Antifungal Activity of β-Carboline Alkaloids Compound and Its Resistance Mechanism on *Peronophythora Litchii*

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**ABSTRACT**

*Peronophythora litchii* (*P. litchii*) is the pathogenic factor of litchi downy blight. Derivatives extracted from harmine officinalis and modified, N-(2-pyridyl)-1-phenyl-9 H-pyrido [3, 4-b] indole-3-formamide (PPPIF), could inhibit *P. litchii* growth and development. Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM) analyses indicated that PPPIF severely damaged the mitochondria, cell wall and endomembrane system of *P. litchii* cells, thus abnormal morphology, as well as deformed mycelia. PPPIF could suppress mycellial growth, sporulation, sporangia germination and germ tube elongation as well. PPPIF also caused a series of physiological and biochemical changes of *P. litchii*, including the serious deviation of extracellular pH, the obvious increase of the content of extracellular reducing sugar and malondialdehyde, the notable decrease of soluble protein and the activity of NADH oxidase. Furthermore, PPPIF seriously raised openness of membrane permeability transporter (MPTP) and markedly decreased the transmembrane potential (Δψm) and the activity of enzyme complex I–complex V in respiratory chain of mitochondria. The current research suggests that PPPIF may act upon the mitochondrion of *P. litchii*, then respiratory chain complex activity was blocked and energy metabolism disrupted or inhibited, resulting in the growth inhibition of *P. litchii*. Above results have strengthened our understanding of *P. litchii* resistance mechanisms and may help in the development of more potent inhibitors against plant diseases in the fields.

**KEYWORDS**

*Peronophythora litchii*; Beta-carboline; antifungal activity; mechanism

**Introduction**

Litchi (*Litchi chinensis* Sonn.), also known as lychee, is an important tropical and subtropical crop widely grown in more than 20 countries around the world, because of its bright appearance, crystal flesh, good taste, rich nutrition, has high commercial value (Jiang et al., 2018; Liu et al., 2011; Zhao et al., 2020). However, its production and quality are restricted by litchi downy blight, caused by the oomycete pathogen *Peronophythora litchii*, which leads to major pre- and post-harvest decay of the fruit and results in considerable economic losses (Situ et al., 2020; Wang and Zhu, 2002; Yi et al., 2008). Nowadays, chemical fungicides such as Metalaxy, Benomyl and Dimethomorph have been widely used to control this disease, there has been increasing fungicide resistance of *P. litchii*, and considering their harmful side-effects on the environment and food safety, which demonstrate the needs for novel, safe, effective, and more environmentally friendly methods to control the disease (Cai et al., 2010; Huang et al., 2021; Jiang et al., 2018; Su et al., 2019; Wang et al., 2010; Wu et al., 2017).

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β-carboline alkaloid is a large group of natural and synthetic indole alkaloids, it and its structural analogues have a wide range of biological activities in the medical and pharmaceutical. β-carboline alkaloid compounds was usually considered as an index of resistance in pathogenic disease control (Nenaah et al., 2010; Sarpeleh et al., 2009). Harmine is a kind of β-carboline, the β-carboline harmine was first isolated in 1847 from seeds of Peganum harmala and Banisteriopsis caapi (Sourkes, 1999), and also present in common plant-derived foods and in human tissues (Guan et al., 2001). Harmine interact with several cell-surface receptors (Glennon et al., 2000), and cyclin-dependent kinases (CDK1, 2, and 5) (Song et al., 2004). Harmine as a potent inhibitor of protein DYRK1A (dual-specificity tyrosine phosphorylation-regulated kinase 1A) (Frost et al., 2011) and yet harmine inhibited phosphorylation of septin 4 (SEPT4) by DYRK1A (Sitz et al., 2008). Harmine has a wide spectrum of pharmacological actions, including antiplasmodial activity (Astulla et al., 2008), and antioxidative action (Kim et al., 1997; Moura et al., 2007). Previous work has shown that harmine alkaloids and related β-carbolines exhibit important antifungal properties (Cruz et al., 2019). To our knowledge, harmine alkaloids or their derivatives have not been previously assayed against *P. litchii*.

Our preliminarily experiments showed that, a β-carboline harmine amide derivative, PPPIF (Figure 1) had a significant inhibitory activity against *P. litchii* (Li et al., 2015). However, there is no information regarding the antifungal mechanism of β-carboline on fungus. The aim of this study was to evaluate the structure, physiological, biochemical changes and mitochondrial function of *P. litchii*, by using electron microscope technology, physiological and biochemical detection technology, to analyze its mechanism.

**Materials and Methods**

**Chemicals and Oomycete Pathogen and Culture Condition**

PPPIF was prepared and obtained as previously reported (Li et al., 2015). Metalaxyl (≥98%) was purchased from Zhejiang Heben Pesticide Chemical Co., Ltd. (Zhejiang, China). All reagents used in the study were analytical grade. The *P. litchii* strain used in this study was obtained from Key Laboratory of Microbial Signals and Disease Control of South China Agricultural University in China. The pathogen was maintained on PDA medium (potato dextrose agar) at 25 ± 1°C and preserved on PDA slant at 4°C. The cultures of *P. litchii* were transferred to a fresh slant every two or three months to avoid viability decreasing. The concentration of sporangia of *P. litchii* that was incubated at 25 ± 1°C for 5 ddays () was adjusted to 5 × 10^5 sporangia/mL use a hemocytometer.

![Figure 1. Structure of the compound N-(2-pyridyl)-1-phenyl-9 pyrido [3, 4-b] indole-3-formamide (PPPIF).]
**Effect on Mycelial Growth of P. Litchii**

The effects of PPPIF on mycelial growth of *P. litchii* were tested in vitro by the agar dilution method with modification (Mohammadi et al., 2015a, 2016). In this method, high concentration of PPPIF (with DMSO) were added to PDA mediums (50 mL) to give desired concentrations of 0, 2.5, 5, 12.5, 25, 50, 100, and 200 μg/mL, which the content of DMSO would less than 0.5% in culture medium, and PDA medium containing of PPPIF was poured into sterilized petri dishes (90 mm diameter). Parallel controls were maintained with distilled water (with 0.05% Tween-80) and DMSO mixed with PDA medium. A 6 mm diameter disc of fungus that had incubated at 25 ± 1°C for 5 d was cut from the periphery of an actively growing culture on PDA plates with a punching needle, and placed at the center of each petri plate with drug-containing medium. Then, culture plates were incubated at 25 ± 1°C for 5 to 7 d until the mycelial growth of the control dishes had reached the edge of the plate. Radial growth of *P. litchii* was recorded by measuring the length of the advancing margin of the colony through the inoculum plug. Each treatment was performed in triplicates. The growth inhibition ratio (I) were calculated according to the following formula:

\[
I(\%) = \frac{D_c - D_t}{D_c} \times 100
\]

Where Dc (cm) was the mean colony diameter for the control sets that had subtracted diameter of disc (6 mm) and Dt (cm) was the mean colony diameter for the treatment sets that had subtracted diameter of disc (6 mm) (Mohammadi et al., 2015b). The EC<sub>50</sub> (the concentration inhibited 50% of the mycelium growth) and EC<sub>95</sub> values (the concentration inhibited 95% of the mycelium growth) were calculated by probit analysis.

**Sporulation Quantity**

A 6 mm diameter disc of fungus that had incubated at 25 ± 1°C for 5 d was cut from the periphery of an actively growing culture on PDA plates with a punching needle, and placed at the center of each petri plate with PPPIF (0, 8.57, 86.22 μg/mL), Metalaxyl (6.32 μg/mL) was used as positive control. Then, culture plates were incubated at 25 ± 1°C for 5 to 7 d until the colony diameter of the control group was greater than 8 cm, the plate was placed under 16 hours(h) light/8 h darkness for 1–2 d to induce sporulation. After sporulation, a 3 mm diameter perforator was used to extract the cake from the central place of the plate and put it into the centrifugal tube in turn. 10 mL sterile water was added, and the spores were eluted by shaking for 2 minutes(min). Four layers of gauze were filtered. The filtrate was centrifuged under 4000 r/min for 5 min. The supernatant was discarded and centrifuged for 5 min. The supernatant was suspended heavily in 10 mL sterile water. Count with a blood count board. Each treatment was performed in triplicates (Chen et al., 2010). The calculation formula is as follows:

Sporangium concentration (one/mL) = N × 10<sup>4</sup> × R (N = Total number of sporangia in four squares; R = Dilution ratio)

**Measurement of Sporangia Germination and Germ Tube Elongation**

In this test, sporangia were collected to 1 mL sterile distilled water containing 0.05% Tween-80 and 0.1% w/v glucose from 5-day-old fungi of *P. litchii*, filtered by four layers sterile cheesecloth to remove hyphae, counted by a hemocytometer (1 × 10<sup>5</sup> sporangia/mL), and used immediately in the next test. An aliquot (100 μL) of *P. litchii* sporangia suspension (1 × 10<sup>5</sup> sporangia/mL) was incubated into 280 μL sterile water containing 0.05% Tween-80 and 0.1% w/v glucose in 24-well plate. Then, the 20 μL amounts of PPPIF (with DMSO) dissolved in 0.05% Tween-80 was, respectively, transferred into the above culture medium to obtain the concentrations of 0, EC<sub>50</sub> and EC<sub>95</sub>. The 24-well plate were incubated at 25 ± 1°C for 8 h, and sporangia morphology were observed under the microscope and recorded. A sporangium was considered germinated when the germ tube was equal to or greater than the diameter of the sporangium. About 200 sporangia were examined microscopically. Germinated sporangia were expressed as a percentage of the total number of evaluated sporangia, and germ tube...
elongation were expressed as a percentage of the length of evaluated sporangia. Each treatment was performed in triplicates. Sporangia germination and germ tube elongation were inhibited by different of treatments was observed microscopically with inverted fluorescence microscope (MF52, Mshot; Guangzhou, China). Sporangia germination indexes (SGI) and germ tube elongation (GTE) were calculated according to the following formula:

\[ R(\%) = \frac{N_g}{N_t} \times 100 \]

\[ R_e(\%) = \frac{R_t}{R_0} \times 100 \]

\[ SGI(\%) = \frac{R_0 - R_e}{R_0} \times 100 \]

\[ GTE(\%) = \frac{L_0 - L_e}{L_0} \times 100 \]

Where \( R \) was the mean rate of sporangia germination, \( N_g \) was the mean number of sporangia germination, \( N_t \) was the mean total number of sporangia, \( R_e \) was the mean correction sporangia germination of treatment, \( R_t \) was the mean rate of sporangia germination of treatment, \( R_0 \) was the mean rate of sporangia germination of control, \( L_0 \) was the length of germ tube of control, \( L_e \) was the length of germ tube of treatment.

**Scanning Electron Microscopy (SEM) Assay**

The 5-day-old fungi of *P. litchii* was treated by PPPIF with different concentrations (0, EC50 = 8.57 μg/mL and EC95 = 86.22 μg/mL) according to the method described by Helal et al. and Yahyazadeh et al. (Helal et al., 2007; Yahyazadeh et al., 2008). Segments with size of 10 × 10 mm were cut from the edge of fungal colonies growing on PDA and promptly placed in 4.0% (v/v) glutaraldehyde at 4°C. Segments of samples were kept in this fixative solution for 12 h, and washed with 0.1 mol/L phosphate buffer (pH 7.2) for 10 min in thrice. Then, they were fixed in 1.0% osmium tetroxide for 2 h or more, and washed with phosphate buffer thrice again. Then, samples were dehydrated in a graded ethanol series (30%, 50%, 70% and 90%, v/v) for 10 min in each alcohol dilution and finally dehydrated in absolute ethanol for 10 min twice. After that, samples were put in liquid carbon dioxide for critical point dried and placed in desiccators until further use. Then, samples were mounted on standard 1/2 in SEM stubs using double-stick adhesive tabs and coated with gold-palladium electroplating (60 s, 1.8 mA, 2.4 kV) in a SC7620 sputter coater (Polaron, England). All samples were observed in a XL-30 SEM (FEI Electron Optics Ltd., Hillsboro, USA) operating at 25 kV.

**Transmission Electron Microscopy (TEM) Assay**

The 5-day-old fungi of *P. litchii* was treated by PPPIF with different concentrations (0, EC50 and EC95) on PDA were observed by TEM (Helal et al., 2007; Tao et al., 2014). Mycelium blocks of *P. litchii* (5 mm diameter) that were treated by PPPIF at different concentrations (0, EC50 and EC95) were fixed in 3% paraformaldehyde and 4% glutaraldehyde mixed solution for more than 4 h, washed with 0.1 mol/L phosphate buffer (pH 7.2) for 10 min in thrice, then post fixed in 1.0% osmium tetroxide for 12 h. After that, the mycelium blocks were washed again with the phosphate buffer for 10 min thrice. All fixation steps were performed at 4°C. Mycelium blocks were dehydrated in a graded ethanol series (30%, 50%, 70%, 80% and 90%) for 10 min and finally dehydrated in absolute ethanol for 10 min twice. The specimens were further treated with absolute acetone twice each for 20 min as a transitional fluid,
then embedded in three changes of spurr's resin and acetone (1:3, 1:1, 3:1) for 4 h each and finally embedded in spurr's resin for 24 h. Samples were embedded in a mold, polymerized at 45°C for 24 h, and then polymerized at 60°C for 24 h. Ultrathin sections (70 nm in thickness) were made by an ultramicrotome with a diamond knife. The sections were mounted on copper grids, and stained with 2% uranyl acetate and lead citrate each for 30 min, after that, the stained sections were observed in a TECNAI G^2 12 TEM (FEI Electron Optics Ltd., Hillsboro, USA) operated at an accelerating voltage of 80 kV.

**Determination of Acidification of External Medium**

The proton pumping activity of *P. litchii* was detected by monitoring the acidification of the external medium by detecting the pH as previously reported method with modification (Manavathu et al., 1999; Tian et al., 2012). 1 mL sporangia suspension (5 × 10^5 sporangia/mL) in liquid PDA (30 mL) was inoculated in flasks. The flasks were then incubated for 5 d at 25 ± 1°C. The medium containing mycelia after 5 d was filtered through filter paper and washed twice with distilled water, the washed mycelia in the weight of 0.5 g was suspended in 20 mL solution containing PPPIF with different concentrations (EC_{50} and EC_{95}). The control was without any PPPIF treatment. The mixtures were incubated at 25 ± 1°C, and the mycelia were filtered using filter paper. The value of the external pH was checked by using digital pH meter (FE20-FiveEasy Plus™, Mettler-Toledo, Zurich, Switzerland) at 0, 2, 4, 8, 12 and 24 h.

**Determination of Soluble Protein**

Soluble protein content was measured by the Bradford method (Bradford, 1976). 1 mL sporangia suspension (5 × 10^5) was inoculated in flasks with liquid PDA (30 mL) for 5 d at 25 ± 1°C. The medium containing mycelia were filtered through filter paper and washed twice with distilled water. Approximately 0.5 g wet weight of the washed mycelia was suspended in 10 mL solution containing PPPIF with different concentrations. The mixtures were incubated at 25 ± 1°C for 3, 6, 12 and 24 h. Mycelia were filtered by using filter paper and recorded fresh weight of mycelia, respectively. Then, mycelia were added 9 volumes of phosphate buffered saline (weight (g): volume (mL) = 1:9), then homogenized in ice bath, 2500 r/min, 10 min, the supernatant was tested. 0.4 mL supernatant and 5 mL Bradford G-250 were added to the test tube, mixed by inversion and measured at 595 nm. Content of soluble protein (CSP) were calculated according to the following formula:

\[
\text{CSP(\mu g/g)} = \frac{V_0/V_T}{M} \times X
\]

Where \(V_0\) mean the total volume of extraction solution, \(V_T\) mean sampling volume, \(M\) mean fresh weight of sample, \(X\) mean soluble protein content of the standard curve.

**Determination of Extracellular Reducing Sugar**

Extracellular reducing sugar content was analyzed by the 3, 5-dinitrosalicylic (DNS) colorimetric method (Miller, 1959; Yuan et al., 2014) with some modifications. 1 mL sporangia suspension (5 × 10^5) was inoculated in flasks with liquid PDA (30 mL) for 5 d at 25 ± 1°C. The medium containing mycelia were filtered through filter paper and washed twice with distilled water. 0.5 g wet weight of the washed mycelia was suspended in 10 mL solution containing PPPIF with different concentrations (EC_{50} and EC_{95}). The control was without any PPPIF. The mixtures were incubated at 25 ± 1°C for 3, 6, 12 and 24 h. Then, they were filtered using filter paper to obtain filtrate. For each of the 2 mL of the filtrate, 1.5 mL of DNS reagent was added. The mixture was heated in boiling water for 5 min until the red brown color was developed and cooled to room temperature in a water bath. The absorbance of the mixture was measured at 520 nm and the concentration of reducing sugars was calculated based on a standard curve obtained with D-glucose. Each assay contained three replicates for each concentration.
Determination of Lipid Peroxidation

1 mL sporangia suspension \((5 \times 10^5)\) was inoculated in flasks with liquid PDA (30 mL) for 5 days at 25 ± 1°C. The medium containing mycelia were filtered through filter paper and washed twice with distilled water. Approximately 0.5 g wet weight of the washed mycelia was suspended in 10 mL solution containing PPPIF with different concentrations \((EC_{50}\) and \(EC_{95}\)). The control was without any PPPIF. The mixtures were incubated at 25 ± 1°C for 3 d. Then, the mycelia were removed by filtration through 0.2 µm pore size membrane and washed twice with distilled water, respectively. The mycelia were used in the malondialdehyde (MDA) assay for lipid peroxidation. For assaying lipid peroxidation, a method based on the reaction of thiobarbituric acid with MDA derived from lipid peroxidation was employed. Detection of thiobarbituric acid species was carried out by a colorimetric assay (Ritter et al., 2008). The lipid peroxidation was expressed as µmol/g fresh weight of mycelia.

Determination of NADH Oxidase Activity

The NOX (NADH oxidase) activity of \(P.\ litchii\) was measured by the method of Kunio Tochikubo’s description with modification. 1 mL sporangia suspension \((5 \times 10^5)\) was inoculated in flasks with liquid PDA (30 mL) for 5 d at 25 ± 1°C. The medium containing mycelia were filtered through filter paper and washed twice with distilled water. The mycelia pellets with uniform size were suspended in 5 mM HEPES-Na buffer \((pH 7.0)\), then was suspended in the solution containing PPPIF with different concentrations \((EC_{50}\) and \(EC_{95}\)) and incubated for 2 h at 25°C. The control was without any PPPIF. The mycelia pellets that were collected by centrifugation at 4000 r/min for 15 min at 4°C were suspended in 0.2 M phosphate buffer \((pH 7.2)\), and homogenized in ice bath, then centrifuged at 12000 r/min, for 30 min at 4°C to obtain the supernatant, the decrease of OD\(_{340}\) indicates the activity of enzyme. Each assay contained three replicates for each concentration.

Extraction and Processing of Mitochondria

The sporangium suspension of \(P.\ litchii\) \((1 \times 10^5/mL)\) was inoculated into 30 mL PDB liquid medium at 25°C, 120 r/min for 5 d, 4000 r/min, centrifugation for 15 min, mycelial precipitation was obtained and washed with sterile water for 3 times. Take 10 g mycelium, add appropriate amount of mitochondrial buffer and quartz sand. The slurry was ground in ice bath and collected. The homogenate was centrifuged at 4°C for 10 min at 2000 r/min. The supernatant was centrifuged (2000 r/min, 10 min). The supernatant was discarded and then centrifuged (12000 r/min, 15 min). After discarding the supernatant, the precipitate was suspended sufficiently by adding a proper amount of buffer. The precipitate obtained by centrifugation at 12000 r/min for 15 min was mitochondria. After extracting the mitochondrial protein, the protein content was adjusted to 50 µg/mL, and the protein content was randomly divided into four groups: blank control group, \(EC_{50}\) (8.57 µg/mL) treatment group, \(EC_{95}\) (86.22 µg/mL) treatment group, Metalaxyl (6.32 µg/mL), 37°C isothermal oscillation for 30 min.

Openness of Mitochondrial Membrane Permeability Transport Pore (MPTP)

The direct result of MPTP opening is mitochondrial swelling, and the measurement of mitochondrial swelling can reflect the degree of MPTP opening. The swelling of mitochondria can result in more transmitted light and less scattered light. The OD\(_{520}\) value was determined by adding 2.9 mL medium to the quartz colorimetric dish, then adding 0.1 mL protein content of 50 µg/mL treated mitochondria. Each treatment was repeated three times. Calculating formula:

\[
\text{Openness of MPTP(\%)} = \frac{OD_c - OD_t}{OD_c} \times 100 \quad (OD_c = \text{the control OD}_{520} \text{ value}; OD_t = \text{the treatment OD}_{520} \text{ value})
\]
**Mitochondrial Transmembrane Potential (Δψm)**

The sporangia of *P. litchii* cultured for 5 d were washed with aseptic water (Pourahmad and O’Brien, 2000), and the sporangia concentration was adjusted to $1 \times 10^5$/mL by using a blood count plate. The sporangia were randomly divided into four groups and shaking treatment at 25°C for 2 h. The treated sporangia were mixed with 5 μL 20 nM Rhl23 and kept at 37°C for 30 min. The sporangia were observed and photographed under fluorescence microscope.

**Mitochondrial Respiratory Chain Complex Activity**

In order to release the mitochondrial respiratory chain complex better, the treated mitochondrial suspension was frozen and thawed repeatedly three times (−80°C/37°C) in a cryogenic refrigerator before determination, which resulted in the rupture of mitochondrial membrane. The activity of mitochondrial respiratory chain complex was determined by ultraviolet spectrophotometry. ATPase is the dissociation state of mitochondrial respiratory chain complex V. It can decompose ATP to produce ADP and inorganic phosphorus to produce energy. The activity of ATPase can be judged by measuring the content of inorganic phosphorus released in the enzymatic reaction. The amount of inorganic phosphorus produced by ATPase decomposition of mitochondrial proteins per milligram per hour is one unit of ATPase activity, and the activity of ATPase is calculated with ATP Enzyme Kit Method.

**Statistical Analysis**

Data were presented as means and standard deviations. ANOVA was used to determine whether there were significant ($P < .05$) treatment effects between groups, and the means were compared using the Duncan’s multiple range test. Differences among different treatments were analyzed using SPSS version 19.0.

**Results**

**Effects of PPPIF on Growth and Development of *P. Litchii***

To evaluate the inhibitory activity, the effects of the PPPIF on *P. litchii* mycelial growth on solid media were determined. The results showed that the PPPIF had high dose-dependent activities and an effective concentration of 50% (EC$_{50}$) value of 8.57 μg/mL (Table 1) against the *P. litchii* mycelial growth. The EC$_{50}$ and EC$_{95}$ of PPPIF treated *P. litchii* were 8.57 μg/mL and 86.22 μg/mL, respectively. The EC$_{50}$ and EC$_{95}$ of the positive control group treated with Metalaxyl were 0.93 μg/mL and 6.32 μg/mL, respectively.

Compared with the blank control, 8.57 μg/mL PPPIF treatment significantly reduced ($P < .05$) the number of spores produced by *P. litchii*, the average inhibition rate of sporangium formation was more than 95%. However, 86.22 μg/mL PPPIF treatment, the average inhibition rate of sporangium formation reached 99.4%, which was similar to that of the positive control (99.71%) and extremely significant compared with the blank control (Table 2).

![Image](https://via.placeholder.com/150)

**Table 1.** The inhibitory activity of PPPIF against *P. litchii*.

| Treatments | $y = a + bx$ | $r$ | EC$_{50}$ (μg/mL) | 95% confidence interval | EC$_{95}$ (μg/mL) | 95% confidence interval |
|------------|--------------|----|------------------|------------------------|------------------|------------------------|
| PPPIF      | $y = -1.531 + 1.641x$ | 0.961 | 8.57 | 5.41–12.92 | 86.22 | 47.10–241.84 |
| Metalaxyl  | $y = 0.060 + 1.979x$ | 0.965 | 0.93 | 0.68–1.26 | 6.32 | 3.97–13.26 |

*EC$_{50}$ and EC$_{95}$ were determined by probit-log analysis.*
Table 2. Inhibitory effect of PPPIF on spores of P. litchii.

| Treatments | Concentration (μg/mL) | Mean sporangium density (number/mL) | Average inhibition rate (%) |
|------------|-----------------------|-----------------------------------|-----------------------------|
| control    | 0                     | $6.06 \times 10^2 \pm 6.73 \times 10^0$ a | --                          |
| PPPIF      | 8.57                  | $3.01 \times 10^4 \pm 4.61 \times 10^3$ b | 95.04 ± 0.94 b              |
| PPPIF      | 86.22                 | $3.65 \times 10^4 \pm 0.00$ c       | 99.40 ± 0.00 a              |
| Metalaxyl  | 6.32                  | $1.78 \times 10^1 \pm 5.9 \times 10^2$ c | 99.71 ± 0.11 a              |

*The date in the table represents the mean of three independent experiments ± standard deviation. Different letters indicate that the means are significantly different at P < 0.05.

Figure 2. Inhibition rate of spores germination and germ tube elongation of P. litchii after PPPIF treatment. Each bar represents the mean of three independent experiments ± standard deviation. Different letters indicate that the means are significantly different at P < 0.05. (CK = 0 μg/mL, metalaxyl = 6.32 μg/mL, EC$_{50}$ = 8.57 μg/mL, EC$_{95}$ = 86.22 μg/mL).

PPPIF exhibited greater inhibition of spore germination and germ tube elongation than the Metalaxyl (Figure 2) of P. litchii, the inhibition rate of spore germination reached 28.32% after 8.57 μg/mL treatment, which was significantly higher than the relative inhibition rate of positive control Metalaxyl, and the inhibition rate of spore germination and germ tube elongation, respectively, reached 72.46% and 80.29% after 86.22 μg/mL treatment, they were significantly higher than those of the positive control group.

To understand the antifungal mechanism of PPPIF, the hyphae morphology of P. litchii treated with PPPIF was examined by Scanning Electron Microscopy (SEM). The untreated P. litchii mycelium showed characteristic morphology with regular cylindrical tube with smooth external surfaces and full oval apexes (Figure 3a). After treated with PPPIF at the concentration of 8.57 μg/mL, the hyphae morphology showed torsion, a little swelling, collapse and spillover, and the surface was uneven, and the surface of the sporangium showed ravine and obvious shrinkage (Figure 3b). After treated with PPPIF at the concentration of 86.22 μg/mL, the mycelium of P. litchii has severe shrinkage and shriveling, most of the surviving mycelia are severely swollen and spillover, and the sporangium also collapse and shriveled (Figure 3c).

Transmission Electron Microscopy (TEM) observation showed that the untreated hyphae of P. litchii displayed a smooth and compact surface, the cytoplasm was abundant and homogeneous, and the organelles of cell wall, cell membrane, vesicle and mitochondria were normal and evenly distributed (Figure 4a). Under the treatment condition of 8.57 μg/mL, the cells showed obvious abnormal changes, the cell wall was obviously thickened, the cell membrane was thickened and the plasmodesm was separated, the matrix in the vesicles was lost, the cell matrix was reduced and concentrated, and the fusion of vacuoles was obvious. The vacuolation (Figure 4b), the intermembrane space of mitochondria (Figure 4b) was narrowed with slight swelling, and the number of cristae was
reduced. Under the treatment condition of 86.22 μg/mL, the abnormality of the cells was serious, the cell wall was thickened and the outer surface was obviously rough, the cell membrane thickening and plasmolysis were obviously accompanied by the formation of a large number of small lysosomes, the vacuolar fusion and vacuolation were serious, and the cells. The matrix was severely absent, electron-dense granules were formed (Figure 4c), mitochondria (Figure 4c) were severely swollen, intermembrane disappeared, and the number of cristae was significantly reduced.

Figure 3. Scanning electron micrographs of the hyphae of *P. litchii* grown on PDA plates with or without PPPIF at 5 d. a = control (0 μg/mL), b = 8.57 μg/mL, c = 86.22 μg/mL.

Figure 4. Transmission electron microscopy (TEM) of the cellular ultrastructure of the hyphae of *P. litchii*. a, b and c, sporangium; a’, b’ and c’, chondriosome of *P. litchii*; a, a’ = control (0 μg/mL), b, b’ = 8.57 μg/mL, c, c’ = 86.22 μg/mL; CW = cell wall, PM = plasma membrane, M = mitochondria, V = vesicle, EDG = Electron dense particle, * = Plasmolysis, # = vacuolation, L = Small lysosome.
The extracellular pH of *P. litchii* cells exposed to PPPIF is presented in Figure 5a. The extracellular pH showed a gentle trend in the control. However, the extracellular pH of *P. litchii* suspensions with PPPIF continued to increase, and significantly higher than that of the control group at 24 h (P < .05), which was similar to that of the positive control group.

The higher concentration of PPPIF, the longer treatment time, the greater deviation of soluble protein content from the blank control. The concentration of soluble protein in the blank control group increased during the whole test period. Under the condition of EC₅₀ concentration treatment, the soluble protein increased rapidly after 3 h, and then increased slowly, which was lower than that of the control group at the same time. After 24 h, the soluble protein was only about half of that of the blank control group, and the difference was obvious. Under the condition of EC₉₅ concentration treatment, the soluble protein increased rapidly after 3 h, which was similar to that of EC₅₀ treatment, then decreased slowly, and was significantly lower than that of control at the same time. After 24 h, the concentration of soluble protein was significantly different from that of blank control, and it was lower than that of positive control at the same time during the whole test period (Figure 5b). The above results showed that PPPIF could induce the increase of bacterial resistance proteins to resist the damage of bacterial cells in a short time, and then the PPPIF interfere with protein biosynthesis was enhanced.

The extracellular reducing sugar contents in *P. litchii* cells with PPPIF continuously decreased during the entire period, whereas those in the untreated cells remained stable. The sugar contents of *P. litchii* cells incubated with PPPIF (concentration of 8.57 µg/mL and 86.22 µg/mL) for 3 h rapidly increased to twice of the control group, and then slowly rising, there was a significant difference between the same period concentration of the control group and that of the positive control group (Figure 6a). At 3 d after treated with PPPIF, with the increase of concentration, malondialdehyde (MDA) content increased. After treated with PPPIF at the concentration of 8.57 µg/mL, the content of

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**Figure 5.** a: Effects of PPPIF treatment on changes in pH content of *P. litchii*; b: Effects of PPPIF treatment on changes in soluble protein content of *P. litchii*. (CK = 0 µg/mL, Metalaxyl = 6.32 µg/mL, EC₅₀ = 8.57 µg/mL, EC₉₅ = 86.22 µg/mL).

**Figure 6.** a: Effect of reducing sugar activity after PPPIF treatment at 3 h; b: Effect of MDA activity after PPPIF treatment at 3 d; c: Effect of reducing NADH oxidase activity after PPPIF treatment at 2 h. Each bar represents the mean of three independent experiments ± standard deviation. Different letters indicate that the means are significantly different at P < .05. (CK = 0 µg/mL, Metalaxyl = 6.32 µg/mL, EC₅₀ = 8.57 µg/mL, EC₉₅ = 86.22 µg/mL).
malondialdehyde was 0.09 mol/g, was significantly higher than the control group (0.07 mol/g), when under the condition of 86.22 μg/mL treatment, the content of MDA was reached up to 0.13 mol/g, extremely remarkable compared with controls, and with positive control was equivalent (Figure 6b).

**Effect of Reducing Sugar, MDA, NADH Oxidase Activity after PPPIF Treatment**

The changes of NADH oxidase activity of P. litchi cells after PPPIF treatment for 2 h were determined by UV spectrophotometer (Figure 6c). The results showed that after the compound PPPIF treatment, the activity of NADH oxidase showed an obvious dose negative correlation. When the concentration was 8.57 μg/mL, the activity of NADH oxidase was 24.81 U/mgpro, which was almost a quarter of the activity of the control group (102.24 U/mgpro), and the difference was significant; when the concentration was increased to 86.22 μg/mL, the activity of NADH oxidase continued to decrease to 17.28 U/mgpro, and the activity of the control group was significantly different, which was comparable to that of the positive control group.

**Influence of PPPIF on Mitochondrial Function of P. Litchii Cells**

After treatment with PPPIF, there was a positive correlation between MPTP openness and treatment concentration (Figure 7). In the treatment of 8.57 μg/mL concentration, the opening degree of MPTP was 64.55%, which was significantly different from that of the control group. When the concentration up to 86.22 μg/mL, the opening degree of MPTP reached 88.18%, which was extremely significant compared with the control group.

Fluorescence microscopy was used to observe the changes of mitochondrial transmembrane potential of P. litchii after treatment with PPPIF for 2 h, which showed that Rh123 was enriched in the mitochondria of sporangia of P. litchii in the control group and emitted bright fluorescence with more bright spots (Figure 8a). When treated at 8.57 μg/mL concentration (Figure 8b), the fluorescence quantity decreased significantly, and the brightness also decreased significantly, compared with the blank control group; when treated at 86.22 μg/mL concentration (Figure 8c), the fluorescence intensity decreased significantly, the fluorescence was less and the brightness was lower than that of 8.57 μg/mL.

Ultraviolet spectrophotometer results show that compared with the blank control group, after treatment with PPPIF for 0.5 h, the activity of respiratory chain complex I–complex IV decreased, showing a dose-negative correlation, with significant difference (Figure 9a–d). When treated with 8.57 μg/mL, the inhibitory effect of PPPIF decreased significantly compared with that of the blank.
control group, and was similar to that of the positive control group. When treated with 86.22 μg/mL, the inhibitory effect of compound PPPIF did not continue to increase significantly and was similar to that of the group at 8.57 μg/mL.

The activity of Na⁺K⁺-ATPase and Ca²⁺Mg²⁺-ATP in complex V of P. litchii mitochondria cells treated with PPPIF was determined and the sum of them was calculated (Figure 10a–c). Compared with the blank control group, ATPase activity of complex V showed a negative dose correlation, and there was a significant difference (Figure 10c). When the concentration of 8.57 μg/mL was treated, it decreased...
significantly compared with the blank control group, which was the same as the positive control group. When the concentration of 86.22 μg/mL was treated, the inhibitory effect continued to increase, and there was a significant difference between the results of 8.57 μg/mL treatment and that of the blank control group, which was extremely significant compared with the results of the blank control group.

Discussion

In recent years, with the continuous improvement of food safety awareness of consumers, the demand for fresh fruits and vegetables without chemical residues has been greatly increased (Turnipseed et al., 2020). Susceptibility of phytophthora disease caused by P. litchii is the most serious problem affecting the litchi industry worldwide. As alternatives to synthetic fungicides, the use of natural antifungal compounds isolated from plants to control P. litchii has been investigated, but few if any satisfactory compounds have been found. It has been reported that some plant active substances can be used to control P. litchii and other plant diseases (Jaysainghe et al., 2004; Malquichagua et al., 2005). Furthermore, structural optimization of novel fungicide by natural metabolites has recently been used for the research and development of new pesticides. PPPIF is a synthetic β-carboline harmine, which belongings to the β-carbolines alkaloids, was isolated from Peganum harmala (Kartal et al., 2003), it was effective on the control of P. litchii infection on litchi leaves (Li et al., 2015). However, the possible mechanisms of PPPIF against P. litchii was not documented. This is a report on the potential agricultural use of the environmentally synthetic compound relying on natural plant against P. litchii.

The inhibition of mycelia growth, sporulation, spore germination and germ tube elongation achieved by PPPIF becomes relevant considering that conidia act as an airborne inoculum, which determines subsequent disease development (Cabrerizo et al., 2017; Elad and Even, 1995). During postharvest litchi management, the reduction of conidial production represents a desirable effect (Zhang et al., 2021), minimizing cosmetic flaws of the unsightly contamination of adjacent fruit and protecting fruit from future infection. It has been reported that β-carbolines as antimicrobial agents related to a broad cause, such as cell cycle, signal transduction enzymes (Di Giorgio et al., 2004), membrane damage (Lala et al., 2004), respiratory chain (Rivas et al., 1999), and programmed cell death (Rosenkranz and Wink, 2007). PPPIF caused membrane disruption in conidia of P. litchii, maybe as a first step in its antimicrobial action. SEM analysis showed that PPPIF caused mycelium surface severe abnormalities and abnormal cellular tissue and organs of P. litchii, similar to that of other reported bioactive compounds (Alonso-Villaverde et al., 2011; Barreto et al., 2016). TEM analysis displayed that the plasma membrane of the hyphae were severely damaged and certain intracellular organelles disappeared. These results were similar to the damages on P. litchii induced by hypothemycin, zeamines or isoliquiritin (Liao et al., 2015; Xu et al., 2013) or on Penicillium italicum induced by Citral (Tao et al., 2014) and Penicilium digitatum Sacc. treated with Clove oil (Yahyazadeh et al., 2008). Moreover, in agreement with plasma membrane per-meabilization assay, both of SEM and
TEM analyses showed that plasma membrane was a target for modification of membrane permeability was mainly through interactions with the negatively charged phospholipids of plasma membrane (Ahuja et al., 2012; Da Rocha Neto et al., 2015).

Cabrerozo, F.M. et al. (Cabrerozo et al., 2017) reported that harmol, a β-carboline, was fungicidal to Botrytis cinerea and fungistatic to Penicillium digitatum affecting conidia membrane permeabilization in both species. Namely, this alkaloid might produce an initial structural disruption and alteration of the membrane, before generating intracellular damage. Several authors have already related antimicrobial action of many agents with membrane permeabilization (Badosa et al., 2009; Cerioni et al., 2010; Makovitzki et al., 2006).

As the most important component of cell metabolism, protein can catalyze a large number of biochemical reactions, and all functional activities inside and outside the cell cannot leave the involvement of proteins. The soluble protein content of treated P. litchii was significantly decreased, indicating that the growth and metabolism of P. litchii were blocked, which may result in the abnormal growth of mycelia and the normal germination of sporangium. Malondialdehyde (MDA), an important lipid peroxidized product, can reflect the extent of membrane lipid peroxidation and membrane system damage (Lin et al., 2017, 2015, 2014). However, the biological activity of NADH oxidase (NOX) has a very important antioxidant function. In the aerobic growth environment of cells, NOX’s participation in the resistance to oxygen toxicity is the main defense barrier for cell metabolism protection (Qing and Fang, 2011). The content of MDA in P. litchii was abnormally increased and the activity of NOX was significantly decreased, which also led to the accumulation of reactive oxygen species (ROS) in cells. When PPPIF entered the mycelium, NOX activity decreased, ROS scavenging capacity of P. litchii cells decreased, and ROS increased, resulting in mitochondrial peroxidation. The abnormal swelling of mitochondrial membrane, the increase of membrane permeability transport pore (MPTP) openness and the decrease of mitochondrial transmembrane potential (Δψm) were observed under TEM. Yan and Sohal (Yan and Sohal, 1998) found that the opening of MPTP could cause the decrease or even collapse of mitochondrial membrane potential (Δψm). Our results also pointed out that the damage of mitochondrial membrane resulted in the dysfunction of mitochondrial membrane.

Guan et al. (Guan and Li, 2006) discovered the bacteriostasis mechanism of terpenoids, mainly affecting the primary energy metabolism, NADH activity of bacteria, and electron transfer in respiratory chain, thus playing the role of bacteriostasis. It reported that harmine inhibited phosphorylation of protein (Frost et al., 2011; Seifert et al., 2008), this is consistent with our research, PPPIF lowered ATPase activity, likely due to inhibition of phosphorylation. If the normal functioning of mitochondrial material and energy metabolism is disturbed, it will lead to energy deficiency of cells, which will lead to dysfunction of cells and organelles. In our present study, the activity of enzyme complex I-complex V in mitochondrial respiratory chain were all inhibited. Many studies have shown that amide fungicides can inhibit the growth of pathogenic bacteria by affecting the respiratory chain electron transport system of pathogenic bacteria, and ultimately lead to their death. Early products such as atrazine hindered the respiration of pathogenic bacteria by inhibiting the succinate dehydrogenase of iron II complex in the respiratory transmission chain (Yang et al., 2008). According to the above results, compound PPPIF may have a target protein in mitochondria.

In conclusion, we report for the first time that β-carboline harmine have significant antifungal properties against P. litchii. The compound PPPIF inhibited the growth of P. litchii mycelium, sporulation and sporangium germination at the apparent level, and caused morphological changes on the cell surface and interior, especially mitochondrial morphological changes. At the physiological and biochemical levels, it caused extracellular pH, cellular A series of changes in exogenous reducing sugar content, malondialdehyde (MDA) content, soluble protein content, NOX activity, etc. However, the most important thing is to cause damage to mitochondrial morphology and function, resulting in obstruction of respiratory chain enzyme activity and inhibition of its energy metabolism, which eventually led to the growth of P. litchii being blocked. These results indicate that PPPIF might be a promising candidate as a new antifungal compound to control plant diseases.
Disclosure Statement

No potential conflict of interest was reported by the author(s).

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References

Ahuja, I., R. Kissen, and A.M. Bones. 2012. Phytoalexins in defense against pathogens. Trends Plant Sci 17(2):73–90. doi: 10.1016/j.tplants.2011.11.002.

Alonso-Villaverde, V., F. Voinesco, O. Viret, J. Spring, and K. Gindro. 2011. The effectiveness of stilbenes in resistant Vitaceae: Ultrastructural and biochemical events during Plasmopara viticola infection process. Plant. Physiol. Bioch 49(3):265–274. doi: 10.1016/j.plaphy.2010.12.010.

Astulla, A., K. Zaima, Y. Matsono, Y. Hirasawa, W. Ekasari, A. Widyawaruyanti, N.C. Zaini, and H. Morita. 2008. Alkaloids from the seeds of Peganum harmala showing antiplasmodial and vasorelaxant activities. J. Nat. Med 62(4):470–472. doi: 10.1007/s11418-008-0259-7.

Badosa, E., R. Ferre, J. Frances, E. Bardaji, L. Feliu, M. Planas, and E. Montesinos. 2009. Sporidical activity of synthetic antifungal undecapeptides and control of Penicillium rot of apples. Appl. Environ. Microbiol 75(17):5563–5569. doi: 10.1128/AEM.00711-09.

Barreto, T.A., S.C.A. Andrade, J.F. Maciel, N.M.O. Arcanjo, M.S. Madruga, B. Meireles, Â.M.T. Cordeiro, E.L. Souza, and M. Magnani. 2016. A chitosan coating containing essential oil from Origanum vulgare L. to control postharvest mold infections and keep the quality of cherry tomato fruit. Front Microbiol. 7:1724. doi: 10.3389/fmicb.2016.01724.

Bradford, M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem 72(1–2):248–254. doi: 10.1016/0003-2697(76)90527-3.

Cabrero, F.M., G.M. Olmedo, L. Cerioni, M.M. Gonz, V.A. Rapisarda, and S.I. Volentini. 2017. Antifungal activity of β-carbolines on Penicillium digitatum and botrytis cinerea. Food Microbiol 62:9–14. doi: 10.1016/j.fm.2016.09.011.

Cai, X.Q., N. Lin, W. Chen, and F.P. Hu. 2010. Control effects on litchi downy blight disease by endophytic bacterial strain TB2 and its pathogenesis-related proteins. Acta Hortic 863(863):631–636. doi: 10.17660/ActaHortic.2010.863.89.

Cerioni, L., S.I. Volentini, F.E. Prado, V.A. Rapisarda, and L. Rodriguez-Montelongo. 2010. Cellular damage induced by a sequential oxidative treatment on Penicillium digitatum. J. Appl. Microbiol 109(4):1441–1449. doi: 10.1111/j.1365-2672.2010.04775.x.

Chen, F.P., P. Han, Z.Z. Zhang, J.L. Liu, and X.L. Liu. 2010. Studies on antifungal activity of fungicide 5-(4-chlorophenyl)-2,3- dimethyl-3-(pyridine-3)-oxazoline against botrytis cinerea. J. Pestic. Sci 1(12):42–48. doi: 10.1080/00949651003724790.

Cruz, K.S., E.S. Lima, M.J.A. Silva, E.S. Souza, A. Montoa, A.M. Pohl, and Souza. 2019. Screening and antifungal activity of a β -Carboline derivative against Cryptococcus neoformans and C. gattii. Int J Microbiol 2019:1–8. doi: 10.1155/2019/7157845.

Da Rocha Neto, A.C., M. Maraschin, and R.M. Di Piero. 2015. Antifungal activity of salicylic acid against Penicillium expansum and its possible mechanisms of action. Int. J. Food Microbiol 215:64–70. doi: 10.1016/j.ijfoodmicro.2015.08.018.

Di Giorgio, C., F. Delmas, E. Ollivier, R. Elias, G. Balansard, and P. Timon-David. 2004. In vitro activity of the beta-carboline alkaloids harmane, harmine, and harmaline toward parasites of the species Leishmania infantum. Exp. Parasitol 14:479–500. doi: 10.1016/j.exppara.2004.04.002.

Elad, Y., and K. Evensen. 1995. Physiological aspects of resistance to Botrytis cinerea. Phytopathology. 85:637–643. doi: 10.1094/Phys-85-637.

Frost, D., B. Meechovoet, T. Wang, S. Gately, M. Giorgetti, I. Shcherbakova, T. Dunkley, and E.M.C. Skoulakis. 2011. Beta-Carboline Compounds, including harmine, inhibit DYRK1A and Tau phosphorylation at multiple Alzheimer’s disease-related sites. PLoS One 6(5):e19264. doi: 10.1371/journal.pone.0019264.
Mohammadi, A., M. Hashemi, and S.M. Hosseini. 2015a. Nanoencapsulation of Zataria multiflora essential oil preparation and characterization with enhanced antifungal activity for controlling Botrytis cinerea, the causal agent of gray mould disease. Innov. Food Sci. Emerg 28:73–80. doi: 10.1016/j.ifset.2014.12.011.

Mohammadi, A., M. Hashemi, and S.M. Hosseini. 2015b. Comparison of antifungal activities of various essential oils on the Phytophthora drechsleri, the causal agent of fruit decay. Iran. J. Microbiol 7(1):31–37. doi: 10.1002/irho.19600450307.

Mohammadi, A., M. Hashemi, and S.M. Hosseini. 2016. Integration between chitosan and Zataria multiflora or Cinnamomum zeylanicum essential oil for controlling Phytophthora drechsleri, the causal agent of cucumber fruit rot. LWT-Food Sci Technol. 65:349–356. doi: 10.1016/j.lwt.2015.08.015.
Moura, D.J., M.F. Richter, J.M. Boeira, J.A.P. Henriques, and J. Saffi. 2007. Antioxidant properties of beta-carboline alkaloids are related to their antimutagenic and antigenotoxic activities. Mutagenesis 22(4):293–302. doi: 10.1093/mutage/gem016.

Nenaah, G. 2010. Antibacterial and antifungal activities of (beta)-carboline alkaloids of Peganum harmala (L) seeds and their combination effects. Fitoterapia 81(7):779–782. doi: 10.1016/j.fitote.2010.04.004.

Pourahmad, J., and P.J. O’Brien. 2000. A comparison of hepatocyte cytotoxic mechanisms for Cu²⁺ and Cd²⁺. Toxicology 143(3):263–273. doi: 10.1016/S0300-483X(99)00178-X.

Qing, S.H., and B.S. Fang. 2011. Recent progress in research of NADH oxidase. J Huaqiao Univ (Nat. Sci) 32(5):554–559.

Ritter, J., A. Golitquer, J.P. Salaün, T. Tonon, J.A. Correa, and P. Potin. 2008. Copper stress induces biosynthesis of octadecanoid and eicosanoid oxygenated derivatives in the brown algal kelp Laminaria digitata. New Phytol 180(4):809–821. doi: 10.1111/j.1469-8137.2009.03157.x.

Seifert, A., L.A. Allan, and P.R. Clarke. 2008. DYRK1A phosphorylates caspase 9 at an inhibitory site and is potently inhibited in human cells by harmane. FEBS J 275(24):6268–6280. doi: 10.1111/j.1742-4658.2008.06751.x.

Sitz, J.H., K. Baumgärtel, B. Hämmerle, C. Papadopoulos, P. Hekerman, F.J. Tejedor, W. Becker, and B. Lutz. 2008. The down syndrome candidate dual-specificity tyrosine phosphorylation-regulated kinase 1A phosphorylates the neurodegeneration-related septin 4. Neuroscience 157(3):596–605. doi: 10.1016/j.neuroscience.2008.09.034.

Song, Y.C., D. Kesuma, J. Wang, Y. Deng, J.N. Duan, J.H. Wang, and R.Z. Qi. 2004. Specific inhibition of cyclin-dependent kinases and cell proliferation by harmine. Biochem. Biophys. Res. Comm 317(1):128–132. doi: 10.1016/j.bbrc.2004.03.019.

Stokke, T.S. 1999. “Rational hope” in the early treatment of Parkinson’s disease. Can. J. Physiol. Pharm 77(6):375–382. doi: 10.1139/cpp-77-6-375.

Su, Z., M. Hu, Z. Gao, M. Li, Z. Yun, Y. Pan, Z. Zhang, and Y. Jiang. 2019. Apple polyphenols delay senescence and maintain edible quality in litchi fruit during storage. Postharvest Biol Tec. 157:110976. doi: 10.1016/j.postharvbio.2019.110976.

Tao, N.G., Q.L. Ou Yang, and L. Jia. 2014. Citral inhibits mycelial growth of Penicillium italicum by a membrane damage mechanism. Food Control. 41:116–121. doi: 10.1016/j.foodcont.2014.01.010.

Tian, J., X.Q. Ban, H. Zeng, J.S. He, Y.X. Chen, Y.W. Wang, and B. Lightowlers. 2012. The mechanism of antifungal action of essential oil from dill (Anethum graveolens L.) on aspergillus flavus. PloS one 7(1):e30147. doi: 10.1371/journal.pone.0030147.

Turnipseed, S.B., and H. Jayasuriya. 2020. Analytical methods for mixed organic chemical residues and contaminants in food. Anal. Bioanal. Chem 412(24):5969–5980. doi: 10.1007/s00216-020-02668-8.

Wang, H.C., H.Y. Sun, G. Stammiller, J.X. Ma, and M.G. Zhou. 2010. Generation and characterization of isolates of penicillium litchii resistant to carboxylic acid amide fungicides. Phytopathology 100(5):522–527. doi: 10.1094/PHYTO-100-5-0522.

Yan, J.D., and X.R. Zhu. 2002. Postharvest diseases of litchi and the research progress on their control. Int. J. Fruit Sci 19:128–131. doi: 10.13925/j.cnki.gxbz.2002.02.014.

Yan, L., and R. Sohal. 1998. Mitochondrial adenine nucleotide translocase is modified oxidatively during aging. P Natl Acad Sci USA 95(22):12896–12901. doi: 10.1073/pnas.95.22.12896.

Yang, J.C., J.B. Zhang, B.S. Chai, and C.L. Liu. 2008. Progress of the development on the novel amides fungicides. Agrochemicals 47(1):6–9.
Yi, C., H.X. Qu, Y.M. Jiang, J. Shi, X.W. Duan, D.C. Joyce, and Y.B. Li. 2008. ATP-induced changes in energy status and membrane integrity of harvested litchi fruit and its relation to pathogen resistance. Phytopathology 156(6):365–371. doi: 10.1111/j.1439-0434.2007.01371.x.

Yuan, Z., G. Cong, and J. Zhang. 2014. Effects of exogenous salicylic acid on polysaccharides production of *Dendrobium officinale*. S. Afr. J. Bot. 95:78–84. doi: 10.1016/j.sajb.2014.08.007.

Zhang, J., F. Zhu, M. Gu, H. Ye, L. Gu, L. Zhan, C. Liu, C. Yan, and G. Feng. 2021. Inhibitory activity and action mechanism of coumoxystrobin against *Phytophthora Litchii*, which causes litchi fruit downy blight. Postharvest Biol Tec. 181:111675. doi: 10.1016/j.postharvbio.2021.111675.

Zhao, L., K. Wang, K. Wang, J. Zhu, and Z. Hu. 2020. Nutrient components, health benefits, and safety of litchi (Litchi chinensis Sonn.): A review. Compr. Rev. Food Sci. Food Saf 19(4):2139–2163. doi: 10.1111/1541-4337.12590.