Study on the inhibitory mechanism of fig leaf extract against postharvest Fusarium in melon

Jun Yao [1.2], Xin Li Geng [2*], He Yun Zheng [2], Zhi Wei Wang [2], Cui Huan Zhang [2], Jing Li[2],Zaituna Maimaiti [2],Yong Qin[1*]

1.College of Horticulture, Xinjiang Agricultural University, Urumqi Xinjiang,830000 ,china;

2. Xinjiang Uygur Autonomous Region Grapevine and Melon Fruit Research Institute, Shanshan Xinjiang, 838200, china

Corresponding Author:

Yong Qin1

College of Horticulture, Xinjiang Agricultural University, Urumqi Xinjiang,830000 ,china

Email address: xjndqinyong@sina.com.

Xin Li Geng 2

Xinjiang Uygur Autonomous Region Grapevine and Melon Fruit Research Institute,Shanshan Xinjiang, 838200, china

Email address:89069899@qq.com

Abstract

The objective of this study was to explore the bacteriostatic mechanism of fig leaf extract against Fusarium and to provide a theoretical basis for the development of new plant-derived fungicides. Methods: The bacteriostatic properties of fig leaf extract were analyzed by the ring of inhibition method. *Fusarium equiseti* was selected as the target for analyzing its bacteriostatic mechanism in terms of mycelial morphology, ultrastructure, cell membrane permeability, plasma membrane ~~plasma~~ peroxidation, reactive oxygen species (ROS) content and changes in the activity of protective enzymes. The effect of this extract was verified in melon, and its components were determined by metabolite analysis using ultraperformance liquid chromatography－mass spectrometry (UPLC－MS). Results: Fig leaf extract had an obvious inhibitory effect on Fusarium, and the difference was significant ($P<0.05$) or highly significant ($P<0.01$). Scanning

Comment [V1]: What species

Formatted: Font: Italic

Comment [V2]: Not clear

and transmission electron microscopy revealed that *F. equiseti* hyphae exhibited obvious folding, twisting and puckering phenomena, resulting in an increase in the cytoplasmic leakage of spores, interstitial plasma, and the concentration of the nucleus, which seriously damaged the integrity of the fungal cell membrane. This phenomenon was confirmed by propidium iodide (PI) and fluorescein diacetate (FAD) staining, cell membrane permeability and malondialdehyde (MDA) content. Fig leaf extract also induced the mycelium to produce excessive H2O2, which led to lipid peroxidation of the cell membrane, promoted the accumulation of MDA, accelerated protein hydrolysis, induced an increase in antioxidant enzyme activity, and disrupted the balance of ROS metabolism; these findings showed that fungal growth was inhibited, ~~which was verified in melons~~. A total of 1540 secondary metabolites were detected by broad-targeted metabolomics, among which the bacteriostatic active substances flavonoids (15.45%), phenolic acids (15%), and alkaloids (10.71%) accounted for a high percentage. Conclusion: Fig leaf extract has the potential to be developed into a plant-derived fungicide as a new means of postharvest pathogen prevention and control in melon.

Keywords: fig leaf extract, Fusarium , mechanism of inhibition, broadly targeted metabolomics,melon

Introduction

China is one of the largest producers of melon (*Cucumis melo*) in the world, with 387,100 hectares accounting for more than 45% of the global total planted area and 14,071,600 tonnes of production, or approximately 50%, according to the World System of Agricultural Organisations (FAO) Database (2022). Melon has attracted much attention as a specialty cash crop. However, melon subjected to long-distance transport and short-term storage will experience a 5-15% loss in the sales process and a 3-5% loss through wholesale, distribution and other intermediate links. Studies have shown that postharvest pathogenic fungal colonies of melons are diverse, ~~with~~ of - these Fusarium being the dominant fungal genus(Zheng et al., 2020). More than 15% of Brazilian melons are reportedly lost to Fusarium fruit rot during export ~~transport~~ (Nogueira et al.,2023). Fusarium is an important postharvest pathogen of fruits and vegetables ~~and can infect fruits and vegetables~~ such as blackthorn plum (Hye et al.,2016), watermelon (Balasubramaniam et al.,2023), winter pumpkin(Kitabayashi et al.,2023), banana (Kanhe et al.,2023), pineapple(Barral rt al.,2019), kiwifruit (Wang er al.,2023) and melon (Nogueira et al.,2023). In

Formatted: Font: Italic

Comment [V3]: Not clear check the sentence

Formatted: Font: Italic

addition to causing postharvest decay, *Fusarium oxysporum* metabolically produces various mycotoxins, such as deoxynivalenol (DON) (Ferreira et al.2018), zearalenone (ZEN)(Marin et al.,2004), fumonisin FB1 (FB1)(Juglal et al.,2002) and other mycotoxins, which are harmful to the safety of fruits and vegetables.

Chemical fungicides can effectively control a variety of postharvest diseases caused by Fusarium, but there are many problems, such as pollution of the environment, pesticide residues harmful to health and drug resistance. Therefore, ~~the researeh and~~and the present study was carried to develop~~ment of~~ new natural plant-derived bacteriostatic agents against Fusarium ~~has become an important direction for food preservation~~causing post harvest disease in melon. Several studies have reported that extracts of *Cunoniaceae* species(Fogliami et al.,2002), Morus alba and *Aegle marmelos* (Naseem et al.,2021), Mesquite(Lopez er al.,2021), bagasse(Hadimani et al.,2023), clove and thyme(Monteiro et al.,2013), and cumin seeds (Wang et al.,2017) have significant inhibitory effects on Fusarium spores. The use and residue of pesticides can be effectively reduced through the application of plant-derived extract preparations. Fig leaves are rich in a variety of functional components, such as flavonoids, polysaccharides, polyphenols and other compounds, which have antioxidant, anti-inflammatory, anticancer and antimicrobial effects(Mustafa,et al.,2019;Mahmoudi et al.,2016). Figs have low leaf utilization(Teruela et al.,2021), and fig leaf extract has high phenolic and antioxidant activities (Stintic et al.,2021). Using fig leaf extract, Ustun E(Ustun et al.,2022)and Arumugam J (Arumugam et al.,2021)synthesized nanoparticles with antioxidant, bacteriostatic and photocatalytic properties. The remarkable bacteriostatic properties of fig leaf extracts have attracted the attention of researchers. Currently, postharvest rot disease in melon caused by *F. oxysporum* is controlled by UV-C radiation (Daniel et al.,2021), heat treatment (Bokshi et al.,2007), bioinducer induction (Sanchez et al.,2009), and pulsed light (pulsed light)(Francisco et al.,2020); however, plant-derived bacteriostatic materials for the biological control of *F. oxysporum* in postharvest melon have rarely been reported. In this study, we used the bacteriostatic properties of fig leaf extract as a plant source material to control postharvest rot disease in melon caused by Fusarium, clarify the bacteriostatic mechanism of Fusarium, propose a new method for the green prevention and control of postharvest rot disease in fruits and vegetables and provide a theoretical basis for the development of a new type of plant source fungicide.

Formatted: Font: Italic

Formatted: Font: Italic

Formatted: Font: Italic

Comment [V4]: Not clear

Comment [V5]: Not clrar

Formatted: Font: Italic

Formatted: Font: Italic

Comment [V6]: ?

Comment [V7]: Not clear check the sentences

Materials & Methods

2.1 Materials

2.1.1 Materials for testing

Xinjiang Early Yellow fig leaves were harvested in November 2022 in Lukqin Town, Shanshan County, Xinjiang, after fruit ripening and harvesting.

Strains H-3, H-5, H-6, H-10, 1 and 2 were isolated ~~for~~ from infected melon storage fungal disease testing and sequenced to obtain the ITS sequence of the strains. After a BLAST search in GenBank to obtain the registration number of similar strains, the sequence of these isolates and the similarity of the reference sequence were 99-100% (as shown in Table 1).

Comment [V8]: Very poor sentences

2.1.2 Test instruments

An ultrasonic cell pulverizer, a rotary evaporator, an ultraclean bench, a fluorescence microscope, an autoclave, an ultraviolet spectrophotometer, a high-speed low-temperature centrifuge, and a constant-temperature shaker were used.

2.2 Experimental methods

2.2.1 Preparation of fig leaf extracts

The preparation method was performed according to the methods ~~of Zhao~~ (described by Zhao et al.,(2018), with slight adjustment. The fig leaves were washed after harvesting, dried naturally, ~~placed in a~~at 60° C in a drying oven, then crushed, and sieved through 80 mesh to obtain a dry powder. Fig leaf powder (5.0 g) was weighed into a 150 mL conical flask~~,~~ for that 100 mL 70% ethanol solution was added, and the powder was soaked at room temperature for 12 h. The mixture was put into the ultrasonic cell crusher for 30 min. The ethanol extracts of fig leaves were filtered with filter paper, the filtrate was put in a rotary evaporator at 70° C and distilled under reduced pressure to obtain the fig leaf concentrate master batch, which was sealed in a 4° C refrigerator for preservation.

2.2.2 Antifungal potential of fig leaf extracts

In the growth rate assay(Chen et al.,1990), the master leaf extract was mixed with potato dextrose agar (PDA) medium at a ratio of 5:1 to create a drug-carrying medium, sterile water was used as a blank control, and each treatment was repeated five times. The isolated and purified H-3, H-5, H-6, H-10, 1 and 2 strains were activated in PDA media at 28° C for 72 h, after which the bacteria were removed from the edge of the colony with a perforator to make a

Comment [V9]: How much per cent

Comment [V10]: Check it is bacteria or fungi

pellet with a diameter of 6 mm. The resulting pellet was subsequently transferred to the drug-loaded PDA Petri dishes and blank control dish and incubated at 28° C for 72 and 96 h. The diameter of the colonies was subsequently measured via the crossover method, after which the inhibition rate of the growth of mycelia was calculated. The relevant formulae were as follows:

Mycelial growth inhibition rate (%) = (mycelial growth diameter of control group - mycelial growth diameter of treatment group)/(mycelial growth diameter of control group - diameter of mycelial cake) × 100

2.2.3 Effect of fig leaf extract on *F. equiseti* cell morphology

In the ultraclean bench with 6 mm sterilized punch in the edge of the activated *F. equiseti* fungi, with a sterilized inoculation needle to pick up complete colonies, inverted to 100 mL potato liquid culture medium, placed in a constant temperature shaker at 28° C incubated for 24 h, and then combined with 20 mL of sterile water as a control or 20 mL of concentrated fig leaf extract as a treatment. The potato glucose liquid medium containing the fig leaf extract was incubated for 24 h, the organisms were collected and washed with phosphate-buffered saline (pH 7.2) 3 times, and the treated samples were put into 3% glutaraldehyde fixative and placed at 4° C for 12 h fixation. The samples were subsequently sent to Guangzhou Jingyan Testing Technology Service Co. for electron microscopic observation.

2.2.4 Effect of fig leaf extract on the cell membrane permeability of F. equiseti

2.2.4.1 PI and FAD staining

Propidium iodide (PI) was added to a solution at a concentration of 400 ~~μ~~μg/mL in PBS, and fluorescein diacetate (FAD) was dissolved in acetone and added to a solution at a concentration of 5 mg/mL. When staining, the spores of *F. equiseti* were made into a 1×106 spore suspension, which was added to the PI or FAD solution and then left in the dark at room temperature for 5 min for staining. Staining was observed and photographed under a fluorescence microscope.

2.2.4.2 Determination of extracellular conductivity

Mycelia of *F. equiseti* pathogenic bacteria samples cultured in a constant temperature shaker at 28° C for 24 h, were, under aseptic conditions, added to a 50 mL sterile ~~centrifuge~~ tube and~~,~~ centrifuged at 3000 g at low speed for 20 min, and then rinsed three times with sterilized deionized water to fully remove the medium, and then an equal amount of mycelia was

Comment [V11]: How you have cutted 6mm bacteria

Comment [V12]: Which drug ?

Formatted: Font: Italic

Formatted: Font: Italic

Comment [V13]: Very poor sentence formation

Formatted: Font: Italic

Formatted: Font: Italic

added to 100 mL sterile deionized water for incubation; subsequently, 1 mL of supernatant was taken from each bottle and centrifuged at 3000 × g for 5 min to measure the conductivity. The mother liquor of the fig leaf extract was subsequently added to potato glucose liquid medium at a concentration of 20%, after which the mixture was incubated at a constant temperature of 1200 r/min at 28° C in a constant temperature shaker. At 0, 0.5, 1.5, 2.5, 3.5, 4.5, 5.5, 6.5, 7.5 and 8.5 h, 1 mL of supernatant was collected and centrifuged at 3000 ×g for 5 min to measure the conductivity of the supernatant. Afterward, the sample was replenished with sterile deionized water, and each test was repeated three times.

2.2.4.3 Determination of enzyme activity

Samples of *F. equiseti* pathogenic bacteria that were cultured ~~in a constant temperature shaker~~ at 28° C for 48 h were taken, fig leaf extract was added to potato dextrose liquid medium on an ultraclean bench, fig leaf extract was filtered through a sterile 0.20 mm microporous membrane, and an equal amount of sterile deionized water was added to the negative control group. The mycelia of the *F. equiseti* pathogen were removed at 0, 3, 6, 12 and 24 h and washed three times with PBS. The samples were subsequently filtered with gauze, and the water was removed. The Samples were frozen in liquid nitrogen and stored at -80° C.

To determine the MDA content, 0.1 g of mycelia was weighed, 1 mL of extraction solution was added, the homogenate was fully ground with a mortar and pestle ~~at low temperature~~, and the mixture was centrifuged at 10000 r/min and 4° C ~~at low temperature~~ for µ10 min. Afterward, 225 µL of the supernatant was collected to measure the MDA content in the mycelia according to the instructions of the kit.

The standard curve was y=0.0391x-0.0076, R2=0.9997, x is the concentration of the standard (nmol/mL), and y is the ΔA. MDA content (nmol/g)=(ΔA+0.0076)÷0.0391×Vti÷W

Determination of protein content: First, 0.1 g of mycelia was weighed, added to 1 mL of distilled water, fully ground and homogenized with a mortar and pestle ~~at low temperature. Afterward, the mixture was~~and centrifuged at 12000 r/min at 4° C at a low temperature for µ10 min. Afterward, 500 µL of the supernatant was taken, and a blank control tube was used to adjust the temperature to zero. The content of the proteins in the mycelia was measured in accordance with the instructions of the kit.

Comment [V14]: ?

Comment [V15]: Very poor sentences

Formatted: Font: Italic

Formatted: Font: Italic

Comment [V16]: Check your conducting expt on fungi not bacteria Check these sentences

Comment [V17]: ?

Comment [V18]: ?

For the standard curve, y=14.253x-0.0007, R2=0.9997, x is the concentration of the standard (mg/mL), and y is the ΔA. Cpr (mg/g fresh weight)=0.07×(ΔA+0.0007)÷W

Determination of hydrogen peroxide content: Mycelia (0.15 g) were weighed and combined with 1.5 mL of extraction solution, fully ground with a mortar and pestle ~~at low temperature~~ until a white powder was formed. The mixture was transferred to an EP tube, 1.5 mL of the extract was centrifuged in an ultralow temperature centrifuge at 10,000 r/min at 4° C for 10 min, and then 1,000 μμL of the supernatant was collected for H2O2 content determination in the mycelium according to the instruction manual of the kit.

Comment [V19]: Very poor sentences

The standard curve was y=0.6674x-0.0155, R2=0.9997, x is the concentration of the standard (μμmol/mL), and y is the ΔA. H_2O_2content (μμmol/mL)=(ΔA+0.0155)÷0.6674×Vti÷W

2.2.4.4 Determination of protective enzyme activity

Superoxide dismutase (SOD) activity: Mycelia (0.1 g) were added to 1 mL of extraction solution, a mortar and pestle was used to fully grind the mixture to white powder, and the homogenate was centrifuged ~~in an ultralow temperature centrifuge~~ at 12000 r/min and 4° C for 10 min. A total of 50 mL of supernatant was collected and processed according to the instructions of the kit for microadjustment to measure SOD activity.

Peroxidase (POD) activity: Mycelia (0.1 g) were weighed, 1 mL of extraction solution was added, the mixture was ground to a white powder with a mortar and pestle at low temperature, and the mixture was centrifuged at 12000 ~~r~~rpm/min and 4° C for 10 min. Afterward, 50 mL of the supernatant was collected to measure the POD activity according to the manufacturer instructions.

POD content (U/g fresh weight) = ΔA×V1÷(W×V2÷V3)÷1÷T

Catalase (CAT) activity: Mycelia (0.1 g) was combined with 1 mL of extraction solution, fully ground with a mortar and pestle at low temperature until a white powder formed, and centrifuged in an ultralow temperature centrifuge at 12000 r/min and 4° C for 10 min. Supernatant (35 mL) was subjected to CAT activity determination according to the manufacturer instructions.

CAT content (μμmol/min/g fresh weight)=[ΔA×Total V counter ÷ (Ɛ×d)×106]÷(W×V sample÷Total V sample)÷T=678×ΔA÷W

2.2.5 Effect of fig leaf extract on the lesion diameter of damaged inoculated fruit

The spore suspension was prepared according to the methods ~~of Bi Yang (~~described by Bi et al.,2006). The PDA medium containing Fusarium was cultured at 28 ° C for 7 d. Approximately 10 mL of sterile water containing 0.05% Tween 20 was added by volume, and the spores of Fusarium on the PDA medium were scraped off with a glass rod transferred into 50 mL triangular flasks, oscillated on a vortex mixer for 15 s, and subsequently filtered through double-layered gauze. The filtrate was counted on a hematology counting plate to calculate the concentration of the spore suspension, which was subsequently diluted to 1×10^6 spores/mL with sterile water to dilute to 1×10^6 spores/mL.

Fruit damage was induced via inoculation according to the methods of described by ~~Bi Yang (~~Bi et al.,2006). The fruits were first rinsed with tap water, soaked in 2% sodium hypochlorite solution by volume for 1 min, rinsed with tap water and dried at room temperature (22±2° C). After surface disinfection of the fruit inoculation site with 70% ethanol solution by volume, five holes (3 mm deep) were punched uniformly in the equatorial part of the fruit with a sterilized hole punch (6 mm in diameter). The holes were inoculated with 20 μμL of the above spore suspension, inoculated with sterile water as the control group, dried and stored at room temperature. The diameter of the spots was measured at 3, 6 and 9 days after inoculation. Three fruits were used for each treatment at each time point, and the results were replicated three times.

2.2.6 LC−MS detection of secondary metabolites in fig leaf extracts

A total of 100 μμL of the fig leaf extract master batch was added to a 2.0 mL centrifuge tube, 100 μμL of 70% methanol internal standard extract was added, and the mixture was vortexed for 15 min and centrifuged at 12000 r/min for 3 min at 4° C. The supernatant was filtered with a microporous filter membrane (0.22 μm) and stored in an injection bottle for LC−MS detection, and the samples were subsequently sent to Wuhan Mavi Metabolic Technology Service Co.

Data processing

The measured values of each indicator were statistically analyzed and plotted using Origin 8.5. The data were subjected to analysis of variance (ANOVA) using SPSS 17.0 data processing software, and the significance of the differences was analyzed using Duncan's multiple comparisons ($P < 0.05$ indicates significant differences).

Results

Formatted: Superscript

Formatted: Superscript

Comment [V20]: ?

Comment [V21]: Very poor sentences

Comment [V22]: ?

Comment [V23]: ?

3.1 Effect of fig leaf extracts on colony diameter

The fig leaf extract showed different inhibitory effects on all postharvest fungal diseases of melon plants. As shown in Figure 1, fig leaf extract had more obvious inhibitory effects on H-3, H-5, H-6, and No. 1 than on other fungal strains, for which the inhibition rates were 46.80%, 42.88%, 45.31%, and 42.34%, respectively. The difference reached a highly significant level ($P<0.01$), whereas the inhibitory effects on H-10 and 2 fungi ~~were inhibited, but the difference~~ was not significant.

3.2 Effect of fig leaf extract on the morphology of *F. equiseti* cells

Scanning electron microscopy revealed that the surface of the mycelia of **F. equiseti** in the control group had fine folds, the mycelia were cylindrical and had a smooth surface and uniform thickness, and the tip of the mycelia was a full semicircle (Fig. 2A, B); however, after 24 h of treatment with fig leaf extract, the surface of the mycelia appeared to have more folds, exhibiting a notable puckering phenomenon accompanied by a small amount of leakage of cytoplasmic substances (Fig. 2C, D). Transmission electron microscopy revealed that in the control group, the cell wall and cell membrane of the fungal hyphae ~~were~~ was clear and structurally intact, the interstitium was uniformly thin and clear, the intracellular cytoplasm was structurally intact, and the nuclei were rounded in the center of the cell, with vacuoles of varying sizes in the vicinity (Fig. 2E, F, and G); however, in the control group, after treatment with fig leaf extract for 24 h (Figs. 2 H, I, and J), the cell membrane was not smooth, and the interstitium was not smooth, exhibiting a small amount of cytoplasmic leakage (Fig. 2C, and D). The cell membrane edges were not smooth, the intercellular plasm was enlarged, the surface of the cell wall was notably rough, leakage of intracellular cytoplasm occurred, the nucleoplasm was condensed, the nucleus was fragmented, and apoptotic vesicles were formed, among other morphological changes.

Therefore, the results of scanning electron microscopy tests revealed that fig leaf extract can increase the extravasation of substances within the cell membrane, resulting in the collapse of the mycelial cell wall and cell membrane due to the leakage of large amounts of cytoplasmic debris, after which the mycelia undergo puckering and nonuniform swelling.

3.3 PI and FAD staining to determine the effect of fig leaf extracts on F. equiseti cell membrane integrity

PI is a nuclear staining reagent that stains DNA and can be used to verify that cell membranes are not damaged; if red fluorescence is observed under a fluorescence microscope

Comment [V24]: At what per cent

Formatted: Font: Italic

Formatted: Font: Bold

Comment [V25]: % age

Comment [V26]: expand

after staining, the plasma membrane is not intact. Similarly, FAD staining can be used to verify whether the cell membrane is intact; if green fluorescence is observed under a fluorescence microscope, the cytoplasmic membrane is intact. As shown in Fig. 3, the number of spores in the control treatment was significantly greater than that in the fig leaf extract treatment (Fig. 3A, D), the number of spores with red fluorescence in the control treatment was significantly lower than that in the fig leaf extract treatment after PI staining (Fig. 3B, E), and the number of spores with green fluorescence in the control treatment was significantly greater than that in the fig leaf extract treatment after FAD staining (Fig. 3C, F); these findings indicate that fig leaf extract treatment damaged the integrity of the F. equiseti cell membrane.

3.4 Effect of fig leaf extract on *F. equiseti* mycelial cell membrane permeability and MDA content

As shown in Figure 4, the changes in cell membrane permeability and MDA content of F. *equiseti* mycelia in both the control and treatment groups showed a gradual increase. The cell membrane permeability increased more quickly in the treated group than in the control group, and there was a transient and rapid increase in the cell membrane permeability in the control group from 23.33±±2.63 μμs/cm to 36.50±1.46 μμs/cm in 0-1.5 h. This difference may be attributed to the fact that after the addition of deionized water to the mycelia of the pathogenic fungus *F. equiseti*, some of the intracellular ions of the fungus were exuded into the solution from the cell due to the change in osmotic pressure. Some of the fungal intracellular ions leaked from the cell into the solution due to the change in osmotic pressure, and the extracellular conductivity generally maintained an equilibrium state from 2.5 to 8.5 h, which was 39.57±0.52 μμs/cm at 8.5 h. In contrast, the cell membrane permeability of the treated group increased rapidly from 24.33±6.33 μμs/cm to 47.33±0.89 μs/cm in the period from 0 h to 2.5 h and then increased at a more gentle rate in the period of 3.5-8.5 h, from 48.07±1.5 μs/cm to 47.33±0.89 μs/cm and from 48.07 ± 1.51 μs/cm to 50.07 ± 1.04 μs/cm, and the differences were all significant ($P<0.05$) after 0.5 h. The MDA content also increased faster in the treated group than in the control group, with the MDA content in the control group increasing slowly from 2.84 ± 0.28 mmol/g to 4.08±0.29 mmol/g; however, in the treated group, the MDA content increased rapidly from 2.89 ± 0.32 mmol/g to 6.22 ± 0.65 mmol/g. The difference reached a highly significant level ($P<0.01$). The increase in MDA content after 24 h was 43.41% and 115.04% in

Formatted: Font: Italic

Formatted: Font: Italic

Comment [V27]: ?

Comment [V28]: ?

Formatted: Font: Italic

Comment [V29]: ?

Comment [V30]: ?

Comment [V31]: ?

Comment [V32]: mM or mmol check

Comment [V33]:

Comment [V34]:

the control and treated groups, respectively. Taken together, these findings indicate that the cell membrane of *F. equiseti* mycelia was disrupted by treatment with fig leaf extract, which resulted in spillage of intracellular fluids, increased conductivity in the extracellular fluid, and a significant increase in MDA content, which indicated lipid peroxidation in the cell membrane of the fungal tissues.

3.5 Effect of fig leaf extract on the protein content of *F. equiseti* mycelia

As shown in Fig. 5, the protein content in the mycelia of the control and treatment groups exhibited opposite trends, with the protein content of the control group showing a gradual increase, where as ~~and the~~ protein content of the treatment group showing a gradual decrease. The protein content in the mycelia of the control group increased from 0.283±0.009 mg/mL to 0.316±0.008 mg/mL, while the protein content in the mycelia of the treated group decreased from 0.281±0.006 mg/mL to 0.264±0.004 mg/mL, exhibiting a significant difference ($P<0.05$) after 6 h of treatment. These findings indicate that fig leaf extract has a significant inhibitory effect on the soluble protein content in *F. equiseti* mycelia.

3.6 Effect of fig leaf extract on reactive oxygen species (ROS) levels and antioxidant enzyme activities in F. *equiseti* mycelia

$H_2O_2$is an important reactive oxygen species, and its content can reflect changes in ROS in mycelia. As shown in Figure 6A, the changes in H_2O_2 concentration in the mycelia of the control and treatment groups were similar and showed a gradual increase. The H_2O_2 concentration in the control group slowly increased from 4.24±0.30 mmol/mg prot to 5.25±0.32 mmol/mg prot, whereas that in the treatment group increased from 4.75±0.17 mmol/mg prot to 7.84±0.08 mmol/mg prot, and the H_2O_2 concentration in the control group and the treatment group increased from 4.75±0.17 to 7.84±0.08 mmol/mg prot by 24 h. The rates of increase in H_2O_2 content were 23.99% and 65.27% in the control and treated groups, respectively. The rate of increase in H_2O_2 content in the treated group was greater than that in the control group, and the differences reached highly significant levels ($P<0.01$) after 3 h of treatment. These findings suggested that fig leaf extract can induce excessive production of H_2O_2 in the mycelia of *F. equiseti*, thus disrupting the normal growth of Fusarium.

CAT, POD and SOD are important antioxidant enzymes in fungi that can scavenge oxygen radicals in the body. The activities of these three enzymes exhibited similar trends, all of which

Formatted: Font: Italic

Formatted: Font: Italic

Formatted: Font: Italic

Formatted: Font: Italic

Comment [V35]:

Comment [V36]:

Comment [V37]:

Comment [V38]:

Comment [V39]:

Formatted: Font: Italic

tended to increase first and then decrease. The SOD activity in the treated group increased rapidly from 2.80±0.29 U/g prot to 3.88±0.30 U/g prot within 0-3 h and then decreased slowly to 2.48±0.09 U/g prot within 24 h. The difference in SOD activity between the control group and the treated group was significant ($P<0.05$) at 3 h, and the SOD activity increased by 38.60% (Figure 6B). Similarly, the POD activity in the treated group increased rapidly from 0.667± 0.058 U/g prot to 2.8±0.2 U/g prot from 0-3 h. At 3 h, the difference between the control and treated groups was significant ($P<0.05$), and the POD activity increased by 320% (Fig. 6C). CAT activity in the treated group increased rapidly from 0.798±0.058 U/g prot to 1.328±0.12 U/g prot from 0-3 h, followed by a slow decrease to 0.945±0.165 U/g prot at 24 h. At 3 h, the difference between the treated and control groups was significant ($P<0.05$), with an increase in CAT activity of 66.41% (Fig. 6D).

3.7 Effect of fig leaf extract on the lesion diameter of damaged inoculated fruit

As shown in Figure 7, the effects of *F. equiseti* inoculation and suppression by fig leaf extract were obvious for two melon varieties, Xizhou Mi 17 and Huang Meng Crisp; the differences between the control group and the treatment group was significant ($P<0.05$) at 6 and 3 days of inoculation, respectively; and the suppression rates reached 29.77% and 8.82%, respectively. With the prolongation of treatment, the effect of fig leaf extract did not weaken, the differences between the control group and the treatment group reached significant levels ($P<0.05$) at 9 days and 6 days after inoculation for Xizhou Mi 17 and Huangmeng Crisp, respectively, and the inhibition rates reached 21.62% and 10.91%, respectively. These findings indicate that fig leaf extract is effective at inhibiting F. oxysporum in postharvest melon and has a long duration of action.

Formatted: Font: Italic

3.8 Compositional analysis of fig leaf extracts

Using UPLC−MS wide-target metabolomics technology, a total of 1540 metabolites were detected and analyzed in fig leaf extracts, of which amino acids and derivatives accounted for 16.04%; flavonoids, 15.45%; phenolic acids, 15%; others, 12.14%; alkaloids, 10.71%; organic acids, 7.73%; lipids, 7.47%; lignin and coumarin, 5.91%; nucleotides and derivatives, 4.61%; terpenoids, 3.18%; quinine, 1.43%; and tannins, 0.32% (Fig. 8). Flavonoids, phenolics, and alkaloids, which exhibit fungicidal activity, were present at relatively high percentages.

Discussion

Plant-derived natural products mainly refer to the secondary metabolites of plants; these metabolites are widely available in nature, are extremely diverse, and include many types of effective antibacterial agents. In the present study, the secondary metabolites in fig leaf extracts were analyzed via UPLC−MS through a broad-targeted metabolomics technique, and it was found that there was a high percentage of bacteriostatically active compounds, such as flavonoids (15.45%), phenolic acids (15%), and alkaloids (10.71%) were observed. This finding is in agreement with the results of ~~SU(~~Su et al.,(2023)on the major bioactive constituents of fig leaves. It has been shown that the flavonoid extracts 2,5-dicyclopentenyl cyclopentanone from marigold ~~can~~ inhibit the growth of watermelon wilt fungus (Du et al.,2017). Flavonoids from bitter ginseng have inhibitory effects on bacteria and fungi (Hadadi et al.,2020). Moreover, flavonoids, ~~as~~ are the important medicinal components in fig leaves, have unique pharmacological effects and a wide range of applications in the fields of food, medicine, and wellness products, which is consistent with the aim of this study.

Comment [V40]: ?

The cell wall and cell membrane play crucial roles in maintaining the morphology of fungi, and plant-derived flavonoids can destroy the integrity and permeability of the cell wall membrane, thus disrupting the cellular morphology of microorganisms (Cushnie et al.,2011). In the present study, significant changes in the morphology and ultrastructure of Fusarium after treatment with fig leaf extract were observed via electron microscopy, which showed leakage of cell contents, crumpling and even collapse of hyphae, and disruption of the integrity of the cell membrane, which led to an increase in the conductivity of the extracellular fluid. PI-FAD double staining also confirmed the disruption of Fusarium cell membrane integrity and a decrease in spore activity. ~~WANG~~(Wang et al.,2017) reported that *Fusarium rotundum* mycelia were severely curved, wrinkled and collapsed after treatment with carvacrol and eugenol, and PI staining revealed that the treatment disrupted the integrity and permeability of the *F. rotundum* cell membranes, which was in agreement with the results of the present study. Thus, fig leaf extract inhibited fungal growth by disrupting Fusarium cell membrane permeability.

Formatted: Font: Italic

Formatted: Font: Italic

Proteins, as the basic material basis of life activities, play an important physiological role in cells, and a reduction in protein content affects the normal physiological function of cells(Yin et al.,2020). Moreover, protein leakage is one of the sign~~s~~ ~~that~~ for the integrity of the cell membrane is disrupted (Baijpai et al.,2013). In this study, the protein content in the mycelia of the control and treatment groups showed opposite trends, with a gradual decrease in the protein

content in the mycelia of the treatment group, which demonstrated that the synthesis of fungal proteins was inhibited. LIU (Liu er al.,2018)demonstrated that an aqueous solution of total ginseng stem and leaf saponins at 10 mg/mL inhibited the synthesis of *Fusarium rotundifolium* proteins, which led to an inhibition of mycelial growth. Therefore, fig leaf extract can achieve bacterial inhibition by inhibiting mycelial protein synthesis.

Formatted: Font: Italic

Membrane peroxidation and oxygen radical reactions maintain a dynamic equilibrium state in fungi to maintain the normal metabolic processes of fungal cells, and once this equilibrium is disturbed, the cell membrane of fungi can be damaged, resulting in cellular injury(Yan et al.2015). Many studies have shown that membrane peroxidation is one of the main reasons for the increase in cell membrane permeability(Chowhan et al.2013;Duma et al.,2012). MDA is an important metabolite in the process of biomembrane peroxidation, and its content in mycelia can reflect the degree of damage to tissue membranes (Guo,et al.,2017). Additionally, it is an important indicator of the degree of lipid peroxidation in fungal cell membranes after drug treatment(Wang et al.,2009). In the present study, the content of MDA in Fusarium mycelium increased significantly after treatment with fig leaf extract, and at the same time, it induced the production of excessive H2O2 in the mycelia, thus disrupting the normal growth of Fusarium.

SOD, CAT and POD are important antioxidant enzymes in fungi that can scavenge oxygen radicals in the body, and changes in the activities of these three enzymes can be used to reflect the process of membranous peroxidation in fungal mycelia(Qu et al.,2017). In this study, the activities of these three protective enzymes tended to increase and then decrease, indicating that in the early stage of fig leaf extract treatment, the defense system of fungal tissues produces many protective enzymes to resist the oxidative damage to tissues caused by excessive ROS; however, as the fig leaf extract treatment concentration increased, the activities of the three enzymes decreased due to the intensification of fungal tissue damage.

Conclusions

Fig leaf extract treatment disrupted the integrity of the Fusarium cell membrane, increased conductivity, disrupted protein synthesis, increased the leakage of cell contents and promoted the accumulation of MDA, which exacerbated the degree of cell membrane damage, induced changes in antioxidant enzyme activities in the organism, and disrupted the balance of reactive oxygen species metabolism. These changes led to the inhibition of fungal growth. The authenticity and reliability of the inhibitory effect of fig leaf extract on Fusarium was confirmed

426 by the damage inoculation test of Fusarium spores. Therefore, this study revealed the inhibitory
427 effect of fig leaf extract on Fusarium and the underlying mechanism and provides a theoretical
428 basis for the use of fig leaf extract as a natural plant-derived fungicide.
429
430 ## References

431 Zheng H Y,Gen X L,Yao j,Zhang C H,Liao X F.2020.Pathogen Identification and Their
432 Diversity of Melon Postharvest Diseases in Xinjiang.Southwest China Journal of Agricultural
433 Sciences. 33(4):788-796.
434 Nogueira G de A,Conrado V S C,Freires A L de A,Souza J J F,Figueiredo F R A,Barroso K
435 A,Araujo M B M,Nascimento L V,Lima J S S,Neto F B,Silva W L,Ambrosio M de Q.
436 2023.Aggressivity of Different Fusarium Species Causing Fruit Rot in Melons in Brazil.Plant
437 Disease 107(3):886-892.
438 Hye W L, Thi T T N, Hyang B L.2016.First Report of Post-Harvest Fruit Rot of Aronia
439 melanocarpa Caused by *Fusarium tricinctum* in Korea.The Korean Journal of Mycology 44:68-
440 71.
441 Balasubramaniam J,Goh K S,Sani S F,Alam M W,Ismail N A,Gleason M,Rosli H .
442 2023.*Fusarium falciforme* and *F. oxysporum* causing postharvest fruit rot of watermelon
443 (Citrullus lanatus) in Malaysia: A first report.Crop Protection 163:106115.
444 Kitabayashi S,Kawaguchi A,Yoshida M,Kami D,Sugiyama K,Kawakami A. 2023.First report of
445 Fusarium ipomoeae and F. citri causing postharvest fruit rot of winter squash (Cucurbita
446 maxima).Journal of general plantpathology 89(1):61-66.
447 Kanhe N R, Abhilasha A L, Girish P. 2019. Eco-friendly management of post harvest rot of
448 Banana (Musa paradisiaca L.).Annals of Plant Protection Sciences 27(3):363-366.
449 Barral B,Chillet M,Lechaudel M,Logan R,Schorr G S.2019.Coumaroyl-isocitric and caffeoyl-
450 isocitric acids as markers of pineapple fruitlet core rot disease.Fruits 74(1):11-17.
451 Wang W Z, He L N, Zhang Z Z,Li W Z,Chen J,Chen T T,Long Y H.2023.Activity of the
452 botanical compound thymol against kiwifruit rot caused by *Fusarium tricinctum* and the
453 underlying mechanisms.Pest Management Science 79(7):2493-2502.
454 Ferreira F M D, Hirooka E Y, Ferreira F D.2018Effect of Zingiber officinale Roscoe essential
455 oil in fungus controland deoxynivalenol production of *Fusarium graminearum* Schwabe in vitro.
456 Food Additives & Contaminants:Part A 35:2168-2174.

Formatted: Font: Italic
Formatted: Font: Italic
Formatted: Font: Italic
Formatted: Font: Italic
Formatted: Font: Italic

Marin S,Velluti A,Ramos A J,Sanchis V.2004.Effect of essential oils on zearalenone and deoxynivalenol production by *Fusarium graminearum* in non-sterilized maize grain. Food Microbiology 21(3):313-318.

Juglal S,Govinden R,Odhav B.2002.Spice oils for the control of co-occurring mycotoxin-producing fungi.Journal of Food Protection 65:683-687.

Fogliani B, Bouraïma-Madjebi S, Medevielle V. 2002.Screening of 50 Cunoniaceae species from New Caledonia for antimicrobial properties. New Zealand Journal of botany 40:511-520.

Naseem H, Hina A, Tehmina A,Waheed A.2021.Phyto-Synthesized Silver Nanoparticles using Leaves Extracts of Morus alba and *Aegle marmelos* Inhibited Fusarium Wilt and Charcoal Rot in Tomato.International Journal of Agriculture and Biology 25(1):165-172.

Lopez-Anchondo A N, Lopez-dela C D, Gutierrez-Reyes E,Castaneda-Ramirez J C,De La Fuente-Salcido N M.2021.Antifungal Activity In Vitro and In Vivo of Mesquite Extract (*Prosopis glandulosa*) Against Phytopathogenic Fungi. Indian Journal of Microbiology 61(1):85-90.

Hadimani S,Supriya D,Roopa K,Soujanya S K,Rakshata V,Netravati A,Akshayakumar V,De B S,Jogaiah S.2023.Biodegradable hybrid biopolymer film based on carboxy methyl cellulose and selenium nanoparticles with antifungal properties to enhance grapes shelf life.International Journal of Biological Macromolecules: Structure, Function and Interactions 237:0141-8130.

Monteiro F P, Ferreira L C, Silva J L. 2013.Influence of Plant Extracts and Essential Oils against Panama Disease (*Fusarium oxysporum* f. sp. cubense) in Banana Seedlings. Journal of Agricultural Science 5:63-74.

Wang Y,Zhang J,Sun Y,Feng J T,Zhang X.2017.Evaluating the Potential Value of Natural Product Cuminic Acid against Plant Pathogenic Fungi in Cucumber. Molecules 22(11):1-10.

Mustafa E, Merve E,Nuraniye E,Mehnet A,Esra U.2019.In vitro evaluation of the chemical composition and various biological activities of ficus carica leaf extracts. Turkish journal of pharmaceutical sciences 16(4):401-409.

Mahmoudi S,Khali M,Benkhaled K,Benamirouche K,Baiti I.2016.Phenolic and Flavonoid Contents, Antioxidant and Antimicrobial Activities of Leaf Extracts from Ten Algerian Ficus carica L.Varieties.Asian Pac J Trop Biomed 6(3):239-245.

Formatted: Font: Italic

Formatted: Font: Italic

Formatted: Font: Italic

Formatted: Font: Italic

Teruela C, Andreu C L, Lopez L D,Sendra E,Hernandez F,Cano L M.2021. Ficus carica fruits, by-products and based products as potential sources of bioactive compounds:A review. Agronomy 11(9):2073-4395.

F Stinic, C G Constantin, A C Butcaru,A C Asnic,C A Mihai.2021.Biochemical assessment of some extracts derived from leaves and fruit of Ficus carica L.Acta Horticulturae 1310:123-128.

Ustun E, Onbas S C, Celik S K.2022.Green Synthesis of Iron Oxide Nanoparticles by Using Ficus Carica Leaf Extract and Its Antioxidant Activity .Biointerface Research in Applied Chemistry 12(2):2108-2116.

Arumugam J , Thambidurai S , Suresh S,Kandasamy M,Pugazhenthiran N,Kumar S K,Muneeswaran T,Quero F.2021.Green synthesis of zinc oxide nanoparticles using Ficus carica leaf extract and their bactericidal and photocatalytic performance evaluation. Chemical Physics Letters 783:139040.

Daniel T, Katia L N, Rosa T S F.2021.Control of Fusarium rot in Galia-melon and preservation of fruit quality with UV-C radiation and hot water treatments.Tropical Plant Pathology 46:350-359.

Bokshi A, Morris S C, McConchie R.2007.Environmentally-safe control of postharvest diseases of melons (Cucumis melo) by integrating heat treatment, safe chemicals, and systemic acquired resistance.New Zealand Journal of Crop and Horticultural Science 35(2):179-186.

Sanchez E A, Tiznado H M E, Ojeda C A J,Valenzuela Q A I,Troncoso R R. 2009. Induction of Enzymes and Phenolic Compounds Related to the Natural Defence Response of Netted Melon Fruit by a Bio-elicitor.Journal of Phytopathology 157(1):24-32.

Francisco O F, Ebenézer de O S , Mônica Maria de A L,Paulo R V R,Andreia H O,Jhonyson A C G,Davila S Z,Patricia N B,Guilherme J Z.2020.Effect of pulsed light on postharvest disease control-related metabolomic variation in melon (Cucumis melo) artificially inoculated with Fusarium pallidoroseum.plos one 15(4):1-18.

Zhao M H ,Liu Z Q,Geng A F,Xie W B.2018.Identification and content determination of active constituents of fig leaves.Chinese Journal of Applied Chemistry 35(11):1391-1398.

Chen N L.1990.Bioassay techniques for pesticides.Beijing: Beijing Agricultural University Press.1990 : 66-68.

Bi Y, Tian S P, Guo Y R. 2006.Sodium silicate reduces Post harvest decay on Hami melons: induced resistance and fungistatic effects. Plant Disease 90:279-283.

SU X J, XU Ying, ZONG C Y,Qian J.2023.Research progress on extraction technology of bioactive components and total flavonoids from fig leaves.Chemical Engineer 37(2):62-65.

Du R, Liu J, Sun P.2017.Inhibitory effect and mechanism of Tagetes erecta L fungicide on Fusarium oxysporumf sp. niveum. Scientific Reports 7:14442.

Hadadi Z, Nematzaden G A, Ghahari S.2020. A study on the antioxidant and antimicrobial activities in the chloroformic and methanolic extracts of 6 important medicinal plants collected from north of Iran . BMC Chem 14:33.

Cushnie T P T, Lamb A J. 2011.Recent advances in understanding the antibacterial properties of flavonoids. International Journal of Antimicrobial Agents 38(2):99-107.

Wang Y, Zhang J, Sun Y,Feng J T,Zhang X .2017.Evaluating the Potential Value of Natural Product Cuminic Acid against Plant Pathogenic Fungi in Cucumber. Molecules 22(11):1-10.

Yin L Z,Chen J H,Wang K Y,Geng Y,Lai W M,Huang X L,Chen D F,Guo H R,Fang J,Chen Z L,Tang L,Huang C,Li N Q,Ouyang P. 2020.Study the antibacterial mechanism of cinnamaldehyde against drug-resistant Aeromonas hydrophila in vitro. Microbial Pathogenesis 145:104208.

Bajpai V K, Sharma A, Baek K H. 2013.Antibacterial mode of action of Cudrania tricuspidata fruit essential oil, affecting membrane permeability and surface characteristics of food-borne pathogens. Food Control 32(2):582-590.

Liu F F,Zhang A H,Lei F J,Xu Y H,Zhang L X.2018.Inhibiting Effects of Total Ginsenosides of Ginseng Stems and Leaves against Fusarium solani and Their Antibacterial Mechanism.Journal of Jilin Agricultural University 40(1):85-91

Yan S Y.2015.The antibacterial activity of ehdophytic fungi from Rhodiolasp.and effects of Se4+ 、Pb2+ on its physiological activity.Shanxi University.

Chowhan N,Singh H P,Batish D R.2013.G-Pinene inhibited germination and earlygrowth involves membrane peroxidation . Protoplasma 250:691-700.

Duma R K,Nenavathu B P,Gangishetty M K,Reddy A V R.2012.Studies on antibacterial activity of ZnO nanoparticles by ROS induced lipid peroxidation . Colloids&Surfaces B Biointerfaces 94:143-150.

Guo Y L, Guo L, Yan W B,Wang Z,Chen W F,Chen M X.2017.Progress in the effect of stress on physiological and chemical characteristics of tea tree chloroplast . Journal of Food Safety and Quality 8(5):1610-1615.

Wang C M, Zhang J,Chen H.2009.Lipid Peroxidation and Membrane Disruption by Natural Compound Eugenolin Mycelia of Botrytiscinerea. Chinese Journal of Pesticide Science 11:104-108.

Qu M Y, Li H T,Cheng H.2017.Antioxidant enzymes of fungi. International Journal of Dermatology and Venereology 43:50-53.