infections, so the infection period was evaluated by the random bagging of fruits in 2015 and 2016. Fruit bagging began at petal fall (14 May), with 100 bagged fruits every 10 days until 12 September. Bagged fruits were collected on 1 October and stored at room temperature (25 ± 1 °C). The incidence of soft rot was calculated according to Equation (2):

$$\text{Incidence (\%)} = \text{No. of diseased fruits/total No. of fruits} \times 100 \quad (2)$$

2.3.3. Experimental Design

The experimental treatments are shown in Table 1. Twenty-seven plots were arranged in a randomized block design. Each plot contained six kiwifruit trees, and only the interior four trees were used for evaluation. The spray volume applied to each tree each time was 1.50 L. No rainfall was observed after 3–4 days of CCF spray.

Table 1. Experimental design of the field experiments.

Treatments	Materials	Concentration (g L^{-1})	Spray time
T1	CCF	1.42	20 May and 1 August
T2		0.71	
T3		0.35	
T4		1.42	1 August and 1 September
T5		0.71	
T6		0.35	
T7	Calcium nitrate	0.50	20 May and 1 August
T8	80% Thiopsin-M WP	1.50	
Control	Irrigation water	-	

Abbreviations: CCF—chitosan composite film

2.4. Sampling and Analysis of Fruit

A total of 100 fruits were randomly collected and divided into two groups from each plot on 1 October and stored at 25 ± 1 °C. Fruits of the first group were used for the determination of the development, quality, resistance-related, and fresh-keeping parameters. Fruits of the other group were used to investigate the softening rate of fruit and the incidence of soft rot.

2.4.1. Soft rot Parameters

The incidence, disease severity index, and control effect of soft rot in kiwifruit were determined according to Equations (3) and (4), respectively. The disease severity scale used was as follows: 0, no disease; 1, cumulative lesion diameter less than 1 cm; 2, cumulative lesion diameter of 1–2 cm; 3, cumulative lesion diameter of 2–3 cm; 4, cumulative lesion diameter of 3–4 cm; 5, cumulative lesion diameter of 4–5 cm; 6, cumulative lesion diameter greater than 5 cm.

$$\text{Disease severity index} = \sum(\text{Disease severity value} \times \text{No. of the fruit within each disease severity value})/(\text{Total No. of fruit} \times \text{the highest disease severity value}) \times 100 \quad (3)$$

$$\text{Control effect (\%)} = (\text{Disease severity value of control} - \text{Disease severity value of treatment})/\text{Disease severity value of control} \times 100 \quad (4)$$

2.4.2. Content and Enzyme Activity of Defense-Related Compounds

Total phenolic and flavonoid contents were determined according to the protocols proposed by Pirie and Mullins [26] but with some modifications. Briefly, a flesh sample of 2.00 g was ground in 20 mL of 1% (v/v) HCl-methyl alcohol at 3 °C and centrifuged (12,000× *g*, 3 °C, 10 min) when extracted for 1 h without light, and the levels of total phenolics and flavonoids in the supernatant were determined at 280 and 325 nm, respectively; HCl-methyl alcohol was the standard. Superoxide dismutase (SOD) activity assay: fruit flesh (2.00 g) was homogenized on ice with 4 mL of 100 mmol L^{-1} sodium phosphate buffer (pH 7.5) containing 5 mmol L^{-1} dithiothreitol and 20 g L^{-1} polyvinylpyrrolidone. The homogenate was centrifuged at 12,000× *g* for 30 min at 3 °C, and the supernatant was used for the enzyme assay. SOD activity was assayed according to Zhu et al. [27] by measuring the ability of the enzyme to inhibit the photochemical reduction of nitro-blue tetrazolium (NBT). Phenylalanine ammonia-lyase (PAL) activity was measured according to Zhou et al. [28]. One unit of PAL activity was defined as the amount of enzyme that caused an increase in absorbance of 0.01 at 290 nm per minute. Polyphenoloxidase (PPO) and peroxidase (POD) activities were analyzed according to Shi et al. [29]. A fresh fruit peel was mixed with 5 mL of ice-cold sodium phosphate buffer (100 mM, pH 7.8). One unit of POD and PPO activities was defined as the amount of enzyme which caused a change of 1 in absorbance per min at 470 and 420 nm, respectively. The results are expressed as U g^{-1} min^{-1} fresh weight (FW).

2.4.3. Development, Quality, and Fresh-Keeping Parameters

Longitudinal and transverse fruit diameter was determined by a digital caliper (Bunker tools Co. Ltd., Shandong, China), and shape index and fruit volume was calculated according to the longitudinal and transverse diameter. Fruit weight was determined using an electronic analytical balance (Precision Scientific Instrument Co. Ltd., Shanghai, China). The soluble solids content was analyzed using a digital refractometer (PAL-1, Yishida Tech Co. Ltd., Beijing, China). Total soluble sugar, dry matter, titratable acidity, and soluble protein were analyzed by the anthrone colorimetric, drying, acid-base titration, and Coomassie brilliant blue methods, respectively. Vitamin C was analyzed using an HPLC system (1260, Agilent, Santa Clara, Calif, USA). Firmness was determined using a sclerometer (GY-4, Aidebao Instrument Co. Ltd., Leqing, China). Softening rate was measured at 5, 10, 15, 20, and 25 days, expressed as a percentage using Equation (5):

$$\text{Softening rate (\%)} = \text{No. of softened fruits/total No. of fruits} \times 100 \quad (5)$$

2.5. Data Analyses

The effective concentration for 50% inhibition value (EC_{50}) of CFF was calculated using a regression equation of $y = a + bx$. All results are expressed as means of three replicates and their standard deviations. A one-way analysis of variance followed by Tukey's HSD was performed. The data were analyzed using SPSS 18.0 (SPSS Inc., Chicago, IL, USA).

3. Results

3.1. In Vitro Inhibitory Effects of CCF

The inhibitory effect of CCF on the mycelial growth of *B. dothidea* and *Phomopsis* sp. is shown in Table 2. CCF had good antifungal activity. The EC_{50} values of CCF for the mycelium growth of *B. dothidea* and *Phomopsis* sp. were 68.11 and 50.34 mg L^{-1}, respectively.

Table 2. The inhibitory effects of CCF on the mycelial growth of *Botryosphaeria dothidea* and *Phomopsis* sp.

Fungal Isolates	Regression Equation	Coefficient of Determination (R^2)	EC_{50} (mg L^{-1})
B. dothidea	$y = 6.0206 + 0.8746x$	0.9980	68.11
Phomopsis sp.	$y = 5.8783 + 0.6764x$	0.9757	50.34

3.2. Preventive Effects of CCF against Soft Rot in Artificially Inoculated Fruit

The preventive effects of CCF against *B. dothidea* and *Phomopsis* sp. are displayed in Table 3. The lesion diameter caused by *B. dothidea* and *Phomopsis* sp. on the fruits sprayed with different concentrations of CCF was smaller than that on the control fruits, meaning that there was a dose-dependent inhibition effect. For inoculation 1 day after spraying, the lesion diameter of 0.35–1.42 g L^{-1} CCF against *B. dothidea* and *Phomopsis* sp. decreased 38.05–75.06% and 52.78–71.30%, respectively. The preventive effect of CCF became more obvious with the delay of pathogen inoculation.

Table 3. The preventive effects of CCF in fruits inoculated with *B. dothidea* or *Phomopsis* sp.

Fungal Isolates	Concentration (g L^{-1})	Lesion Diameter (cm)		
		Inoculation 1 Day After Spray	Inoculation 3 Days After Spray	Inoculation 7 Days After Spray
B. dothidea	1.42	0.97 ± 0.12 [d]	0.82 ± 0.09 [d]	0.79 ± 0.05 [d]
	0.71	1.58 ± 0.09 [c]	1.37 ± 0.07 [c]	1.17 ± 0.08 [c]
	0.35	2.41 ± 0.07 [b]	2.09 ± 0.09 [b]	1.73 ± 0.07 [b]
	Control	3.89 ± 0.13 [a]	3.89 ± 0.13 [a]	3.89 ± 0.13 [a]
Phomopsis sp.	1.42	0.62 ± 0.04 [c]	0.54 ± 0.06 [b,c]	0.37 ± 0.04 [c]
	0.71	0.87 ± 0.05 [c]	0.63 ± 0.05 [b]	0.52 ± 0.05 [b]
	0.35	1.02 ± 0.04 [b]	0.75 ± 0.05 [b]	0.63 ± 0.05 [b]
	Control	2.16 ± 0.10 [a]	2.16 ± 0.10 [a]	2.16 ± 0.10 [a]

[a] Values indicate the mean ± SD, $n = 3$. Upper case letters indicate significant differences at 5% level ($p < 0.05$).

3.3. Curative Effects of CCF against Soft Rot in Artificially Inoculated Fruit

As shown in Table 4, the lesion diameter development in fruits inoculated with both *B. dothidea* and *Phomopsis* sp. was remarkably inhibited by spraying different concentrations of CCF after inoculation. Regarding spraying CCF 7 days after inoculation, the lesion diameter of 0.35–1.42 g L^{-1} CCF on *B. dothidea* and *Phomopsis* sp. decreased 33.64–65.70% and 50.00–62.61%, respectively. The earlier the inoculation time of pathogens, the more obvious the inhibition effect of CCF.

Table 4. The curative effects of CCF in fruits inoculated with *B. dothidea* or *Phomopsis* sp.

Fungal Isolates	Concentration (g L^{-1})	Lesion diameter (cm)		
		Spray 1 Day After Inoculation	Spray 3 Days After Inoculation	Spray 7 Days After Inoculation
B. dothidea	1.42	0.37 ± 0.10 [d]	0.84 ± 0.11 [d]	1.68 ± 0.09 [d]
	0.71	0.83 ± 0.10 [c]	1.29 ± 0.10 [c]	2.31 ± 0.07 [c]
	0.35	1.68 ± 0.07 [b]	2.10 ± 0.10 [b]	3.25 ± 0.07 [b]
	Control	4.90 ± 0.13 [a]	4.90 ± 0.13 [a]	4.90 ± 0.13 [a]
Phomopsis sp.	1.42	0.00 ± 0.00 [c]	0.41 ± 0.04 [c]	0.83 ± 0.04 [c]
	0.71	0.35 ± 0.06 [b]	0.76 ± 0.04 [b,c]	0.92 ± 0.04 [c]
	0.35	0.45 ± 0.06 [b]	0.94 ± 0.04 [b]	1.11 ± 0.04 [b]
	Control	2.22 ± 0.07 [a]	2.22 ± 0.07 [a]	2.22 ± 0.07 [a]

[a] Values indicate the mean ± SD, $n = 3$. Upper case letters indicate significant differences at 5% level ($p < 0.05$).

3.4. Infection Period

The infection period of pathogenic fungi was confirmed by randomly bagging fruits after petal fall in 2015 and 2016. As shown in Figure 1, the incidence rate of soft rot significantly increased during 24/5–13/6 and there was a slight increase at 02/8–12/8. Therefore, the pathogenic fungi of soft rot had one clear infection period on the cultivar Guichang, which was 10–30 days (24/5–13/6) after petal fall.

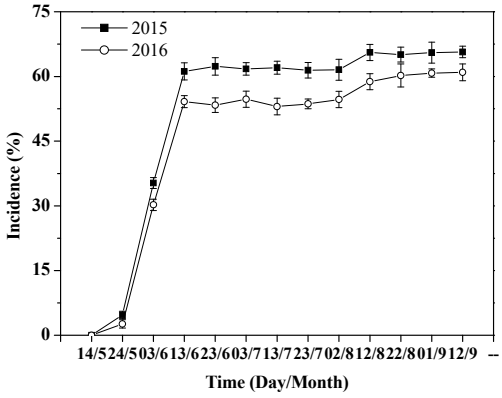

Figure 1. Incidence of soft rot in bagged fruits. Error bars are the standard deviations of the mean ($n = 3$).

3.5. Incidence of Soft Rot in Kiwifruit

As illustrated in Table 5, treatments sprayed with different concentrations of CCF before pathogen infection significantly ($p < 0.05$) reduced the incidence and disease severity index of soft rot in kiwifruit. The control effects of treatments sprayed with 0.35–1.42 g L^{-1} CCF on 20 May and 1 August as well as on 1 August and 1 September were 60.75–82.13% and 38.35–64.85% after 30 days of storage, respectively. In the same groups, the control effects of three concentrations reached a significant difference ($p < 0.05$) and showed a dose-dependent control effect. This indicates that the critical period of soft rot control was 20 May and 1 August. Moreover, the samples treated by 0.71–1.42 g L^{-1} CCF on 20 May and 1 August displayed higher control effects against soft rot than calcium nitrate (T7) and fungicide (Thiopsin-M, T8).

Table 5. The effects of CCF application before the pathogen infection on the control of soft rot in kiwifruit.

Treatments	Incidence (%)	Disease Severity Value	Control Effect (%)
T1	17.78 ± 3.85 ^{f}	6.67 ± 1.11 ^{e}	82.13 ± 2.86 ^{a}
T2	26.67 ± 3.85 ^{e}	10.37 ± 0.64 ^{d,e}	71.94 ± 1.01 ^{b}
T3	35.56 ± 3.85 ^{c,d}	14.44 ± 1.11 ^{cd}	60.75 ± 2.91 ^{c}
T4	33.33 ± 0.00 ^{d,e}	12.96 ± 0.65 ^{d}	64.85 ± 0.68 ^{c}
T5	42.22 ± 3.85 ^{b,c}	18.15 ± 1.28 ^{b,c}	50.70 ± 1.52 ^{d}
T6	46.67 ± 6.67 ^{b}	22.59 ± 1.70 ^{b}	38.35 ± 5.90 ^{e}
T7	48.89 ± 3.85 ^{b}	21.11 ± 2.94 ^{b}	42.73 ± 5.89 ^{e}
T8	31.11 ± 3.85 ^{de}	12.59 ± 0.64 ^{d}	65.72 ± 3.37 ^{b,c}
Control	71.11 ± 3.85 ^{a}	36.67 ± 2.04 ^{a}	

^{a} Values indicate the mean ± SD, $n = 3$. Lower case letters indicate significant differences at 5% level ($p < 0.05$).

3.6. Defense-Related Compounds and Enzyme Activity

As shown in Figure 2a, the content of total phenolics in all treatments sprayed with 0.35–1.42 g L^{-1} CCF before pathogen infection gradually increased during storage. Total flavonoids increased at first and then decreased during storage (Figure 2b). The spray of CCF on 20 May and 1 August increased total flavonoids in fruits during storage. The activity of SOD in fruits during storage was enhanced by CCF and calcium nitrate before pathogen infection (Figure 2c). The fruit SOD activity of 0.71–1.42 g L^{-1} CCF treatments on 20 May and 1 August was significantly higher than that of other treatments. As shown in Figure 2d, the fruit PAL activity of the treatments sprayed with CCF before pathogen infection and calcium nitrate increased gradually and peaked in 21 days, while other treatments peaked in 14 days. The spray of CCF and calcium nitrate before pathogen infection increased PAL activity in fruits,

as compared with fungicide and control during storage. The spray of both CCF and calcium nitrate before pathogen infection effectively increased the activity of PPO and POD in fruits. The spray of 0.35–1.42 g L^{-1} CCF on 20 May and 1 August significantly ($p < 0.01$) increased PPO and POD activities in fruits during storage.

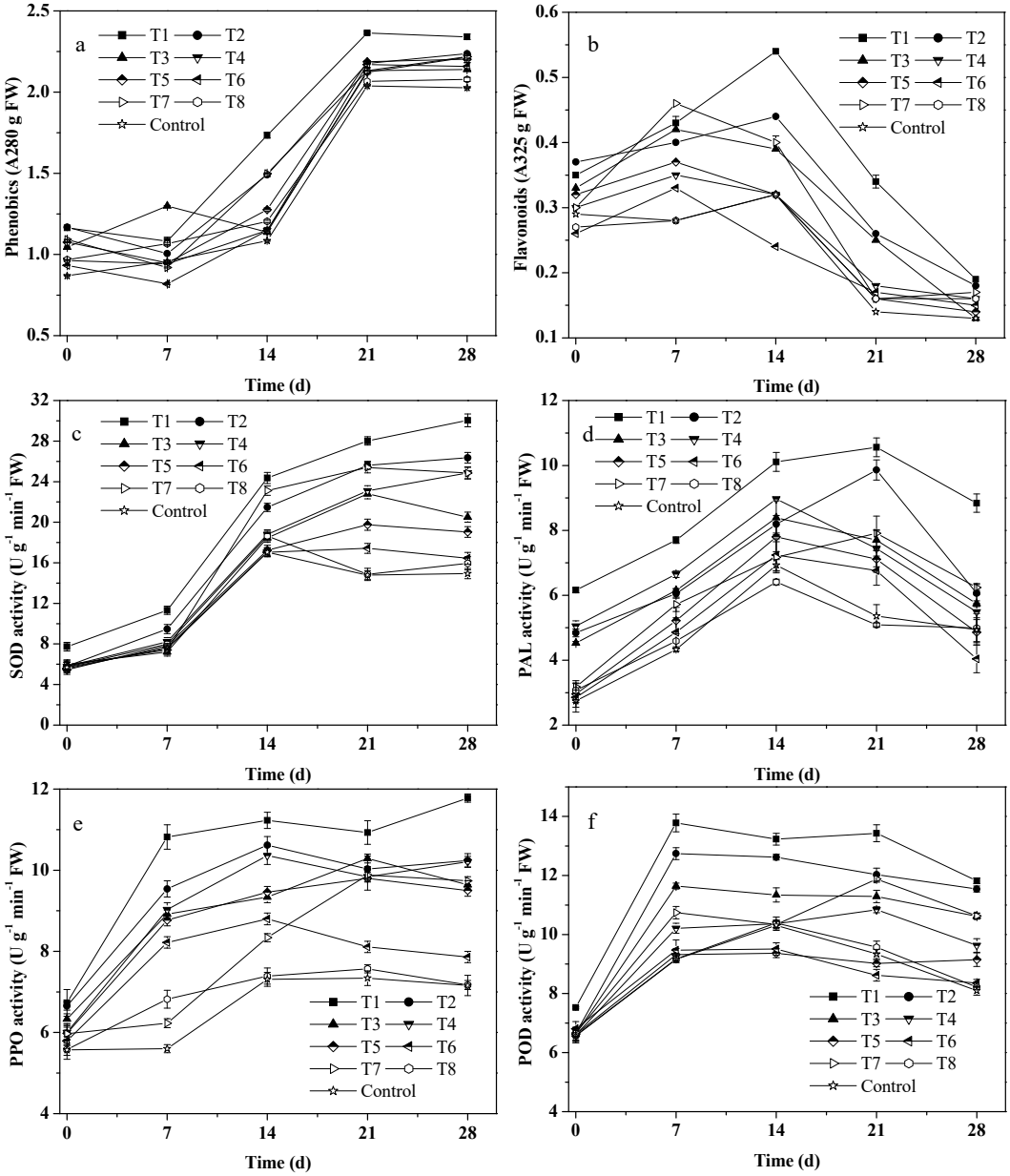

Figure 2. The effects of CCF application before the pathogen infection on changes in total phenolic (**a**) and total flavonoid (**b**) contents, superoxide dismutase (SOD) (**c**), phenylalanine ammonia-lyase (PAL) (**d**), polyphenoloxidase (PPO) (**e**), and peroxidase (POD) (**f**) activity of kiwifruit during storage. Values indicate the means of the replicates; error bars indicate the standard deviations of the mean ($n = 3$).

3.7. Growth and Quality Parameters

As shown in Table 6, the spray of 1.42 g L^{-1} CCF on 20 May and 1 August could significantly ($p < 0.05$) increase the longitudinal diameter compared with the control. The transverse diameter and shape index of the kiwifruit cultivar Guichang showed no significant ($p < 0.05$) differences in all treatments. Single fruit volume was significantly ($p < 0.05$) increased by spraying 0.71–1.42 g L^{-1}

CCF on 20 May and 1 August and 1.42 g L^{-1} CCF on 1 August and 1 September, compared with other treatments. The spray of 0.35–1.42 g L^{-1} CCF on 20 May and 1 August and 0.71–1.42 g L^{-1} CCF on 1 August and 1 September significantly ($p < 0.05$) increased single fruit weight, compared with other treatments.

Table 6. The effects of CCF application on the development of kiwifruit.

Treatments	Diameter (mm)			Shape Index	Fruit Volume (cm^3)	Fruit Weight (g)
	Longitudinal	Transverse 1	Transverse 2			
T1	77.53 ± 2.31 [a]	53.71 ± 4.36 [a]	40.98 ± 0.53 [a]	1.64 ± 0.10 [a,b]	71.42 ± 1.77 [a]	81.07 ± 1.36 [a]
T2	76.42 ± 0.73 [a,b]	50.70 ± 0.65 [a,b]	41.58 ± 0.74 [a]	1.66 ± 0.02 [a]	67.45 ± 1.92 [b]	77.76 ± 1.63 [b]
T3	74.55 ± 0.12 [c]	51.89 ± 0.92 [a,b]	40.84 ± 1.27 [a]	1.61 ± 0.04 [a,b]	66.15 ± 1.94 [c]	75.85 ± 1.58 [d]
T4	75.66 ± 0.32 [b,c]	51.89 ± 0.93 [a,b]	41.06 ± 1.56 [a]	1.63 ± 0.05 [a,b]	67.51 ± 1.48 [b]	77.03 ± 1.41 [c]
T5	72.48 ± 0.44 [d]	51.80 ± 0.52 [a,b]	42.02 ± 0.36 [a]	1.55 ± 0.02 [b]	66.04 ± 1.32 [c]	75.83 ± 1.50 [d]
T6	72.54 ± 0.48 [d]	52.67 ± 0.70 [a,b]	40.84 ± 3.46 [a]	1.55 ± 0.08 [b]	65.34 ± 1.94 [c]	74.67 ± 1.09 [e]
T7	75.36 ± 0.43 [b,c]	50.27 ± 0.68 [a,b]	41.07 ± 0.57 [a]	1.65 ± 0.03 [a,b]	65.14 ± 1.74 [c]	74.63 ± 1.12 [e]
T8	75.14 ± 0.68 [b,c]	49.75 ± 2.77 [b]	41.86 ± 0.50 [a]	1.64 ± 0.06 [a,b]	65.53 ± 1.10 [c]	74.35 ± 1.46 [e]
Control	75.25 ± 1.18 [b,c]	50.38 ± 0.85 [a,b]	41.02 ± 0.61 [a]	1.65 ± 0.01 [a,b]	65.13 ± 1.89 [c]	74.19 ± 1.20 [e]

[a] Values indicate the mean ± SD, $n = 3$. Lower case letters indicate significant differences at 5% level ($p < 0.05$).

The quality parameters in fruits are displayed in Table 7. After spraying 0.35–1.42 g L^{-1} CCF on 20 May and 1 August and 0.71–1.42 g L^{-1} on 1 August and 1 September, vitamin C, soluble solids, dry matter, total soluble sugar, and soluble protein of kiwifruit were significantly ($p < 0.05$) enhanced and titratable acidity significantly ($p < 0.05$) decreased compared with control. Moreover, the aforementioned effects of CCF application on 20 May and 1 August were more significant than those on 1 August and 1 September. The calcium nitrate of 0.50 g L^{-1} also improved the quality of the kiwifruit, but its effects were lower than those of 0.35–1.42 g L^{-1} CCF on 20 May and 1 August.

Table 7. The effects of CCF application on the quality of kiwifruit.

Treatments	Vitamin C (g kg^{-1})	Soluble Solids (%)	Dry Matter (%)	Total Soluble Sugar (%)	Titratable Acidity (%)	Soluble Protein(%)
T1	1.90 ± 0.01 [a]	15.53 ± 0.06 [a]	19.67 ± 0.10 [a]	12.64 ± 0.10 [a]	1.01 ± 0.01 [g]	1.82 ± 0.02 [a]
T2	1.86 ± 0.02 [b]	14.70 ± 0.10 [b]	19.18 ± 0.02 [b]	12.32 ± 0.07 [b]	1.09 ± 0.00 [f]	1.79 ± 0.00 [a,b]
T3	1.81 ± 0.00 [c]	14.23 ± 0.06 [c]	18.12 ± 0.03 [d]	12.14 ± 0.04 [b,c]	1.11 ± 0.01 [e]	1.75 ± 0.00 [b]
T4	1.82 ± 0.01 [c]	14.20 ± 0.10 [c]	18.98 ± 0.01 [c]	12.19 ± 0.01 [b,c]	1.09 ± 0.01 [f]	1.76 ± 0.01 [b]
T5	1.81 ± 0.01 [c]	13.97 ± 0.06 [d]	18.09 ± 0.06 [d]	12.02 ± 0.02 [c,d]	1.14 ± 0.01 [d]	1.73 ± 0.01 [b,c]
T6	1.81 ± 0.00 [c]	13.80 ± 0.00 [e]	17.42 ± 0.12 [e]	11.45 ± 0.42 [e]	1.18 ± 0.01 [c]	1.70 ± 0.01 [c]
T7	1.81 ± 0.00 [c]	13.83 ± 0.06 [e]	17.48 ± 0.10 [e]	11.82 ± 0.01 [d]	1.18 ± 0.00 [c]	1.47 ± 0.02 [d]
T8	1.81 ± 0.01 [c]	13.80 ± 0.00 [e]	16.72 ± 0.14 [f]	11.74 ± 0.12 [d]	1.22 ± 0.01 [b]	1.41 ± 0.02 [e]
Control	1.80 ± 0.00 [c,d]	13.63 ± 0.06 [f]	16.57 ± 0.12 [f]	11.37 ± 0.01 [e]	1.27 ± 0.00 [a]	1.39 ± 0.03 [e]

[a] Values indicate the mean ± SD, $n = 3$. Lower case letters indicate significant differences at 5% level ($p < 0.05$).

3.8. Firmness and Softening Rate

As shown in Figure 3a, the spray of both CCF and calcium nitrate on 20 May and 1 August delayed the decrease of fruit firmness compared with controls. The spray of CCF on 20 May and 1 August showed a better effect, which followed the continuous calcium nitrate. Consistent with firmness (Figure 3b), the spray of CCF and calcium nitrate on 20 May and 1 August delayed the increase of softening, and the softening rates of the fungicide and control treatments drastically increased during storage and reached 93.78% and 100% after 15 days, respectively.

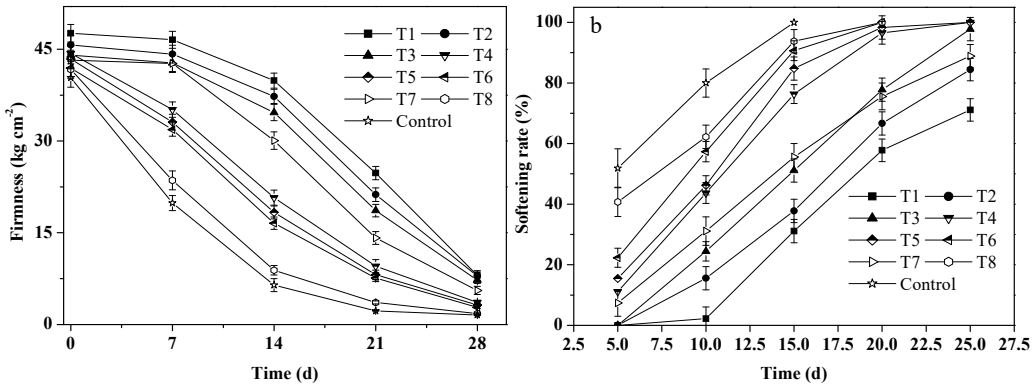

Figure 3. The effects of CCF application before the pathogen infection peak on the firmness (**a**) and softening rate (**b**) of kiwifruit during storage. Values indicate the means of the replicates; error bars indicate the standard deviations of the mean (n = 3).

4. Discussion

Previous studies have shown that chitosan has antifungal activity on various fungal pathogens [17,30–32]. The antifungal activity of CCF mainly originated from the synergy of chitosan and ferulic acid (for detailed results, see Supplementary Table S3,S6–S8). The results presented here show that CCF sprayed on the fruit surface could prevent pathogen infection and hence played a better preventive role. It is possible that the CCF had a preferable film-forming property and antimicrobial activity (for detailed results, see Supplementary Table S3,S6–S8), so that it could form an invisible and fungicidal protective film on the surface of the fruits. The results here also demonstrate that the spray of CCF could preferably control the soft rot of kiwifruit even when the pathogen had infected the fruits.

The pathogenic fungi of soft rot had one clear infection period in the kiwifruit cultivar Guichang, which was 10–30 days after petal fall. A similar result was also observed by Luongo et al. [9]. The results in this study have provided useful information for the control of soft rot in the kiwifruit cultivar Guichang. This study indicates that the spray of different doses of CCF before pathogen infection significantly ($p < 0.01$) reduced the incidence rate and disease severity index of soft rot. In addition, CCF had a good film-forming property and antifungal activity. A natural barrier was probably formed on the fruit and leaf surface of kiwifruit by 0.35–1.42 g L^{-1} CCF application, which prevented pathogen infection. Our results are consistent with previous studies with chitosan or oligochitosan applications on tomato, peach, pear, strawberry, and jujube [19,20,32–34].

Inducing disease resistance is an effective approach to improve the degree to which plants and postharvest fruits use their own defense mechanisms [29]. Kim et al. [35] indicated that phenolic compounds were significantly increased by chitosan treatment. Similar to the previous study, we found that the content of total phenolics and flavonoids in kiwifruit was increased by CCF application before pathogen infection. In the enzymatic system, SOD was the first reactive oxygen species-metabolizing enzyme, by which O_2^- can be metabolized to H_2O_2 and O_2 [36]. The spray of 0.35–1.42 g L^{-1} CCF increased in the SOD activity and enhanced the ability of scavenging reactive oxygen free radicals. Many phenolic compounds in the plant can be synthesized through the metabolism of the phenylpropanoid pathway, and PAL is the key enzyme in this pathway [2]. In this study, the spray of CCF before pathogen infection greatly enhanced PAL activity in kiwifruit, and the content of total phenolics was also significantly increased. These results indicate that the phenylpropanoid pathway is activated by CCF application, resulting in an increase in phenolics. Similarly, previous studies also reported that chitosan increased PAL activity and the content of total phenolics, which provide defense for plant cells to avoid pathogen infection [20,29,32,34,37].

PPO and POD are important defense enzymes associated with disease resistance in plants. They participate in the oxidation of phenolics to toxic quinones and are involved in active oxygen metabolism in plants [32]. The results in this study show that activities of PPO and POD in kiwifruit increased

by CCF application before pathogen infection. This result is supported by previous studies showing that PPO and POD accumulation in apple, peach, and jujube is related to enhancing levels of induced resistance triggered by chitosan [32,34,38]. Of note, the effects of spraying 0.71 and 1.42 g L^{-1} on 20 May and 1 August for eliciting plant defense responses were more than those of calcium nitrate of 0.50 g L^{-1}. In fact, 2.83 g L^{-1} CCF contained calcium nitrate of 0.50 g L^{-1}. The results here show that the mixed application of chitosan and calcium obviously had synergistic effects to induce the enhancement of defense responses in kiwifruit. Figure 4 shows our prediction of the possible action modes of CCF. Adsorption mechanism (1): a natural barrier was probably formed on the fruit and leaf surface by spraying 0.35–1.42 g L^{-1} CCF before pathogen infection, which prevented pathogen infection. Absorption mechanism (2): the compositions of CCF were absorbed into kiwifruit tissues and induced disease resistance by participating in the metabolism.

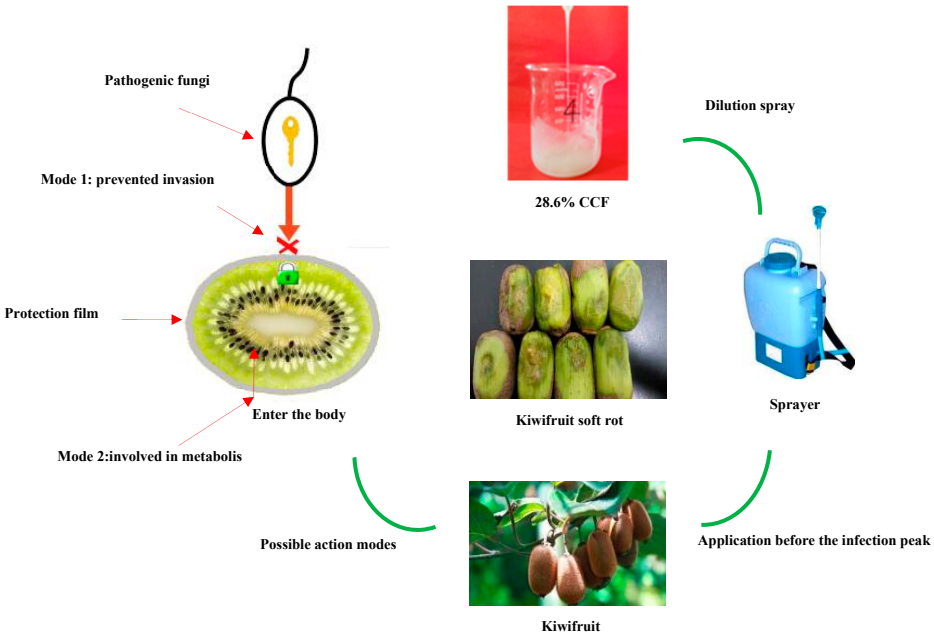

Figure 4. The possible action mode of CCF application before pathogen infection for control of soft rot in kiwifruit.

Boonlertnirun et al. [18] indicated that chitosan could enhance the content of dry matter in several ornamental plants, and another report demonstrated that chitosan is a plant nutrient which can improve quality [39,40]. The results of this study indicate that the chitosan composite film application before the pathogen infection could help to improve the quality and yield of kiwifruit. The results here show that the spray of CCF before infection was better than calcium nitrate treatment in increasing firmness and decreasing softening rates in kiwifruit. The possible reasons are that the CCF had antifungal activity as well as barrier properties to reduce decay and softening. Our results are similar to previous findings on preharvest chitosan and calcium treatments of fruits [25,41]. Therefore, chitosan composite film application before pathogen infection could maintain fruit firmness and reduce weight loss of kiwifruit during storage.

5. Conclusions

In conclusion, the present study indicates that the spraying of CCF before pathogen infection had a positive effect on controlling soft rot in kiwifruit. CCF had an inhibitory effect of mycelial growth of *B. dothidea* and *Phomopsis* sp., as well as preventive and curative effects against soft rot in kiwifruit. The spray of CCF before pathogen infection enhanced defense responses, including the levels of resistance compounds and the activities of defense enzymes such as SOD, PAL, PPO, and POD. Moreover, the spray of CCF might increase the yield, improve the quality, and prolong the shelf life of kiwifruit.

These findings demonstrate that the application of CCF before pathogen infection could be a promising strategy for controlling postharvest disease and improving the quality of kiwifruit.

Supplementary Materials: The following are available online at http://www.mdpi.com/2073-4395/10/2/265/s1, Table S1: The film-forming effects of different concentrations of chitosan and dextrin, Table S2: The film-forming effects of different mixture ratios of chitosan-dextrin, Table S3: The inhibition of two film materials to *B. dothidea* and *Phomopsis* sp., Table S4: The inhibition of different mixture ratios of chitosan-dextrin to *B. dothidea*, Table S5: The inhibition of different mixture ratios of chitosan-dextrin to *Phomopsis* sp., Table S6: The inhibition of different antifungal materials to *B. dothidea*, Table S7: The inhibition of different antifungal materials to *Phomopsis* sp., Table S8: The inhibition of two 19% film (Natamycinpty or Ferulic acid+8:10) to *B. dothidea* and *Phomopsis* sp., Table S9: The inhibition of two different film to *B. dothidea* and *Phomopsis* sp., Table S10: The film-forming effects of different content glycol and glycerol to chitosan-dextrin film, Table S11: The film-forming effects of different content organosilicon to chitosan-dextrin film, Table S12: The storage period of different content sodium benzoate and salicylic acid to chitosan-dextrin film, Table S13: The inhibition of different CCFs to mycelial growth of *B. dothidea* and *Phomopsis* sp.

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