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Application of 6-Pentyl-α-Pyrone in the Nutrient Solution Used in Tomato Soilless Cultivation to Inhibit *Fusarium oxysporum* HF-26 Growth and Development

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Abstract: Soilless cultivation has increased in China. Like vegetables or ornamental plants, tomatoes can be cultivated in soilless culture systems. Fusarium wilt (FW) is an economic tomato disease. The nature and amount of volatile organic compounds in *Trichoderma asperellum* PT-15 were investigated, and 6-pentyl-α-pyrone (6-PP) was detected. Furthermore, the effect of 6-PP on *Fusarium oxysporum* HF-26 was evaluated. Results revealed that 25 mg/L 6-PP was the optimal concentration inhibiting *F. oxysporum* HF-26 and that the content of fusaric acid decreased considerably compared to that of the control. *FUB1*, *FUB4*, and *FUB10* toxin synthesis and transport genes were downregulated. Additionally, *VelA*, *velB*, and *LaeA* genes were downregulated, reducing *F. oxysporum* mycelial growth and hyphae formation. 6-PP was added to the soilless culture solution in a greenhouse experiment. The results showed that an antifungal–nutrient solution containing 25 mg/L 6-PP significantly suppressed FW with 70.71% efficacy and a 27.23% disease index (DI), which were higher efficacy and lower DI than that of the control. Furthermore, treatment with an antifungal–nutrient solution containing 6-PP increased the levels of defence enzymes 24 h post-inoculation (hpi) compared with those at other time periods. The relative expression levels of the *PR1*, *NPR1*, *PR2*, and *PR5* genes were considerably upregulated at 24 hpi.

Keywords: soilless culture; tomato fusarium wilt; *Trichoderma asperellum* PT-15; 6-pentyl-α-pyrone; antifungal–nutrient solution

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1. Introduction

Soilless culture is the cultivation of plants without soil and includes hydroponics, aerosol, and substrate culture [1]. Substrate soilless culture uses solid, non-soil substrate material to support crops and provide nutrients; it has become the primary method in greenhouse vegetable cultivation because it is not affected by terrain, prevents soil-borne diseases, reduces pesticide use, and improves crop quality [2,3]. However, the continuous use of raw materials causes problems associated with continuous cropping, such as the accumulation of soil-borne diseases. Several studies have reported that Fusarium wilt (FW), caused by *Fusarium* spp., is the primary soil-borne disease in substrate vegetables production due to the continuous use of substrates. FW occurs in cucumbers, tomatoes, watermelons, eggplants, peppers, lettuce, and carnations, which are mainly infected with

Fusarium oxysporum [4–8]. Currently, FW limits the production of vegetables in soilless media [6].

Tomatoes can be cultivated in soilless culture systems [9]. FW, caused by *Fusarium oxysporum*, is the primary disease of economic importance in soilless cultivation systems, usually occurring in the middle-to-late growth stages (90–120 d) [4,10]. Various biological agents, such as bacteria, fungi, and their metabolites, have been used to control FW in tomato, cucumber, watermelon, and other crops [5,8,11]. The use of microbial volatile organic compounds (VOCs) is a biological control strategy for plant disease management. VOCs are a large group of carbon-based chemicals with low molecular weight, polarity, boiling point, and high vapour pressure [12]. VOCs are often lipophilic and belong to different chemical classes, such as alcohols, benzenoids, aldehydes, alkenes, acids, esters, ketones, thiols, and their derivatives [13,14]. Many of these volatile compounds have biotechnological applications in agriculture and industry [15]. There is an increase in the use of VOCs from various *Trichoderma* species as fungicides. The genus *Trichoderma* has been used as a biocontrol agent to enhance plant growth since the 1930s [16]. *Trichoderma* species produce various VOCs [17,18]. However, 6-pentyl-α-pyrone, known as pyrone 6-pentyl-2H-pyran-2-one (6-PP), is the most common substance metabolised and volatilised by *Trichoderma* [19]. It is a type of compound with a six membered lactone ring skeleton, and its physical and chemical properties are similar to olefins and aromatic compounds. α- Pyranone, an important substructure of various natural products, exhibited extensive diversity in structure and function and played an important role in microbial metabolism and defense processes [20]. *Trichoderma* has various actions against phytoparasitic fungi, such as restricting spore germination, mycelial pigmentation, and hyphal growth [21]. Furthermore, 6-PP reduces the fusaric acid and mycotoxin deoxynivalenol production by *Fusarium moniliform* and *graminearum*, respectively [22,23]. Additionally, 6-PP is a post-harvest food-grade volatile compound [24]. In commercial production, large amounts of 6-PP can be produced by *Trichoderma asperellum* via forced aeration in a solid-state fermentation system [25].

Currently, substrate replacement is the primary method used to eliminate the problems associated with continuous cropping in substrate cultivation. However, the method wastes substrate and increases production costs [26,27]. Therefore, it is important to develop new methods of preventing soil-borne diseases and alleviating problems associated with continuous cropping. Lin et al. (2014) [28] reported that waste materials from the vinegar industry, after stacking and fermentation, are used singly or in combination with different proportions (volume ratios) of peat and vermiculite to ameliorate cucumber FW considerably. Moreover, the microbiota that develops in a multiple parallel mineralisation system (MPM) can suppress *F. oxysporum* and *Bremia lactucae*. A previous study revealed that adding MPM culture solution to non-soil carriers (rockwool, rice husk charcoal, and vermiculite) in soilless cultures reduced diseases and increased plant growth [29]. However, there are few reports on the control of FW in soilless cultivation, particularly the application of the antibiotic substance 6-PP in nutrient solutions.

In our previous study, *T. asperellum* PT-15 inhibited Verticillium wilt in potatoes [30]. The present study aimed to determine the type and quantity of VOCs in *T. asperellum* PT-15, identify the target substance 6-PP, and test the inhibitory effects of 6-PP on *F. oxysporum* HF-26 cells. Additionally, the present study aimed to determine the inhibitory effect of 6-PP added to the nutrient solution of the soilless culture on tomato FW to develop an environmentally friendly fungicidal nutrient solution for soilless culture media. The present study is expected to contribute to preventing and controlling FW and the problems associated with continuous cropping in soilless cultivation.

2. Materials and Methods

2.1. Fungal Isolates and Plants

T. asperellum PT-15 (GenBank: OQ675158) was obtained from the Fungus Preservation Collection of the Inner Mongolia Agricultural University, Hohhot, China. *F. oxysporum* HF-

26 (GenBank: OQ675162) was isolated from FW-infected tomato plants planted in soilless planting media at the Yimin Hongtai Agriculture Co., Ltd., Hohhot, China. *T. asperellum* PT-15 and *F. oxysporum* HF-26 were cultured in potato dextrose agar (PDA) and stored at 25 °C in the dark for 7 days, and then cultured in potato dextrose broth on a rotator at 180 rpm and 25 °C for 7 days in the dark for conidial production. The concentration of conidial suspensions was adjusted to 1×10^7 conidia mL^{-1} using a hemocytometer (Bioevopeak Co., Ltd., Jinan, China) with sterile distilled water for inoculation.

6-pentyl-α-pyrone was bought from Shanghai Macklin Biochemical Co., Ltd. (Shanghai, China).

Tomato plants (variety1617) were purchased from Mengmiao Agricultural Technology Co., Ltd., Hohhot, China.

2.2. Collection of T. asperellum PT-15 Volatile Compounds and Gas Chromatography-Mass Spectrometry

In our previous study, *T. asperellum* PT-15 had the highest inhibitory activity against Verticillium wilt in potatoes [29]. Therefore, in the present study, *T. asperellum* PT-15 was analysed to identify VOCs using purge-and-trap gas chromatography-mass spectrometry (GC-MS) to extract 6-PP. *T. asperellum* PT-15 grown in PDA for 7 days was used for the study. The control group comprised plates with PDA only.

2.2.1. Sample Extraction

Following the method by [31] for sample extraction, 1 g mycelium was prepared and put into a 15 mL extraction vial. Next, 5 mL boiling distilled water was added, and the mycelium was extracted and absorbed at 600 r/min, placed in a water bath at 80 °C for 60 min, and analysed for 10 min using GC/MS.

2.2.2. Gas Chromatography Conditions

A chromatographic column DB-WAX (30 m × 0.25 mm × 0.25 μm [Agilent, Santa Clara, CA, USA]) at 240 °C inlet temperature was used. Helium (purity > 99.999%) was used as the carrier gas, the column flow rate was 1 mL min^{-1}, and the split ratio was 10:1. The heating procedure was 35 °C for 5 min, at the rate of 2 °C min^{-1} to 100 °C, kept for 1 min; and heated at a rate of 5 °C min^{-1} to 240 °C for 2 min.

2.2.3. Mass Spectrometry Conditions

The mass spectrometer (Oxford Instruments, Abingdon, England) was operated in 230 °C Ion source temperature, 240 °C interface temperature, 70 eV electron ionization mode, 1161 V electron multiplier voltage, and 35–550 u mass scan range in full scan mode. Chemical identification of the compounds was performed by comparing spectra the results with library mass spectra from the National Institutes of Standards and Technology, EPA-NIH, and Wiley libraries. If no match was found, manual mass spectral interpretation was used. Quantification was conducted using a toluene-d 8 internal standard by calculating the peak area ratio of the compound of interest to that of the internal standard, which was added at a known concentration. Volatile compounds in the control (PDA only) were removed from the VOCs profiles.

2.3. Effect of 6-PP on F. oxysporum HF-26 Mycelial Growth

PDA media was amended with 0 (control), 10, 20, and 25 mg L^{-1} 6-PP. An 8 mm mycelial plug taken from the leading edge of 7-day-old *F. oxysporum* HF-26 colonies was inoculated into the centre of the plate with amended PDA medium and incubated in a growth chamber at 25 °C for 7 days. Colony diameter was determined by measuring the average of two perpendicular lines on each plate. Data on each concentration was measured on the three replicates.

2.4. Effect of 6-PP on F. oxysporum HF-26 Fusaric Acid Synthesis

Mycelium (2 g) was placed in a 15 mL centrifuge tube, and 5 mL ethyl acetate was added, centrifuged at 5000 r/min for 10 min, and ultrasonicated at low temperature for 15 min. The supernatant was placed into another 15 mL centrifuge tube. The extraction was repeated thrice, and the supernatant from the three replicates was composited, freeze-dried at low temperature, dissolved in 0.5 mL chromatographic grade methanol at a constant volume, shaken and mixed for 5 min, and filtered through a 0.22 μm membrane for testing. The prepared samples were analysed using an Agilent 1200 High-performance liquid chromatography system (G1313A, Shanghai Weisu Biotechnology Co., Ltd., Shanghai, China). For the test conditions, the detection was done using a DAD C18 column (250 × 4.6 mm; 0.5 μm [Shanghai Weisu Biotechnology Co., Ltd.]) with a column temperature of 25 °C, flow rate of 1 mL/min, a wavelength of 270 nm, moving phase solvent used was methanol: 0.1% formic acid water at 90:10 (V:V), and the sample size was 20 μL.

2.5. Real-Time Quantitative Reverse Transcription Polymerase Chain Reaction

Real-time quantitative reverse transcription polymerase chain reaction (qRT-PCR was performed on *F. oxysporum* HF-26 to examine differences in the transcript levels of genes associated with the biosynthesis of fusaric acid [32,33] and pigments [34]. The mycelia of the *F. oxysporum* HF-26 isolate were prepared as described in Section 2.1. Total RNA was isolated from *F. oxysporum* HF-26 mycelia using an RNA extraction kit (TaKaRa Bio Inc., Shiga, Japan) according to the manufacturer's protocol. First-strand cDNA was generated from RNA using the Primer Script RT Master Mix (TaKaRa Bio Inc.). In the present study, the actin gene was used as an internal control, and all primers used for qRT-PCR are listed in Table S1. qRT-PCR was carried out in a 10 μL reaction mixtures containing 6 μL TB Green ® Premix Ex Taq™ (Tli RNaseH Plus; TaKaRa Bio Inc.), 0.4 μL of each primer, and 0.8 μL templated DNA. All qRT-PCRs were performed using a QuantStudio5 real-time detection system (Thermo Fisher Scientific, Waltham, MA, USA). Each sample was analysed twice in three independent biological experiments. With a related actin gene (Foxq13729) as the reference gene, relative expression levels of target genes were calculated according to the $2^{-\Delta\Delta Ct}$ method [35].

2.6. Greenhouse Experiments

Based on the above results, the 6-PP optimal concentration for inhibiting *F. oxysporum* HF-26 was selected and added to the soilless culture nutrient solution considering the rates used for the Hoagland nutrient solution [36]. The new nutrient solution formula had 1360, 100, 500, 270, 3.0, 1.6, 0.28, 0.12,0.10, 20, and 25 mg/L of Ca $(NO_3)_2$, KNO_3, $MgSO_4$, $NH_4H_2PO_4$, H_3BO_3, $MnSO_4$, $ZnSO_4{\cdot}7H_2O$, $CuSO_4{\cdot}5H_2O$, Na_2MoO_4, Na-Fe Ethylenediaminetetraacetic acid, and 6-PP, respectively. The solution pH was adjusted to 6.5–7.0, and the solution electrical conductivity ranged between 1.8–2.0 S/m.

Tomato plants were planted in a soilless cultivation system dominated by Rockwool and regularly watered with a nutrient solution. After 20 days of growth in the system, tomatoes were inoculated with a spore suspension with 1×10^7 conidia mL^{-1}, and FW incidence was recorded after 20 days. Three groups were established for this experiment. The standard nutrient solution without 6-PP was used as a negative control, and the nutrient solution containing carbendazim [4] was used as the positive control. The nutrient solution containing 6-PP was the treatment group. Each group had three replicates, with 15 tomato plants per replicate.

FW infection was determined using four rating scales, as described as follows [4]:

0: No infection.

1: Slight infection of approximately 25% with one or two leaves turning yellow.

2: Moderate infection with two or three leaves turning yellow and 50% wilting.

3: Extensive infection with all plant leaves turning yellow, 75% wilting, and growth inhibited.

4: Complete infection with all plant leaves turning yellow, wilting, and plant death.

The disease index (DI) was then calculated as follows [4]:

$$\text{DI} = \sum \frac{\text{scale} \times \text{number of plants infected}}{\text{highest scale} \times \text{total number of plants}} \times 100$$

$$\text{Prevention or curative effects } (\%) = \frac{\text{disease index of the control} - \text{disease index of fungicide treatment}}{\text{disease index of the control}} \times 100$$

2.7. Determination of Reactive Oxygen Species and the Activity of Defence-Related Enzymes and Genes

To determine the effects of the nutrient solution containing 6-PP on defence-related enzymes and genes related to the tomato disease defence mechanism, the content of related defence-related enzymes and the relative expression levels of defence-related genes in tomato leaves was determined at 0, 12, 24, and 48 h post-inoculation (hpi) with 1×10^7 conidia mL^{-1}. H_2O_2 accumulation and catalase (CAT), peroxidase (POD), and superoxide dismutase (SOD) activities were measured according to the operating instructions of the H_2O_2, CAT, POD, and SOD respective test kits purchased from Sino Best Biological Technology Co., Ltd. Shanghai, China. The relative expression levels of defence genes were determined using qRT-PCR, as described in Section 2.5. The genes and primers used in the present study are listed in Table S1. The nutrient solution containing 6-PP was used in the treated group, and the standard nutrient solution (without 6-PP) was used in the control group.

The experiments were replicated thrice, with six leaves per replicate.

According to the operating instruction.

2.8. Statistical Analysis

Data from repeated experiments were combined for homogeneity before analysis. All data were processed and analysed using SPSS software (version 25.0; SPSS Inc., Chicago, IL, USA). When significant results ($\alpha = 0.05$) were obtained after analysis of variance, means were separated using Fisher's least significant difference.

3. Results

3.1. Analysis of VOCs Produced by T. asperellum PT-15

Based on the mass spectral properties, 55 unique VOCs were identified, including alcohols, aldehydes, ketones, esters, alkenes, alkanes, alkynes, organic acids, benzenes, terpenes (Table 1), and 6-PP (Figure S1).

Table 1. Volatile organic compounds identified from *Trichoderma asperellum* PT-15 by gas chromatography-mass spectrometry.

Serial Number	Compound	Retention Time (min)	Area (%)	Molecular Formula	Molecular Weight
1	1-Propanamine	3.78	0.02	C_3H_9N	59.11
2	2-Methyl-pentanal	4.06	1.28	$C_6H_{12}O$	100.16
3	2-Methyloctanal	4.30	1.31	$C_9H_{18}O$	142.24
4	Ethanol	4.53	21.94	C_2H_6O	46.07
5	Glycylglycine	4.55	20.84	$C_4H_8N_2O_3$	132.12
6	Pentanal	5.16	0.11	$C_5H_{10}O$	86.13
7	4-Hexyl-1,3-oxazolidin-2-one	5.37	0.66	$C_9H_{16}NO_2$	170.23
8	Ethyl propionate	4.74	0.02	$C_5H_{10}O_2$	102.13
9	Acetonitrile	5.67	0.27	C_2H_3N	41.05

Table 1. *Cont.*

Serial Number	Compound	Retention Time (min)	Area (%)	Molecular Formula	Molecular Weight
10	N-Methyl-2-cyanopyrrole	7.91	0.05	$C_6H_6N_2$	106.13
11	N-Butyl propionate	8.06	0.74	$C_7H1_4O_2$	130.18
12	2-Propanone	8.46	1.57	C_3H_6O	58.08
13	7,7-Dimethylcycloheptatriene	8.60	6.46	C_9H_{12}	120.19
14	8,9-Epithio-1-p-menthene	8.96	0.00	$C_{10}H_{16}S$	168.3
15	Methane-sulfonic anhydride	9.28	0.02	$C_2H_6O_5S_2$	174.2
16	3-Methyl-1-Butanol	9.35	13.46	$C_5H_{12}O$	88.15
17	Ethyl hexanoate	9.70	0.04	$C_8H_{16}O_2$	144.21
18	1,2-Ethanediol,diethylether	10.10	0.23	$C_6H_{12}O_2$	116.16
19	(S)-1,1-Diethoxy-2-propanamine	10.19	0.76	$C_7H_{17}NO_2$	147.13
20	3-Octanone	10.22	4.26	$C_8H_{16}O$	128.21
21	Styrene	10.32	0.19	C_8H_8	104.14
22	Trans-β-methyl-styrene-α,β-d(2)	10.57	0.07	$C_9H_8D_2$	120.19
23	Acetoin	10.83	0.17	$C_4H_8O_2$	88.11
24	Propanoic acid	11.09	0.14	$C_3H_6O_2$	74
25	Tetradecane	12.14	0.31	$C_{14}H_3O$	198.39
26	Acetic acid	13.19	0.05	$C_2H_4O_2$	61.04
27	Ethanethioic acid S-ethyl ester	13.26	0.08	C_4H_8OS	104.17
28	3-Furaldehyde	13.39	0.09	$C_5H_4O_2$	96.08
29	3-Methyl Pentadecane	14.46	0.04	$C_{16}H_{34}$	226.44
30	9-Oxabicyclo[6.1.0]non-2-ene	15.50	0.03	$C_8H_{12}O$	124.18
31	2-Phenylethanal	15.52	0.01	C_8H_8O	120.15
32	1-Cyano-5-benzoyloxy-β-D-ribofuranose	15.84	0.16	$C_{13}H_{13}NO_5$	263.25
33	Hexacosane	16.11	0.05	$C_{26}H_{54}$	366.71
34	1-Eicosanol	16.25	0.04	$C_{20}H_{42}O$	298.55
35	3-Methylthio propanol	16.46	0.02	$C_4H_{10}OS$	106.19
36	Chavicol	16.52	0.02	$C_9H_{10}O$	134.18
37	Methoxy-phenyl-Oxime	16.65	0.08	$C_8H_9NO_2$	151.16
38	Naphthalene	16.96	0.07	$C_{10}H_8$	128.18
39	Isophthalaldehyde	17.78	0.06	$C_8H_6O_2$	134.13
40	2,3-diethoxybutane	17.89	0.03	$C_8H_{18}O_2$	146.23
41	2-Methyl-Naphthalene	18.15	0.01	$C_{11}H_{10}$	142.2
42	Methylnaphthalene	18.24	0.02	$C_{11}H_{10}$	142.2
43	Phenylethyl Alcohol	18.68	0.63	$C_8H_{10}O$	122.18
44	2,5-Furandicarboxaldehyde	19.51	0.02	$C_6H_4O_3$	124.09
45	2-Methyl-butane	20.70	0.03	C_5H_{12}	72.15
46	3-Carbamoyl phthalide	21.13	0.01	$C_9H_7NO_3$	177.16
47	6 amyl α pyrone	21.51	0.65	$C_{10}H_{14}O_2$	166.22
48	1-(2,4-dimethylphenyl)-Ethanone	22.07	0.03	$C_{10}H_{12}O$	148.2
49	2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-Pyran-4-one	22.25	0.04	$C_6H_8O_4$	144.12
50	2-(4-fluoro-3-methoxycarbonylphenyl)-4-Nitrobenzoic acid	22.56	0.01	$C_{15}H_{10}FNO_6$	319.24
51	5-Hydroxymethylfurfural	24.68	0.13	$C_6H_6O_3$	126.11
52	Cis-3-acetoxy-4-ethoxycarbonylamino-1-thia-cyclopentane	25.69	0.02	$C_9H_{15}NO_4S$	233.28
53	1-Methoxy-2-ethoxycyclobutanone	26.12	0.06	$C_7H_{12}O_3$	144.17
54	4,4-Dimethyl-5-(1-methylene-2-phenyl-2-propenyl)-1,3-dioxolan-2-one	27.26	0.07	$C_{15}H_{16}O_3$	244.29
55	Methyl 1,3-dihydro-2H-isobenzofuran-4-carboxylate	27.54	0.13	$C_{10}H_{10}O_3$	178.19

3.2. Effect of 6-PP on Inhibition of F. oxysporum HF-26 Growth

The effects of different 6-PP concentrations on the growth of *F. oxysporum* HF-26 mycelia are shown in Figure 1. 6-PP inhibited *F. oxysporum* HF-26 mycelial growth (Figure 1A) and 25 mg/L 6-PP had the strongest inhibitory effect compared with other treatments, inhibiting mycelial growth by 65.88% (Figure 1B). However, 10 and 20 mg/L 6-PP did not affect *F. oxysporum* HF-26 mycelial growth. Additionally, 25 mg/L 6-PP may affect hyphae growth and formation, as indicated by the red circle in Figure 1A.

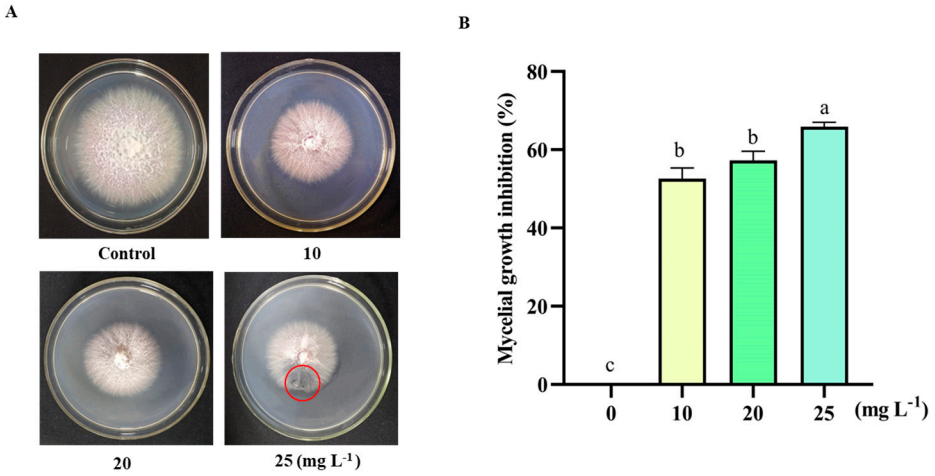

Figure 1. Effect of different concentrations of 6−pentyl−α−pyrone (6-PP) on the growth of *Fusarium. oxysporum* HF-26 mycelia (**A**). (**B**) Effect of different concentrations of 6-PP on the *F. oxysporum* mycelial growth inhibition rate. The red circle was the abnormal part of mycelium growth. The significant differences between genes were marked by letters above bars. Bars (means ± Standard deviation, n = 3) followed by the same lowercase letters are not significantly different at $p < 0.05$.

3.3. Effect of 6-PP on Pigments

Fusaric acid is a non-specific toxin secreted by *Fusarium*, which is an important pathogenic factor and related to plant wilt when *Fusarium* infects host plants [37] 6-PP at 25 mg/L severely affected the *F. oxysporum* HF-26 pigment synthesis. Therefore, the present study determined whether 6-PP affects fusaric acid synthesis (Figure 2A). The results showed that fusaric acid content considerably decreased in treated plates compared with that in the control (Figure 2B).

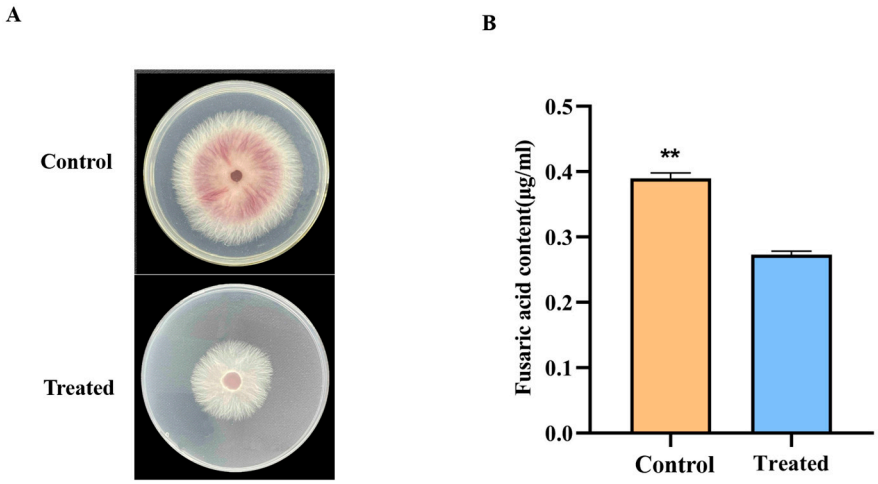

Figure 2. Effect of 25 mg/L 6-pentyl-α-pyrone on pigment (and fusaric acid synthesis (**A**,**B**) were effects of 6-pentyl-α-pyrone on pigment and fusaric synthesis respectively The asterisk represents the difference in the comparison of the two sets of data.

3.4. Relative Expression Levels of Fusarium Acid Synthesis and Transport Genes

The *FUBT* genes involved in toxin synthesis and transport in *Fusarium* belong to the major facilitator superfamily of genes [38]. We modified the code of the velvet protein family complex genes (*VelA*, *VeB*, and *VelC*) to control mycelial growth and hyphae formation in *F. oxysporum*. Deletion of this gene affects *F. oxysporum* growth, development, and invasive ability. Like *LaeA*, which regulate *F. oxysporum* growth, velvet protein family complex genes regulate the colonisation and establishment of *F. oxysporum* in tomato plant vessels and

plays an important role in the development of vascular wilt symptoms [39]. *FUB1*, *FUB4*, and *FUB10* toxin synthesis and transport genes were downregulated in 6-PP treatments compared with those in the control (Figure 3). The most significant downregulation was that of *FUB10*, which was downregulated 0.28-fold that of the control. Furthermore, to control the mycelial growth and hyphae formation of *F. oxysporum*, *VelA*, *velB*, and *LaeA* genes were downregulated, revealing that 25 mg/L 6-PP downregulates genes related to toxin synthesis and transportation, inhibits mycelial growth and hyphae formation, and may decrease fusaric acid synthesis (Figures 2 and 3).

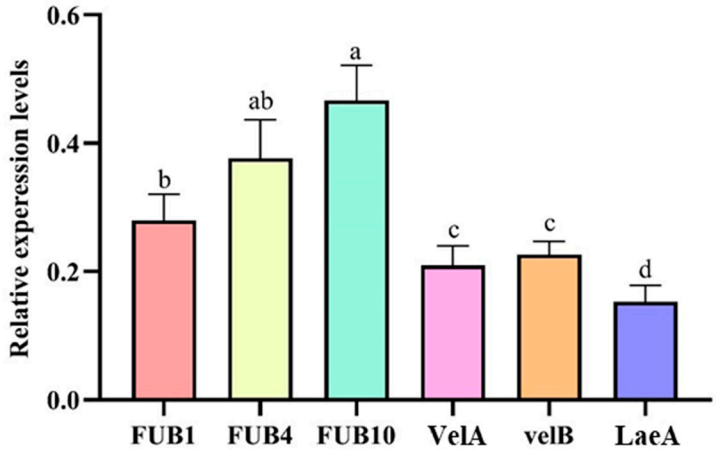

Figure 3. Effect of 6-pentyl-α-pyrone on inhibition of genes related to toxin synthesis and transportation and mycelial growth and hyphae formation. The relative expression of genes related to toxin synthesis and transportation and mycelial growth and hyphae formation were measured using real-time quantitative reverse transcription polymerase chain reaction. Letters above bars indicate the significant differences between genes. Bars (means ± Standard deviation, n = 3) followed by the same lowercase letters are not significantly different at $p < 0.05$.

3.5. Effect of the Nutrient Solution Containing 6-PP on the Control of Tomato FW in Soilless Culture

The effects of the nutrient solution containing 6-PP on *F. oxysporum* were evaluated under greenhouse conditions (Table 2, Figure 4). The present study demonstrated that a fungicidal nutrient solution containing 25 mg/L 6-PP markedly suppressed FW in tomatoes and the disease index was 27.23, which is lower than that of the standard nutrient solution (Figure 4C, Table 2). The fungicidal nutrient solution containing 25 mg/L 6-PP had 70.71% efficacy (Table 2), which is consistent with that of a reported liquid media adding carbendazim in a hydroponic system [4].

Table 2. Effect of soilless culture nutrient solution with 6-pentyl-α-pyrone (6-PP) on the control of Fusarium wilt in tomatoes.

Nutrient Solution	Concentration in Soilless Culture Nutrition	Disease Index (%)	Efficacy (%)
Containing 6-PP	25 mg/mL	27.23 ± 1.23 ^{b}	70.71 ± 1.31 ^{a}
Containing carbendazim	25 mg/mL	29.98 ± 1.04 ^{b}	70.96 ± 1.01 ^{a}
Common	——	92.98 ± 1.16 ^{a}	——

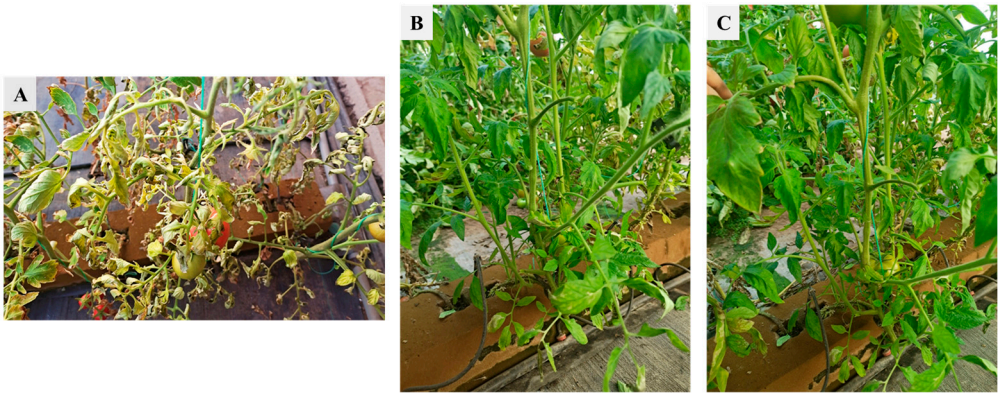

Figure 4. Effect of fungicidal nutrient solution containing 6-PP on the control of tomato *Fusarium* wilt. (**A**) was the control group using the common nutrient solution and then inoculated with the concentration at 1×10^7 spores/mL of spore suspension. (**B**,**C**) were groups treated with 6-PP and carbendazim, respectively.

3.6. Determination of Defence-Related Enzymes

Reactive oxygen species (ROS) are common secondary messengers in various cellular physiological and biochemical processes [40]. We determined the H_2O_2, CAT, POD, and SOD contents at 0, 12, 24, and 48 hpi in the leaves of tomatoes subjected to a fungicidal nutrient solution containing 6-PP, and the enzymes were expressed in various microorganisms. These enzymes play an important role in the removal of ROS and defence against pathogenic infections [41]. Results from the present study revealed that after treatment with a fungicidal nutrient solution containing 6-PP, the content of these defence-related enzymes increased at 24 hpi compared with those at other time points (Figure 5), indicating that 6-PP induced FW resistance in tomato plants.

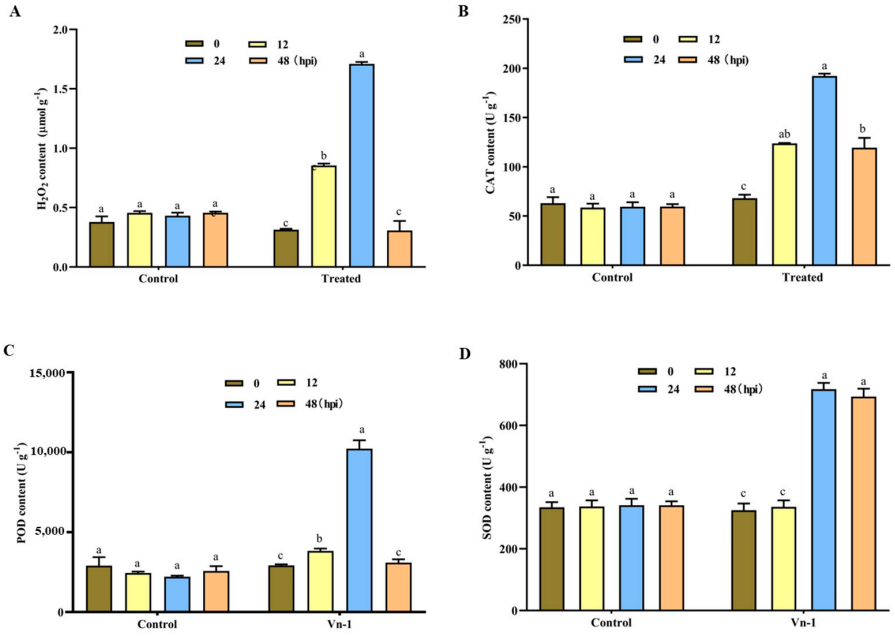

Figure 5. Effect of treatment with nutrient solution containing 6−pentyl−α−pyrone on H_2O_2 accumulation and the contents of defence-related enzymes in tomato leaves: (**A**) H_2O_2, (**B**) catalase, (**C**) peroxidase, and (**D**) superoxide dismutase activities. Letters above bars show the significant differences in different time points. Bars (means ± Standard deviation, n = 3) followed by the different letters are significantly different at $p < 0.05$.

3.7. Effect of Treatment with a Nutrient Solution Containing 6-PP on the Relative Expression of Defence-Related Genes

In the present study, we determined the relative expression of defence-related genes in tomato leaves. Pathogenesis-related proteins (PRs) defence genes were tested after treatment with a fungicidal nutrient solution containing 6-PP, and the relative expression levels of *PR1*, *NPR1*, *PR2*, and *PR5* were markedly upregulated at 24 hpi (Figure 6), suggesting that the expression of genes related to disease resistance was activated by 6-pp, resulting in improved disease resistance in tomatoes.

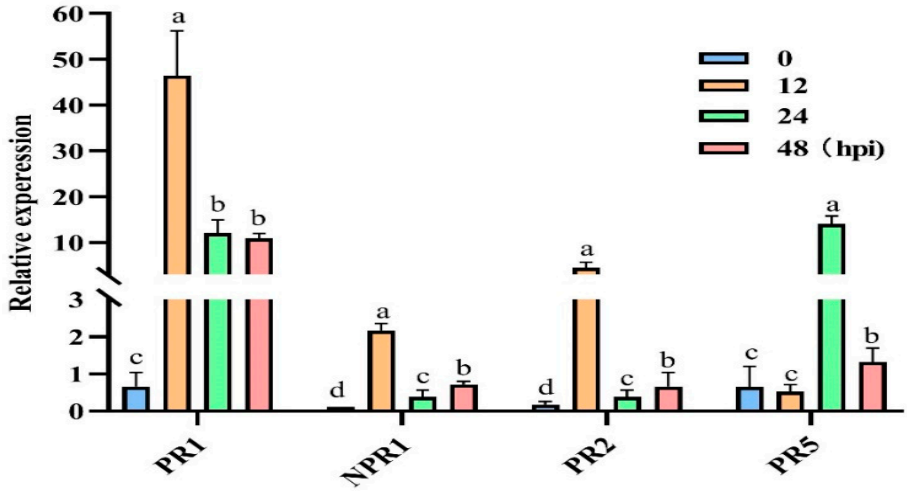

Figure 6. Relative expression of defence-related genes after treatment with a fungicidal nutrient solution containing the 6-pentyl-α-pyrone at different times in tomatoes. Bars with the same lowercase letters are not significantly different according to the least significant difference multiple comparisons at $p < 0.05$.

4. Discussion

Tomatoes (*Lycopersicum esculentum*), belonging to the Solanaceae family, are commercially cultivated globally under indoor and outdoor conditions [42]. In our previous study, the control efficiency of *T. asperellum* PT-15 against potato Verticillium wilt in the field was 61.97% [30]. In the present study, we investigated the types of VOCs in *T. asperellum* PT-15 using GC-MS. We identified 6-PP as the target substance to control FW in soilless tomato cultivation. We added 6-PP at an optimal concentration which could severely inhibit *F. oxysporum* in a soilless culture nutrient solution, and developed a fungicidal nutrient solution with 6-PP that control tomato FW in soilless culture.

6-PP is a key bioactive compound secreted by various *Trichoderma* species [22]. 6-PP has antibacterial activities is used in the biocontrol of various diseases, such as tobacco mosaic virus, black point disease of wheat, and late wilt of maize [43,44]. In the present study, 6-PP was identified in the VOCs from *T. asperellum* PT-15 (Table 1 and Figure S1), and its effect on *F. oxysporum* HF-26 mycelia growth was determined. The results indicated that 25 mg/L 6-PP has the strongest inhibitory effect on *F. oxysporum* HF-26 and could affect hyphae growth and formation (Figure 1). However, 10 and 20 mg/L 6-PP did not affect *F. oxysporum* HF-26 mycelial growth; but markedly affected the *F. oxysporum* HF-26 pigment synthesis and decreased fusaric acid synthesis (Figure 2). The *FUBT* gene involved in toxin transport in *F. oxysporum* belongs to the major facilitator superfamily transporter genes, is located downstream of the polyketo synthase gene, and involved in fusaric acid synthesis. *FUB4* and *FUB11* genes regulates fusaric acid synthesis and transport [45]. Additionally, the deletion of genes (*VelA*, *VeB*, and *VelC*) encoding the velvet protein family complex, which regulates *F. oxysporum* mycelium growth and hyphae formation affects its growth, development, and invasion ability. The *LaeA* gene regulates *F. oxysporum* growth and play an important role in catheter colonisation and vascular

wilt development in tomato plants [46]. *FUB1*, *FUB4*, and *FUB10* genes regulating toxin synthesis and transport were downregulated in 6-PP-treated plants compared with those in the control, and *FUB10* had the most significant downregulation (0.28-fold). Moreover, *VelA*, *velB*, and *LaeA* genes were downregulated, reducing *F. oxysporum* mycelial growth and hyphae formation, demonstrating that 6-pp has a strong inhibitory effect on *F. oxysporum*, consistent with the results described above.

In soilless cultivation systems prochloraz and carbendazim fungicides are added to the soilless culture nutrient solution to control tomato FW [4]. In the present study, 6-PP was added to a soilless culture nutrient solution owing to its inhibitory effect on *F. oxysporum* HF-26 growth and development. The results demonstrated that an antifungal–nutrient solution containing 6-PP suppresses FW with a 27.23 disease index, which was lower than that of the control. Furthermore, the antifungal–nutrient solution containing 6-PP used in the present study had 70.71% efficacy, consistent with that reported for antifungal–nutrient solutions containing prochloraz and carbendazim (Figure 4, Table 2). When plants are attacked by pests, fungi, and other adverse conditions ROS and defence-related enzymes are activated and plant proline-rich proteins are produced and accumulate in plant tissues improving plant resistance to pathogen infection and adapting to adverse conditions [47]. In the present study, treatment with an antifungal–nutrient solution containing 6-PP markedly increased the content of defence-related enzymes at 24 hpi compared with that at other time points. Moreover, 6-PP inhibited *Fusarium* growth and hyphae formation, and also activated *PR* genes and related defense enzymes in tomato (Figure 7). Therefore, 6-PP can be added to soilless culture nutrient solution to prevent and control tomato *Fusarium* wilt.

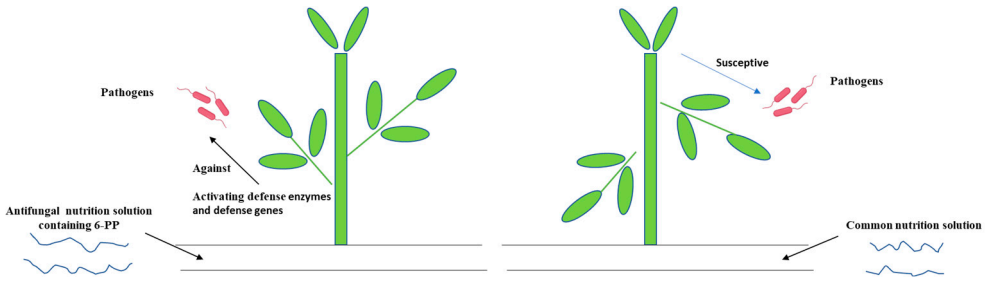

Figure 7. Mechanism of antifungal nutrition solution containing 6-pentyl-α-pyrone suppressing Fusarium wilt.

5. Conclusions

Soilless cultivation has been used throughout China. However, diseases such as FW and root rot limit the use of soilless cultures. Additionally, prolonged use of chemical pesticides can cause serious environmental pollution and toxicity to humans and animals. 6-PP has broad application prospects. We have developed and applied an innovative antifungal soilless culture nutrient solution containing 6-PP. This method has been applied to pepper, cucumber, eggplant, and in hydroponic systems, but not to tomatoes. The specific application sites were the Tongchuang Base and Modern Agricultural Garden in Hohhot, Inner Mongolia, China. We hope our antifungal–nutrient solution can be applied in other locations and contribute to the sustainable development of environmentally friendly soilless cultivation technologies.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy13051210/s1, Figure S1: Peaks of volatile substances detection of *Trichoderma asperellum* PT-15; Table S1: Primers used in the study.

Author Contributions: Conceptualization, J.H. and H.Z.; methodology, J.H., B.D. and D.W. (Dong Wang); software, D.W. (Dalai Wuyun) and X.X.; validation, D.W. (Dalai Wuyun) and X.X.; formal analysis, W.Q. and Z.Z.; investigation, W.Q.; data curation, J.H; writing—original draft preparation, J.H.; writing—review and editing, H.Z.; supervision, H.Z.; project administration, H.Z.; funding acquisition, H.Z. All authors have read and agreed to the published version of the manuscript.

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