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Potential use of cuminic acid as a botanical fungicide against *Valsa mali*

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ABSTRACT

Valsa canker caused by *Valsa mali* is commonly present in eastern Asia and cause large economic losses. Because of limited agricultural measures and chemical residues of commonly used fungicides there is an urgent need of alternative plant protecting agents. On this background the activity of cuminic acid, a plant extract from the seed of *Cuminum cyminum* L, was assessed. The median effective concentration (EC_{50}) values for inhibition of mycelial growth of seven *V. mali* strains ranged from 3.046 to 8.342 μg/mL, with an average EC_{50} value of 4.956 ± 0.281 μg/mL. The antifungal activity was the direct activity of cuminic acid instead of the influence on the pH of media by cuminic acid. After treated with cuminic acid, mycelia dissolved with decreased branches and swelling; cell membrane permeability increased while pectinases activity decreased significantly. Moreover, peroxidase (POD) activity of the apple leaves increased after treated with cuminic acid. Importantly, on detached branches of apple tree, cuminic acid exhibited both protective and curative activity. These results indicated that cuminic acid not only showed the antifungal activity, but also could improve the defense capacity of the plants. Taken together, cuminic acid showed the potential as a natural alternative to commercial fungicides or a lead compound to develop new fungicides for the control of Valsa canker.

1. Introduction

Apple Valsa canker caused by the necrotrophic ascomycete *Valsa mali* Miyabe & G. Yamada, is one of the most destructive diseases, especially in eastern Asia, bringing serious barriers in apple production in China, Korea and Japan [1–3]. For example, in Shaanxi province of China, the incidence of apple Valsa canker ranged from 30 to 90% [4]. The pathogen can infect trees throughout the year by damage or injury to the bark, such as fruit scars, fresh pruning wounds and freeze injury [5]. More seriously, the pathogen causes extensive necrotic lesions on apple branches and trunks, and lead to death of twigs, limbs, and, finally, the entire tree, which seriously reduced the yield and quality of apple [2,6].

In practice, application of fungicide is still the major method for the control of Valsa canker. Benzimidazole fungicides benomyl and thiophanate-methyl were most commonly used fungicides to control Valsa canker [7]. However, these fungicides could prevent the infection of *V. mali*, but exhibited limited ability to inhibit the expansion of the formed lesion [8]. Moreover, different level of fungicide resistance to benzimidazoles in different pathogens had occurred and increased due to the repeated and high concentrations use [9]. Subsequently, asomate, an organic arsenic fungicide, was used for the control of Valsa canker. Unfortunately, due to their threaten to human safety and environment, it was banned for the use in controlling Valsa canker [10,11]. Then coumoxystrobin, a novel fungicide of the strobilurin group discovered and patented by Shenyang Study Institute of Chemical Industry, has been demonstrated more effective than azoxystrobin and amobam against *V. mali* [12]. Although resistance to coumoxystrobin has not been reported in *V. mali*, considering the risk of resistance of plant pathogens to strobilurin fungicides, explore alternative fungicides with novel mode of action is urgently required [13,14]. At present, plant extracts or phytochemicals, such as essential oils, flavour compounds, terpenoids, glucosinolates and chitosan have been paid more attention [15,16]. Firstly, these compounds exhibited the ability to provide attractive alternatives to currently used synthetic fungicides. Secondly, such bioactive chemicals are generally safe due to their low toxicity, easy biodegradability, and minimum residues in the environment. Thirdly, it could be used as lead

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compounds for the development of new pesticides [17–19]. In addition to screen natural products from plants, assess their activity and further explore the action mechanism are more important [20].

Cuminic acid (p-isopropyl benzoic acid), belongs to the chemical group of benzoic acid, was extracted from the seed of *Cuminum cyminum* L [21,22]. Benzoic acid was commonly used as food preservative in China, while few reports about the food preservative activity of cuminic acid are available in literature. Interestingly, previous studies had shown that cuminic acid exhibited potential antifungal activity on several plant pathogens, such as *Phytophthora capsici* Leonian, *Rhizoctonia cerealis* van der Hoeven, and *Gaeumannomyces graminis* var tritici, especially against *Sclerotinia sclerotiorum*. The mycelia growth of *P. capsici*, *S. sclerotiorum*, and *R. cerealis* were completely inhibited when treated with cuminic acid at 200 μg/mL. In greenhouse experiments, over 50% efficacy against *Blumeria graminis* and *S. sclerotiorum* was obtained when applied with cuminic acid at 1000 μg/mL, which was equal to the efficacy by procymidone against *S. sclerotiorum* at 100 μg/mL [22,23]. Moreover, the EC_{50} values of cuminic acid against *S. sclerotiorum* and *P. capsici* for mycelial growth were only 7.3 μg/mL and 19.7 μg/mL, respectively, which were lower than the EC_{50} value compared with natural compound eugenol reported previously [24]. To our knowledge, no studies are available on the activity or the action mechanism of cuminic acid against *V.mali*.

Therefore, the objectives of this study were to (a) compare the sensitivity of *V. mali* to cuminic acid with other fungicides, (b) research the correlation between the pH value of the media and the antifungal activity of cuminic acid, (c) evaluate the effect of cuminic acid on the morphological and physiological characteristics of *V. mali*, and (d) assess the protective and curative activity of cuminic acid against *V. mali* on detached apple tree branches. Additionally, the activity of defense-related enzymes POD and PAL (phenylalanine ammonia-lyase) in apple tree leaves treated with cuminic acid was also determined. These results will provide new information for further investigation on the action mechanism of cuminic acid against *V. mali* and other phytopathogens.

2. Materials and methods

2.1. Fungicides, media and strains

Cuminic acid (98%) in technical grade was purchased from Jianglai Biotechnology Company (Shanghai, China) and dissolved in 10 ml methanol to 100 mg/ml for stock solution. Carbendazim (98%) and coumoxystrobin (96%) provided by Shenyang Study Institute of Chemical Industry (Shenyang, China) were dissolved in 0.1 mol/L hydrochloric acid (HCl) and 10 ml methanol at 10 mg/ml as stock solutions and stored at 4 °C in the dark, respectively.

Potato dextrose agar (PDA),a nutrient-rich medium, was prepared with 200 g of potato, 16 g of agar, and 20 g of dextrose per liter of distilled water [22].

Seven single-spore pure strains of *V. mali* (Table 1) which were collected from Shaanxi Province of China, were kindly provided by the laboratory of Integrated Management of Plant Disease in College of Plant Protection, Northwest A & F University and maintained on PDA slants at 4 °C.

2.2. Sensitivity to cuminic acid

To evaluate the sensitivity of mycelial growth to cuminic acid, seven *V. mali* strains were used to determine the EC_{50} and EC_{95} values. PDA plates were amended with cuminic acid at the final concentrations of 0, 1.5625, 3.125, 6.25, 12.5, 25, and 50 μg/mL. Inverted mycelial plugs (5 mm in diameter) taken from the periphery of 4-day-old colonies were transferred to the center of the amended PDA plates. After 4 days of incubation in a growth chamber at 25 °C, colony diameters were measured by measuring the average diameter in two perpendicular directions. The EC_{50} and EC_{95} values were calculated by regressing percentage growth inhibition against the log of fungicide concentration [22]. There were three PDA plates for each strain and the experiment was repeated three times.

In sensitivity test, carbendazim and coumoxystrobin were used as control fungicides. Fungicide concentrations were: 0, 0.0625, 0.125, 0.25, 0.5, 1, and 2 μg/mL for carbendazim and 0, 1.5625, 3.125, 6.25, 12.5, 25, and 50 μg/mL for coumoxystrobin. The EC_{50} and EC_{95} values were calculated as above.

2.3. Correlation between the media pH and the antifungal activity of cuminic acid

To investigate whether the antifungal activity of cuminic acid was correlated with the influence on the media pH, two experiments were conducted as following. Briefly, 250-ml flasks containing 100 ml PDB (PDA without agar) were amended with cuminic acid at the ultimate concentration of 0, 25, 50 and 100 μg/mL. Then the pH of the amended PDB was determined by pH meter (PHS-3C, Shanghai).

Furthermore, sensitivity to cuminic acid for mycelial growth in PDA with different pH was determined. Briefly, PDA media were amended with HCl to obtain the final pH values of 4, 5, 6, and 7. Then the EC_{50} values of cuminic acid for the seven strains were determined as above. Three replicates per concentration were used and all the experiments above were conducted three times.

2.4. Effect of cuminic acid on mycelial morphology

Mycelia plugs taken from the margin of an actively growing colony of the strain 06-5 randomly selected were transferred mycelia-side down on PDA plates containing EC_{50} value of cuminic acid. Plates without cuminic acid were used as control. After 4 days at 25 °C in a growth chamber, the margin of medium area

Table 1
Sensitivity to cuminic acid and other fungicides.

Strains	EC_{50} (μg/mL) for			EC_{95} (μg/mL) for		
	Cuminic acid	Carbendazim	Coumoxystrobin	Cuminic acid	Carbendazim	Coumoxystrobin
08–9	4.574cd[a]	0.1014b	1.172d	21.35bc	0.5441b	20.08a
06–5	6.237b	0.1345b	3.161b	27.17b	0.6312a	16.15b
07–2	8.342a	0.1273a	2.665bc	39.18a	0.4337c	17.92 ab
06–8	3.046e	0.0983b	2.000c	18.62c	0.4271c	19.33a
07–14	5.213c	0.2110a	4.470a	24.44b	0.3987c	18.25 ab
09–17	3.155e	0.1562 ab	2.899b	19.23c	0.4925bc	17.36 ab
08–21	4.125d	0.1927a	1.982c	22.97bc	0.4111c	20.18a

[a] Mean values followed by the same letter within the same column were not significantly different in LSD (least significant difference) tests at P = 0.05.

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(10 mm × 10 mm) was cut and placed on slide glass. The mycelial morphology of *V. mali* was observed by scanning electron microscope (SEM, JSM-6360LV, Japan) [22]. Three replicates were used and the experiment was repeated twice.

2.5. Effect of cuminic acid on cell membrane permeability

For each of the three strains 08-9, 06-5, and 07-2 (randomly selected), ten mycelial plugs were transferred to 250-mL flasks containing 100 mL of PDB. After the flasks were shaken at 175 rpm and 25 °C for 72 h, partial flasks were amended with cuminic acid at their EC_{50} values. Flasks without cuminic acid were used as control. After the flasks were shaken for additional 24 h, mycelia were collected. Then 0.5 g of fresh mycelia per sample was suspended in 25 mL of distilled water. Conductivity of the distilled water was measured after 0.5, 1, 2, 3, 4, and 5 h with a conductivity meter (CON510 Eutech/Oakton, Singapore). After 5 h, the mycelia were boiled for 5 min to measure the final conductivity [25]. Each treatment had three replicates, and the experiment was performed twice. The relative conductivity of mycelia was calculated as follows:

Relative conductivity = Conductivity at different times/Final conductivity × 100%

2.6. Oxalic acid content and pectinases activity

Oxalic acid content was determined according to a previous study with minor modifications [25]. Mycelial plugs taken from the margin of 2-day-old colonies were placed in 250-ml flasks (Ten plugs per flask) containing 100 ml PDB treated with cuminic acid at the EC_{50} value. Flasks without treatment were used as control. After incubation on a rotary shaker at 175 rpm and 25 °C for 4 days, the mycelia were collected and the filtrate were centrifuged at 1500 rpm for 10 min. Absorbance of the supernatants was measured at 510 nm with a spectrophotometer. Oxalic acid content was calculated with the standard curve. Three flasks per treatment were used and the experiment was repeated three times.

Pectinases activity was determined as reported by Wang et al. (2007) [26]. To establish the standard curve of pectinases, different volumes (0, 0.2, 0.4, 0.6, 0.8 or 1.0 mL) of D-galacturonic acid solution (2 mg/mL) were then added, and double-distilled water was used to increase the volume to 1 mL. Then 0.5 ml citrate buffer (pH 4.8) and 3.0 ml 3,5-dinitrosalicylic acid were added to the tube, and boiled for 5 min. After natural cooling, double-distilled water was used to increase the volume to 10 mL. Absorbance was measured at 540 nm with a spectrophotometer. Finally, the standard curve was established by plotting absorbance against D-galacturonic acid concentration.

For determination of pectinases activity, filtrate collected above were centrifuged at 12000 rpm and 4°Cfor 5 min. Then the pectinases activity in the supernatants was determined with the standard curve. Three flasks per treatment were used and the experiment was repeated three times.

2.7. POD and PAL activity of the apple leaves

Apple leaves of similar growth stage on 2-year-old trees of *Malus domestica* 'Fuji' were sprayed with water, cuminic acid at 500, 1000 and 2000 μg/mL until run-off. After 72 h, leaves were cut down and broken on ice. POD and PAL activity were determined using commercial kits (Jiancheng, Nanjing) according to the manufacturer's instructions. One unit of POD activity was defined as a change of one in absorbance per min; one unit of PAL activity was defined as the increase of one in absorbance per h [25]. Five leaves per treatment were used and the experiment was repeated twice.

2.8. Protective and curative activity

In vivo protective and curative activity of cuminic acid was assessed on detached twigs of apple trees (10-year-old trees of *Malus domestica* 'Fuji'). Twigs were cut into 10 cm segments and wounded with a hole puncher (5 mm in diameter) to remove the bark. For protective activity, the wounded twigs were daubed with water (control), cuminic acid at 1000, or 2000 μg/mL, and carbendazim at 400 μg/mL (recommended dosage) using a writing brush until run-off. Then mycelial plugs taken from the margin of the colony of *V. mali* strain 06-5 (randomly selected) were inoculated after fungicide treatment for 24 h. For curative activity, fungicides were daubed after mycelial plugs were inoculated for 24 h. After the treated twigs were placed in greenhouse at 25 °C and 80% relative humidity for 72 h, lesion diameters were measured in two perpendicular directions and the lesion area was calculated [3]. Each treatment had three plants with nine twigs (three twigs cut from one plant) and the experiment was repeated twice.

Lesion area (cm^2) = 1/4 × ∏ × length of long lesion × length of short lesion.

2.9. Statistical analysis

Statistical analysis was conducted using SPSS 14.0 (SPSS Inc., Chicago, IL) according to previous studies [25]. The EC_{50} values of the strains were calculated by linear regression of the log of the colony diameter versus fungicide concentration. When the ANOVA (analysis of variance) was significant (P = 0.05), means were separated with Fisher's protected least significant difference (PLSD).

3. Results

3.1. Sensitivity to cuminic acid

The EC_{50} values for cuminic acid in inhibiting mycelial growth of the seven *V. mali* strains were different from each other, ranged from 3.046 to 8.342 μg/mL, and the mean EC_{50} value was 4.956 ± 0.281 μg/ml. The EC_{50} values for carbendazim and coumoxystrobin inhibiting mycelial growth on PDA plates of the seven *V. mali* strains ranged from 0.098 to 0.211 μg/mL and 1.172–4.470 μg/mL, with average EC_{50} values of 0.146 μg/mL and 2.621 μg/mL, respectively (Table 1). In addition, the EC_{95} values for cuminic acid were lower than 100 μg/mL, which were nearly equal to that for coumoxystrobin.

3.2. Correlation between the media pH and the antifungal activity of cuminic acid

Cuminic acid belongs to the chemical group of benzoic acid. The pH value of the PDB without treatment was 6.896. With the increased concentration of cuminic acid, the pH value of PDB decreased (Table 2). However, there was no significant difference among the EC_{50} values of the seven strains whether the pH value of

Table 2
The pH value of PDB treated with different concentrations of cuminic acid.

Concentration of cuminic acid (μg/mL)	pH value of media
0.000	6.896a[a]
25.00	6.234b
50.00	5.792c
100.0	5.113d

[a] Mean values followed by the same letter within the same column were not significantly different in LSD (least significant difference) tests at P = 0.05.

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Table 3
EC_{50} values of the seven strains for cuminic acid determined with different pH of PDA.

Strains	EC_{50} (μg/mL) for cuminic acid in PDA with different pH			
	pH = 4	pH = 5	pH = 6	pH = 7
08–9	4.687a[a]	4.323a	4.172a	4.354a
06–5	6.038a	6.341a	6.416a	6.307a
07–2	8.342a	8.127a	8.665a	8.545a
06–8	3.016a	3.089a	3.333a	3.422a
07–14	5.312a	5.121a	5.407a	5.520a
09–17	3.152a	3.156a	3.699a	3.437a
08–21	4.134a	4.592a	3.985a	4.228a

[a] Mean values followed by the same letter within the same row were not significantly different in LSD (least significant difference) tests at P = 0.05.

PDA was 4, 5, 6, or 7, respectively (Table 3).

3.3. Effect of cuminic acid on mycelial morphology

The mycelial morphology of *V. mali* treated or untreated with cuminic acid was observed by SEM. The mycelia without treatment were natural (Fig. 1a), while after treated with cuminic acid at the concentration of EC_{50} value, mycelia dissolved, swelling and the offshoot of top decreased (Fig. 1b,c,d).

3.4. Effect of cuminic acid on cell membrane permeability

With cuminic acid treatment or not, the relative conductivity of the strains increased over time. After treated with cuminic acid, the relative conductivity was always higher for the three strains than the corresponding untreated control (Fig. 2). These indicated that the cell membrane permeability enhanced after treated with cuminic acid.

3.5. Oxalic acid content and pectinases activity

Oxalic acid content was calculated by absorbance at 510 nm of inoculated PDB treated or untreated with cuminic acid. There was no significant difference between the oxalic acid contents of *V. mali* strains 08-9, 06-5, and 07-2 treated or untreated with cuminic acid, respectively (Fig. 3a). However, pectinases activity of the mycelia treated with cuminic acid were significantly lower than control (Fig. 3b).

3.6. POD and PAL activity of the apple leaves

With the increased concentration of cuminic acid, POD activity of the apple leaves increased gradually (Fig. 3c). However, there was no significant difference between the PAL activity whether the leaves were treated with water or cuminic acid (Fig. 3d).

3.7. Protective and curative activity

On detached twigs of apple trees, the strain 06-5 generated large lesions when the twigs were treated with water. When treated with cuminic acid at 2000 μg/mL before inoculation, 71.71% efficacy was obtained, which was nearly equal to the efficacy of carbendazim at 400 μg/mL (Table 4). Moreover, 66.96% efficacy was obtained when treated with cuminic acid at 2000 μg/mL after inoculation. In addition, cuminic acid at 1000 μg/mL also exhibited over 45% protective and curative activity against Valsa canker (Fig. 4). These indicated that cuminic acid had both protective and curative activity.

4. Discussion

The apple-tree canker caused by *V. mali* is one of the most destructive diseases in eastern Asia, especially in China, Korea and Japan [1–3]. Although application of fungicide is still the main

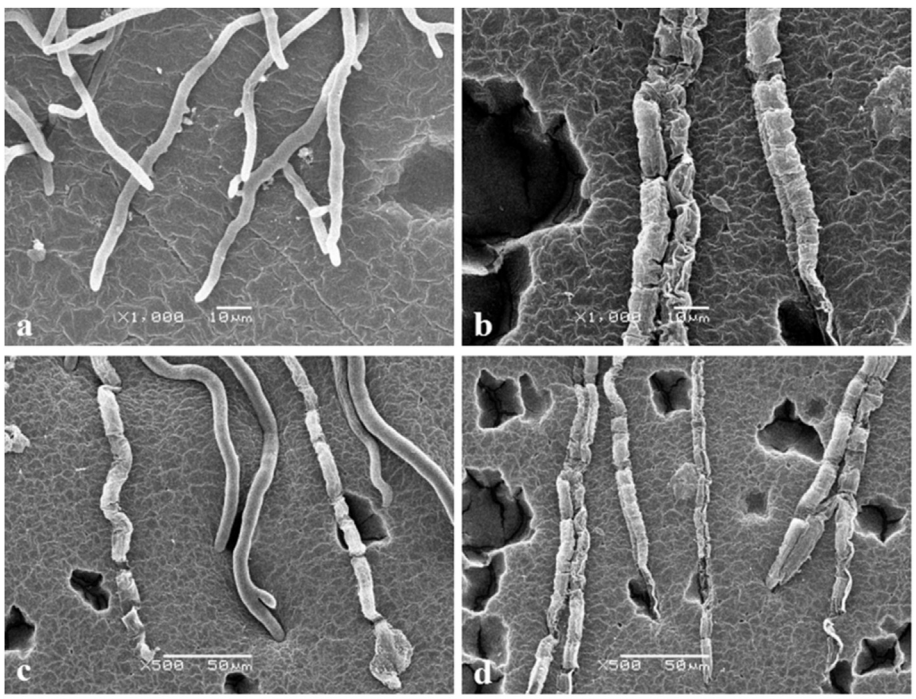

Fig. 1. The mycelial morphology of *V. mali* treated or untreated with cuminic acid. **a**: Plates without treatment; **b,c,d**: Plates treated with cuminic acid at the concentration of EC_{50} value.

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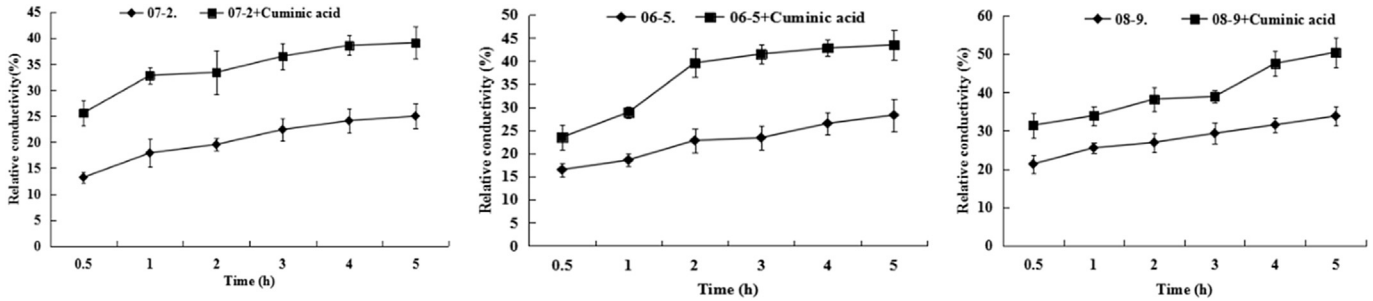

Fig. 2. Relative conductivity of mycelia of three wide-type strains 08-9, 06-5, and 07-2 with or without cuminic acid treatment. Bars denote the stand error of three experiments.

method for control of apple tree Valsa canker, continuous and extensive use of a signal chemical may lead to undesirable effects such as environmental pollution, residue toxicity, and the risk of fungicide resistance. On this background, natural compounds extracted from plants are more popular due to their specific antifungal activity, easy degradation, and human safety [27–29]. *Cuminum cyminum* L mainly distributed in India, Iran, Turkey and the northwest of China and commonly known as cumin. Cumin seeds are commonly used as a cooking spice throughout the world. In addition, cumin seeds have been used in medicine to treat stomach colds, abdominal pain and hypopepsia, and the main component in cumin seed was cuminic acid, which had been demonstrated to exhibit antifungal activity against several plant pathogens [21].

In the present study, the activity of cuminic acid against *V. mali* was assessed. The mean EC_{50} value for cuminic acid in inhibiting mycelial growth of the seven *V. mali* strains was lower than 5 μg/mL, which was even lower than that against *S. Sclerotiorum* [22]. Cuminic acid belongs to the chemical group of benzoic acid [22]. In the correlation between pH and cuminic acid experiment, pH value of PDB media was significantly reduced by cuminic acid, which was in agreement with previous study that intracellular pH of yeast was reduced by benzoic acid [30]. These could be explained by the same mode of action between cuminic acid and benzoic acid. Interestingly, the sensitivity of *V. mali* to cuminic acid could not be influenced by pH. When pH = 3, the PDA media can not be solidified, therefor our efforts to determine the sensitivity to cuminic acid were unsuccessful. These results suggested that although cuminic acid could influence the pH of the media, the antifungal activity of cuminic acid was exhibited by itself.

Benzoic acid as food preservative had been commonly used. It inhibited the absorption of amino acids by interfering with the cell

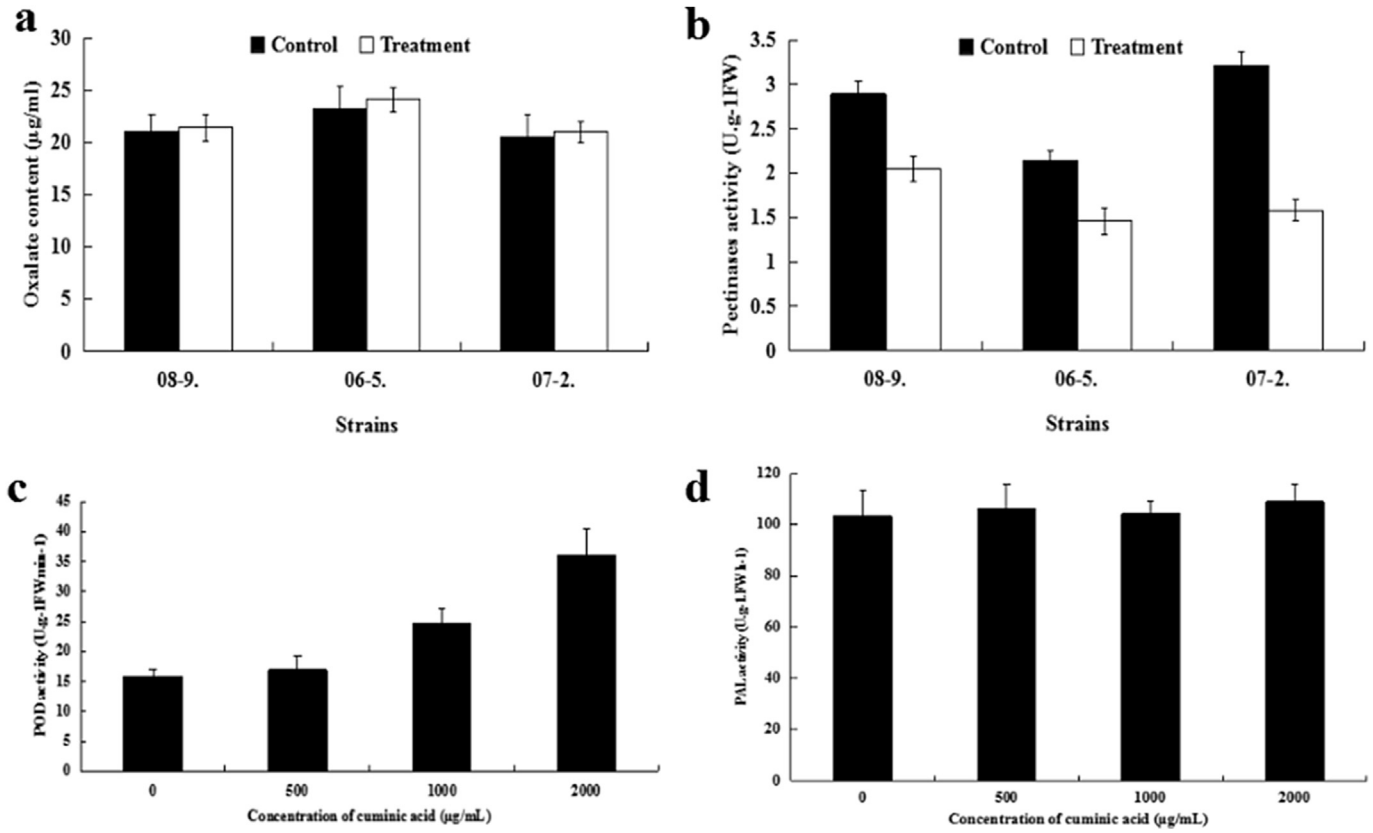

Fig. 3. (a) Oxalic acid content and (b) pectinases activity of mycelia of three wide-type strains 08-9, 06-5, and 07-2; (c) POD and (d) PAL activity of the apple leaves with or without cuminic acid treatment. Bars denote the stand error of three experiments.

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Table 4
Protective and curative activity of cuminic acid in controlling *V. mali* strain 06-5 on detached branches of apple tree.

Treatment	Protective activity		Curative activity	
	Lesion area (cm^2)	Control efficacy (%)	Lesion area (cm^2)	Control efficacy (%)
Cuminic acid (1000 μg/mL)	2.442b[a]	47.70b[b]	2.693b	45.32c
Cuminic acid (2000 μg/mL)	1.321c	71.71a	1.627c	66.96b
Carbendazim (400 μg/mL)	1.242c	73.40a	1.415c	71.27a
Water control	4.669a	–	4.925a	–

[a] Values followed by the same letter within the same column were not different according to Fisher's least significant difference (LSD) (P = 0.05).
[b] Control efficacy = [(Lesion area of control − Lesion area of treated group)/(Lesion area of control)] × 100%.

membrane permeability [22]. In the present work, cell membrane permeability increased after treated with cuminic acid, indicating that cuminic acid could damage the membrane structure of *V. mali* and cause the intracellular plasma leakage through imperfect membrane. Previous studies had demonstrated that oxalic acid was a key pathogenicity determinant and an elicitor of plant programmed cell death during *S. sclerotiorum* disease development. In contrast, oxalate-deficient mutants of *S. sclerotiorum* were nonpathogenic and unable to develop sclerotia [31–35]. In addition, pectinases was one of the most important cell wall hydrolases. Pectinases secreted by *V. mali* play an important role during their infection of apple [36]. In this study, oxalic acid content did not differ while pectinases activity decreased after treated with cuminic acid, indicating that cuminic acid might inhibit the infection capacity and the infection mechanism might be different between *S. sclerotiorum* and *V. mali*.

Previous studies had demonstrated that induction of resistance to pathogens or herbivores was generally regulated by a network of signal transduction pathways in which salicylic acid (SA) and jasmonic acid (JA) function as key signaling molecules [37–39]. SA had been widely recognized as one of the signal molecules involved in systemic acquired resistance (SAR) and hypersensitive reaction. The role of SA as one of the endogenous signaling molecules involved in SAR signal transduction pathways had been confirmed in tobacco, cucumber, arabidopsis and other plants [40]. JA not only induces plants to produce secondary metabolites and volatile compounds, but also induces physiological changes in plants which lead to the formation of defensive structure and increase the physical defense capacity of plants [41]. In addition, there is evidence that POD and PAL are key enzymes involved in plant defense. In the presence of hydrogen peroxide, POD could oxidize phenols to quinines [42,43]. PAL is the indicator of phenolic production rate in the phenylpropanoid pathway and could be induced by various adversities [44,45]. POD activity increased while PAL activity did not differ when treated or untreated with cuminic acid, which was consistent with the previous research that cuminic acid could also increase the POD activity of pepper leaves [22]. The work to research whether cuminic acid had the same function of inducing plant resistance as SA or JA is valuable.

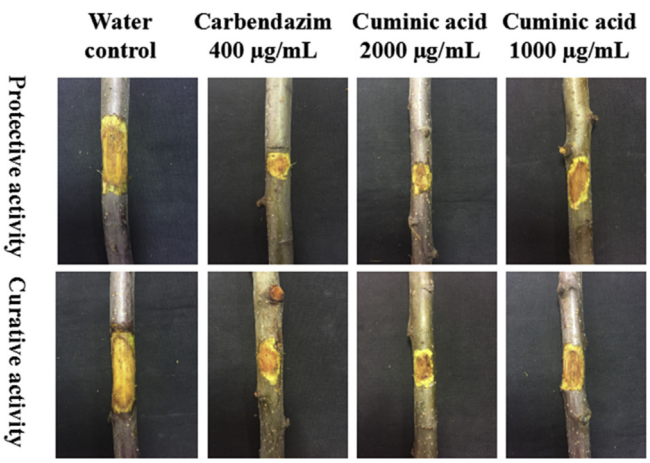

Fig. 4. Protective and curative activity of cuminic acid against Valsa canker.

Compounds extracted from plants have been regarded as an attractive, environmental-sound alternative for disease management [46–48]. Moreover, natural compounds are often considered to be lead compounds for the synthesis of novel fungicides. For example, strobilurin fungicides (Azoxystrobin, picoxystrobin) were synthesized based on the structure of strobilurin A [49]; pyrroles compounds fenpiclonil and fludioxonil were synthesized based on the chemical structure of natural compound pyrrolnitrin [50]. Although the chemical structure of cuminic acid was simple, it exhibited broad-spectrum antifungal activity. More importantly, this not only provided the opportunity that cuminic acid could be used as lead compound to synthesis new antifungal compounds, but also to find new target for synthesis drugs with novel mode of action.

In summary, the current study confirmed that cuminic acid extracted from the seed of *Cuminum cyminum* L exhibits the antifungal activity and could be used as botanical fungicide. To our knowledge, this is the first report on the antifungal activity of cuminic acid against *V. mali* and biochemical responses will provide new information on the action mechanism of cuminic acid. Work to explore the mechanism of cuminic acid and synthesis of new antifungal drugs based on the structure of cuminic acid is underway in our laboratory.

Conflict of interest

This manuscript does not contain any conflict of interest.

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