INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
19F–144/2020/23–1–75–81
DOI: 10.17957/IJAB/15.1260
http://www.fspublishers.org

Full Length Article

Inhibition of Ethanol Extracts of *Psoralea corylifolia* and *Sophora flavescens* on *Phytophthora nicotianae* and their Compound Effect

Qiang Li[1], Mei-Ling Dai[1], Yan Xie[2], Chen-Bing Yan[1], Jia-Hong Liu[2], Yun-Ping Zhang[1], Bing-Fu Yao[1] and Yi-Yang Zhang[1*]

[1]*College of Agronomy, Hunan Agriculture University, Changsha, China 410128*
[2]*Qujing Branch of Yunnan Provincial Tobacco Company, Qujing, Yunnan, China 655000*
[*]For correspondence: cnhncs@163.com; zqiangli@126.com
Received 19 August 2019; Accepted 31 August 2019; Published 08 January 2020

Abstract

Phytophthora nicotianae is pathogen of tobacco black shank disease, leading to great economic losses each year. At present, controlling of tobacco black shank disease mainly depends on chemical fungicides, which may cause environmental and health problems. In this research, we carried out several experiments to investigate the effects of ethanol extracts from *Psoralea corylifolia* and *Sophora flavescens* on mycelial growth, sporangium and zoospore release of *P. nicotianae*, hoping to provide a new method for the control of tobacco black shank disease. The results showed that, extract of *P. corylifolia* and *S. flavescens* both showed good antifungal effects on *P. nicotianae*, with mycelial growth inhibition rates of 95.95% and 92.12% at 40 mg/mL and the EC_{50} values were 1.96 mg/mL and 6.37 mg/mL, respectively. The concentration of *P. corylifolia* extract completely inhibited sporangium production and zoospores release was 1.96 mg/mL and 0.98 mg/mL and the values of *S. flavescens* extract were 12.74 mg/mL and 6.37 mg/mL, which were much lower than 40 mg/mL. This phenomenon suggests that inhibitory effects of two plant extracts on *P. nicotianae* may mainly achieved by inhibiting sporangia production and zoospore release. The mycelium morphology was damaged in different degrees after treated by plant extracts, as extract of *P. corylifolia* led to wall rupture and mycelial adhesion, while extract of *S. flavescens* led to mycelial enlargement and distortion. The combinations showed better antifungal effect than single agent, which may be due to more diverse antifungal components in combinations.

Keywords: *P. nicotianae*; Plant extracts; Inhibition; Mycelial growth; Sporangial production; Zoospore release

Introduction

Tobacco black shank, caused by the infection of *Phytophthora nicotianae*, is a devastating soil-borne disease of flue-cured tobacco worldwide, leading to great economic losses each year (Zhang *et al.* 2017). *P. nicotianae* mainly distributed within 0–5 cm of soil surface and infected tobacco plants mainly from stem base and root. Black spots appeared at base of infected tobacco stem and extended around whole stem, and the leaves of the infected plant turned yellow from the bottom up. After rain encounters hot sun and high temperature, whole plant leaf withers suddenly, when serious in lower leaf often produces circular black spot, come on later period pith ministry is dry shrink show "disc shape", finally whole tobacco plant dies. Sporangium is the main reproductive structure in asexual reproduction of *P. nicotianae*, which can release zoospores under natural conditions, and sporangium and zoospores can be spread by rain or irrigation water to cause reinfection (Tan *et al.* 2018). *P. nicotianae* can live in soil for a long time in form of mycelium, sporangium and zoospore, especially in the presence of host residual tissues, which can live more than 3 years.

Chemical fungicides are the main means of controlling tobacco black shank disease, such as metalaxyl, dimethomorph, aliette, dexon and their mixture (Cui *et al.* 2018). Continuous use of chemical fungicides will cause environmental pollution, pesticide residues, and also lead to fungicide resistance of *P. nicotianae* (Hu *et al.* 2008; Cui *et al.* 2018). Due to the continuous variation of *P. nicotianae* and its ability of surviving in the soil without tobacco plants for many years, it is difficult to control black shank though use of host resistance (Li *et al.* 2017; Gallup *et al.* 2018), as well as Rotation and field hygiene management (Sullivan *et al.* 2005; Fang *et al.* 2016; Tan *et al.* 2018). With the improvement of people's awareness of environmental protection and the increase of demand for pollution-free agricultural products, the research on antibacterial activity of plant extracts and fungicidal pesticides from plant sources has become the focus of scholars. Mycelial growth of

To cite this paper: Li Q, ML Dai, Y Xie, CB Yan, JH Liu, YP Zhang, BF Yao, YY Zhang (2020). Inhibition of ethanol extracts of *Psoralea corylifolia* and *Sophora flavescens* on *Phytophthora nicotianae* and their compound effect. *Intl J Agric Biol* 23:75–81

Pythium ultimum was remarkably inhibited by crude methanol extract from stems of *V. agnus-castus*. Both methanolic crude and Purified extract of *Eclipta alba* revealed antifungal activity against Sorghum Pathogens, e.g., *Fusarium thapsinum*, *Alternaria alternata*, *Epicoccum sorghinum*, and *Curvularia lunata* (Boregowda *et al*. 2019). Dichloromethane extracts from *Desmos chinensis* exhibited high inhibitory activity against *Rhizoctonia solani*, pathogenic bacteria of rice sheath blight (Plodpai *et al*. 2013) and wild pepper extract successfully controlled *A. alternata* on post-harvest cherry tomato (Pane *et al*. 2016).

Psoralea corylifolia L. is a leguminous annual herb; its seed commonly used as medicine to treat osteoporosis (Weng *et al*. 2015), fatty liver (Seo *et al.* 2016) and diabetes (Seo *et al*. 2014) and has been proved to have an excellent inhibitory effect on many kinds of plant pathogens (Guan *et al*. 2007). Root of *Sophora flavescens* is a Chinese traditional medicine and has been proved to have anti-tumor (Huang *et al*. 2019), antiviral (Zhang *et al*. 2018) and anti-inflammatory (Ma *et al*. 2018) effects, also show significant inhibition on plant bacterial and fungal pathogens (Ni *et al*. 2017; Pan *et al*. 2019). The inhibitory effects of *P. corylifolia* and *S. flavescens* on *P. nicotianae* are rarely reported. The effects of ethanol extract from *P. corylifolia* and *S. flavescens* on mycelium growth, sporangium and zoospore release of *P. nicotianae* were studied in this experiment. This study provides a theoretical reference for the research and development of plant-based pesticides for tobacco black shank.

Materials and Methods

Fungal pathogen

P. nicotianae (labeled with GFP) used in this experiment was obtained from 'Key Laboratory of Crop Stress Biology in Arid Areas' led by Dr. Shan Weixing at the Northwest Agriculture and Forestry University in China.

Plant material

The seed of *P. corylifolia* was collected from hillside with a warm and humid climate in Xishuangbanna, China. Fresh root of *S. flavescens* was collected from farm located in Enshi, China. Seed and root were air dried at indoor temperature, then crushed to fine powder. Plant powder (10 g) was added into 100 mL ethanol (95%) and was extracted by using ultrasonic method for 30 min. The mixture of ethanol and powder was filtered for 3 times to obtain filtrate. The filtrate was concentrated by vacuum rotary evaporator to obtain paste, which was fixed in volume to l g/mL and kept at 4℃ for later use.

In vitro antifungal experiment

The mycelium growth rate method was used to determine inhibitory effects of plant extracts on *P. nicotianae* according to the method proposed in the references by (Svecova *et al*. 2013). Under aseptic conditions, the above ethanol extracts were thoroughly mixed with OA culture medium sterilized and cooled to about 50℃. 5 concentrations (40, 20, 10, 5, 1 mg/mL) containing ethanol extract medium with a volume of 20 mL were finally prepared. Culture dish added the same amount of ethanol (The final concentration of ethanol in culture dish was 3.8%) solution alone served as control (CK). The experiment was conducted twice.

Mycelium growth inhibition test was conducted by inoculating in the center of each petri dish 1 piece of 0.5 cm agar with mycelium cut from the edge of fungal colonies. The diameter of colonies was measured by the cross method after culture in a 26ºC incubator for 5 days. All treatments and controls were replicated 4 times. The percentage of inhibition was calculated by formula as follows:

$$Percentage\ of\ growth\ inhibition(\%) = \frac{(D-0.5cm)-(d-0.5cm)}{(D-0.5cm)} \times 100 \quad (1)$$

Where *D* and *d* represent the colony diameter of treatments and control, respectively.

Toxicity calculation method: The percentage of growth inhibition was converted into probability value by checking the biostatistical probability value conversion table. Toxicity regression equation was established as: $y=a+bx$, and the EC_{50} (median effect concentration) was calculated. Where *y* represents the probability value of colony growth inhibition rate and *x* represent Logarithms of different mass concentrations of extracts, respectively.

In vitro sporangium germination and zoospore release test

In vitro experiment was conducted to determine the inhibitory effects on sporangium germination and zoospore release according to the method proposed by Mulugeta *et al.* (2019). Under aseptic conditions, ethanol extract was evenly mixed with sterilized 10%V8 culture solution. Five treatment concentrations ($1/2EC_{50}$, $1EC_{50}$, $2EC_{50}$ and $3EC_{50}$) containing ethanol extract fluid medium were finally prepared. Control was a negative control with sterile water (CK). The experiment was repeated twice.

Ten pieces of fresh cultured mycelia of *P. nicotianae* (about 2×2 mm in size) were transferred to 10% V8 fluid medium ethanol extract (including control), and cultured in 26ºC incubators for 3 days at dark conditions. Mycelium was washed with sterile water after culture solution was poured, and suspended by adding 20 mL sterile water in petri dish and then was cultured in a 26ºC incubator after adding 5 drops of the filtered and sterilized soil extraction. After that, changed the water every 12 h and added soil extract, the number of sporangia was observed under 10×20 optical

microscope after 4 times of water changing, while each colony was randomly examined in 3 fields.

Mycelium stimulated by the above method and producing a large number of sporangia were placed in a refrigerator at 4ºC for 30 min, then treated in incubator at 26ºC for 30 min until the zoospores were released. The suspension of 4 L zoospores was absorbed into clean glass slides with a micro-sampler and dragged into short thin bands. The number of zoospores was calculated under a 10×10 optical microscope and repeated 4 times. The following formula was used to calculate the Zoospore concentration (zoospores/mL) of suspension:

$$Zoospore\ concentration = \frac{Mean\ number\ of\ zoospores\ in\ 4\mu L\ suspension}{4} \times 1000$$

Observation of mycelium morphology by electron microscope

P. nicotianae was cultured using the same method as above. An experiment with 2 treatments (extracts of *P. corylifolia* and *S. flavescens* with a concentration of EC_{50}) was carried out, control with the same amount of 95% ethanol (CK). After culture in a 26℃ incubator for 5 days, mycelium was cut from the edge of fungal colonies. The morphological changes of mycelia were observed under SEM according to the method proposed by Plodpai *et al.* (2013).

Evaluation of antifungal effect of ethanol extracts compound *in vitro*

According to Yang *et al.* (2013), Synergistic ratio (SR) was used to determine antifungal activity of different complex combinations. SR<0.5 was antagonistic, SR=0.5~1.5 was additive and SR > l.5 was synergistic. Five compounds (1:9, 3:7, 5:5, 7:3, 9:1) (v/v) were prepared by mixing the ethanol extract mother liquor prepared above by volume ratio. Five concentrations (10, 7.5, 5, 2.5, 1 mg/mL) containing ethanol extract medium with 20 mL were finally prepared according to the above method, control with the same amount of 95% ethanol (CK). The mycelium growth rate method was applied to determine the inhibitory effects of ethanol extracts compound on *P. nicotianae* according to Svecova (Svecova *et al*. 2013). The experiment was repeated twice.

Percentage of inhibition of each compound to *P. nicotianae* was calculated according to formula (1), the measured value of EC_{50} was calculated by the toxicity regression equation and the theoretical EC_{50} was calculated by the following formula:

$$y_1 = \frac{a+b}{a/P + \mathrm{b/S}}$$

Where y_1 represent theoretical $EC_{50,}$ a and b represent the volume fraction of the two components in the mixture; B and S represent the EC_{50} of extracts of *P. corylifolia* and *S. flavescens*.

The synergistic ratio (SR) was calculated by the following formula:

$$SR = \frac{y_1}{y_2}$$

Where y_1 and y_2 represent theoretical EC_{50} and measured EC_{50}.

Statistical data analyses

The data were analyzed by means of One-way analysis of variance (ANOVA). Significance of the treatments was tested using Least Significant Difference (LSD) with 95 and 99% confidence level. The analysis was done by SPSS 22.0.

Results

Inhibitory effect of plant extracts on *P. nicotianae*

The ethanol extracts of the two plants showed significant inhibitory effects on mycelium growth of *P. nicotianae* and the inhibition rate increased with the rise of concentration (Fig. 1). Ethanol extract of *P. corylifolia* showed a better Inhibitory effect on *P. nicotianae*, with an inhibition rate of 95.95% and 45.05% at concentration of 40 mg/mL and 1 mg/mL, respectively. The antifungal effect of 40 mg/mL *S. flavescens* ethanol extract was similar to *P. corylifolia*. With the decrease of concentration, the antifungal effect of *S. flavescens* ethanol extract decreased significantly and the inhibition rate of 1 mg/mL *S. flavescens* extract was only 24.10%. The results showed that ethanol extracts of two plants had good antifungal effects and could effectively inhibit mycelium growth of *P. nicotianae*.

According to inhibition rates of five concentrations of extracts of two plants on *P. nicotianae*, toxicity regression equations were obtained (Table 1). The determination coefficients of toxicity regression equations were above 0.8, which indicating that regression equations had a high degree

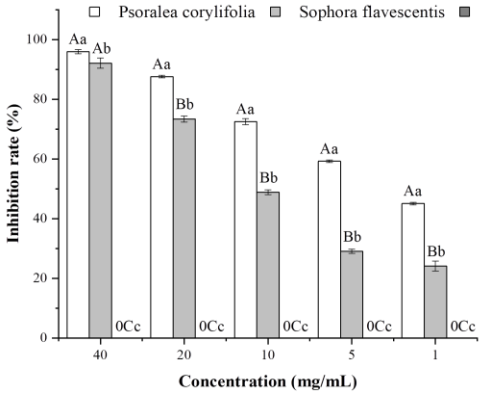

Fig. 1: Inhibitory effect of 2 plant extracts on *P. nicotianae*
Note: Different capital and small letters following data represent a significant level at 0. 05 and 0. 01, respectively (LSD determination). The follows are as the same

Table 1: Comparison of toxicity of 2 plant extracts on *P. nicotianae*

Plant extract	Toxicity regression equation	R^2	EC_{50}(mg/mL)
P. corylifolia	y=1.1523x+4.662	0.9065	1.96
S. flavescentis	y=1.3009x+3.9537	0.8342	6.37

Table 2: Effect of plant extracts on sporangium production of *P. nicotianae*

P. corylifolia (mg/mL)	Sporangium yield* (spores)	Inhibition rate (%)	*S. flavescentis* (mg/mL)	Sporangium yield(spores)	Inhibition rate (%)
0.5×EC_{50}0.98	0.03±0.02 B	96.03±1.64A	0.5×EC_{50}(3.19)	0.18±0.03B	77.43±3.72B
1×EC_{50} (1.96)	0.00±0.00 B	100.00±0.00A	1×EC_{50}(6.37)	0.03±0.01C	96.29±2.93A
2×EC_{50} (3.92)	0.00±0.00 B	100.00±0.00A	2×EC_{50}(12.74)	0.00±0.00C	100.00±0.00A
3×EC_{50} (5.88)	0.00±0.00 B	100.00±0.00A	3×EC_{50}(19.11)	0.00±0.00C	100.00±0.00A
CK(0.00)	0.72±0.11A	0.00±0.00B	CK (0.00)	0.72±0.11A	0.00±0.00 C

Note: * Sporangium yield of single mycelium in single view

Table 3: Effect of plant extracts on zoospore release of *P. nicotianae*

P. corylifolia (mg/mL)	Zoospore density(spores/mL)	Inhibitionrate(%)	*S.flavescentis*(mg/mL)	Zoospore density (spores/mL)	Inhibition rate (%)
0.5×EC_{50}(0.98)	0.00±0.00B	100.00±0.00A	0.5×EC_{50}(3.19)	417.00±0.00B	98.48.00±0.52A
1×EC_{50} (1.96)	0.00±0.00B	100.00±0.00A	1×EC_{50}(6.37)	0.00±0.00B	100.00±0.00A
2×EC_{50} (3.92)	0.00±0.00B	100.00±0.00A	2×EC_{50}(12.74)	0.00±0.00B	100.00 ±0.00A
3×EC_{50} (5.88)	0.00±0.00B	100.00±0.00A	3×EC_{50}(19.11)	0.00±0.00B	100.00±0.00A
CK(0.00)	27500.00±3699.00A	0.00±0.00B	CK(0.00)	27500.00±0.00A	0.00±0.00B

of fitting. The EC_{50} values of ethanol extracts from two plants were calculated according to the toxicity regression equation (Table 1), the EC_{50} value of ethanol extracts from *P. corylifolia* was 1.96 mg/mL, and the EC_{50} value of *S. flavescentis* was 6.37 mg/mL.

Effects of ethanol extracts from two plants on sporangium production of *P. nicotianae*

Ethanol extracts from two plants significantly inhibited the sporangium production of *P. nicotianae* ($P < 0.01$) and inhibition rate increased with increasing of concentration of extracts (Table 2). Ethanol extracts of *P. corylifolia* show better inhibitory effect on sporangium yield, reaching 96.03% at 1/2EC_{50} (0.98 mg/mL) and 100% at 1EC_{50} (1.96 mg/mL).

Effects of two plant extracts on the release of zoospores of *P. nicotianae*

Ethanol extracts from the two plants inhibited the release of zoospores of *P. nicotianae* significantly ($P < 0.01$) (Table 3). The ethanol extract of *P. corylifolia* showed improved inhibition effect on the release of zoospores, with an inhibition rate of 100% at 1/2EC50 (0.98 mg/mL). The ethanol extract of *S. flavescens* with a concentration of 6.37 mg/mL (1EC_{50}) inhibited the release of zoospores of *P. nicotianae* by 100%. The results showed that ethanol extracts of two plants could significantly inhibit release of zoospores at low concentration.

Effects of plant extracts on mycelial morphology of *P. nicotianae*

Compared with the control group, the morphology of mycelia of *P. nicotianae* was significantly deformed by ethanol extracts from two plants. The mycelium morphology of control group was normal (Fig. 2A and B) and mycelium was thin, uniform, smooth and non-septal. Mycelial adhesion (Fig. 2D) and mycelial wall rupture (Fig. 2C) occurred in *P. nicotianae* treated with *P. corylifolia* extract. Mycelium of *P. nicotianae* treated with ethanol extract of *S. flavescentis* were enlarged (Fig. 2E) and distorted (Fig. 2F). Data showed that ethanol extracts from plants had noticeable destructive effect on the mycelia growth of *P. nicotianae*.

In vitro antifungal effect of ethanol extracts compounds

The optimal compound ratio of *P. corylifolia* and *S. flavesne* extract was 9:1(v:v) and the antifungal rate was still 40.72% at the lowest treatment concentration (1 mg/mL), followed by 1:9, which is meaningfully better than other three combinations. The toxicity regression equation was obtained based on the inhibition rate of each combination (Table 4). The determination coefficients of the toxicity regression equation were all above 0.9, indicating that regression equation had a high degree of fitting. According to toxicity regression equation, the EC_{50} values of each combination were calculated (Table 4). The EC_{50} value of combination 9:1 was the smallest, followed by combination 1:9. The synergistic ratio (SR) of the five compound combinations of *P. corylifolia* and *S. flavesne* was greater than 0.5, indicating that antifungal effect of compound combination was better than single agent. The SR value of combination 1:9 was greater than 1.5, shown a synergistic effect, while the other combinations showed an additive effect.

Discussion

Though Chemical fungicides are effective methods to control fungal diseases, continuous use of chemical fungicides will cause environmental and health problems

Table 4: Synergistic evaluation on the inhibition effect of plant extracts combination on *P. nicotianae*

Compound ratio(v/v)	Concentration (mg/mL)	Mycelial growth diameter (cm)	Inhibition rate (%)	Toxicity regression equation	**Measured** EC_{50} (mg/mL)	Theoretical EC_{50} (mg/mL)	Synergistic ratio
1:9	10	1.45s	81.65b	y=1.4785x+4.21	3.42	5.20	1.52
	7.5	2.72o	65.61f	R^2=0.9419			
	5	3.62k	54.22j				
	2.5	4.78fg	39.45no				
	1	5.98c	24.26r				
3:7	10	3.12m	60.55s	y=1.0731x+4.0957	6.96	3.80	0.55
	7.5	3.83j	51.48k	R^2=0.9608			
	5	4.85f	38.61o				
	2.5	5.43d	31.22q				
	1	6.35b	19.62s				
5:5	10	2.83n	64.14g	y=1.1712x+4.1008	5.86	3.00	0.51
	7.5	3.52k	55.49j	R^2=0.9690			
	5	4.62h	41.56m				
	2.5	5.33d	32.49q				
	1	6.33b	19.83s				
7:3	10	2.25q	71.52d	y=1.1178x+4.3368	3.92	2.47	0.63
	7.5	2.78no	64.77fg	R^2=0.9109			
	5	4.10i	48.10l				
	2.5	5.05e	36.08p				
	1	6.08c	23.00r				
9:1	10	1.05t	86.71a	y=1.2710x+4.7036	1.71	2.11	1.23
	7.5	1.77r	77.64c	R^2=0.9731			
	5	2.37p	70.04e				
	2.5	3.38l	57.17i				
	1	4.68gh	40.72mn				
CK	0	7.40a	0.00t				

(Hu *et al.* 2008; Cui *et al.* 2018). Consequently, the search for eco-friendly alternative fungicides has become the research hotspot of scholars. With the aim of reducing risk of chemical fungicides to environment and health, soil disinfection (Serrano-Perez *et al.* 2017; Zhou *et al.* 2019), biological control (Bagy *et al.* 2019; Myo *et al.* 2019), plant extracts (Ncama *et al.* 2019; Boregowda *et al.* 2019), rational rotation (Stewart *et al.* 2014), resistance induction (Swain *et al.* 2019; Wang *et al.* 2019) and disease resistance breeding (Songsomboon *et al.* 2019) have been used to manage crop fungal diseases.

In recent years, there are many reports of plant extracts in control of fungal, bacterial and viral diseases. Jing *et al.* (2017) conveyed that eugenol extract from *Syringa oblata* exhibited strong antifungal activity *in vitro* on *P. nicotianae*, by destroying the integrity of mycelial and spore membranes. Further *in vivo* experiments established that Eugenol preparation reduced the occurrence of tobacco black shank disease. Yuan *et al.* (2012) testified that *Toxicodendron syivestre* methanol extract showed significant inhibitory effects *in vitro* and *in vivo*, and methyl gallate was proved to be the main antibacterial active compound. The EC_{50} of methyl gallate on *R. solanacearum* was 8.3 mg/L. *In vivo* experiments on tomato plants showed that, at the concentration of 0.2 g/L, methyl gallate showed comparable effect in controlling bacterial wilt disease with the bactericide streptomycin sulfate at the concentration of 0.5 g/L. Vuko *et al.* (2019) described that there is a reduction of virus infection in the local and systemic host plants, by treated with essential oil of *Micromeria croatica* Schott before inoculation. β-caryophyllene and caryophyllene oxide from extract from *Micromeria croatica* which have been proved to be main

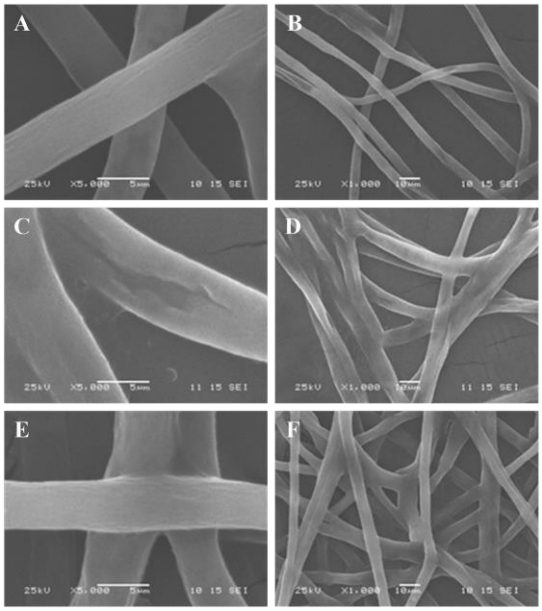

Fig. 2: Scan electron microscopy micrographs of *P. nicotianae*. Control with 3.8% ethanol, showing healthy mycelia (**A** and **B**). The mycelia treated with the *P. corylifolia* extract at a concentration of $_{EC50}$ (1.96 mg/mL) shows mycelial wall rupture (**C**) and mycelial adhesion (**D**). The mycelia treated with the *S. flavescentis* extract at a concentration of EC_{50}(6.37 mg/mL) were enlarged (**E**) and distorted (**F**)

active ingredient, activating plant defense through GA and JA signalling pathways.

The present study showed that, ethanol extracts from *P. corylifolia* and *S. flavesne* inhibited the growth of *P. nicotianae* in different degrees. The *P. corylifolia* extracts showed a higher effect in inhibiting mycelial growth of the pathogen compared with *S. flavesne* extract, with EC_{50} values as 1.96 mg/mL and 6.37 mg/mL, respectively. Many scholars have confidence in that inhibition of plant extracts on pathogens and disease control may due to secondary metabolites in plants. (Aldehydes, phenolic, alcohols, alkaloids, flavonoids and terpenoids), Which control plant diseases through increase cell membrane permeability (Jing *et al*. 2017), disrupt the cell membrane (Elshafie *et al*. 2019), suppressed conidia germination (Pane *et al*. 2016), activating plant defense (Vuko *et al*. 2019), reduced sclerotial germination (Ma *et al*. 2016), affect biofilm structural development through reducing polysaccharide and protein synthesis in the EPS matrix (Catto *et al*. 2019).

In this study, two plant extracts had significant inhibitory effects on sporangium production and zoospore release of *P. nicotianae* and the inhibitory effects were concentration-dependent. It is noteworthy that inhibiting effect of two plant extracts on release of zoospores is better than sporangia production. Sporangia production and zoospore release were inhibited by 100%, at the *P. corylifolia* extract concentration of 1.96 mg/mL and 0.98 mg/mL and at the *S. flavesne* extract concentration of 12.74 mg/mL and 6.37 mg/mL, which was far below 100% inhibitory concentration of mycelial growth. This phenomenon suggests that inhibitory effects of two plant extracts on *P. nicotianae* may have been achieved by inhibiting sporangia production and zoospore release. The results of the scanning electron microscope revealed some of inhibitory mechanisms of two plant extracts. The mycelium morphology was damaged in different degrees after treated by plant extracts, such as local swelling and distortion of mycelium, mycelial adhesion, and mycelial wall rupture. *P. corylifolia* extract can destroy the mycelium of Phytophthora tobacco at low concentration, and cause mycelium breakage and disruption, which indicates that *P. corylifolia* extract has lethal effect on the mycelium of *P. nicotianae*. *S. flavescens* extract has no lethal effect, but it can inhibit the growth of colonies by distorting mycelium expansion and distorting adhesion. Similar results also be taken in previous studies (Ma *et al*. 2016).The antifungal effect of the compound combination was better than that of the single agent, that may be due to more diverse antifungal components in combinations (Wang *et al*. 2018).

Conclusion

The antifungal effects of *P. corylifolia* and *S. flavescens* extracts on *P. nicotianae* were evaluated *in vitro*, nevertheless, the mechanism of antifungal activity has not been elucidated, preliminary. In addition, *in vivo* antifungal and field experiments should be carried out to provide more robust support for further applications.

Acknowledgements

The authors acknowledge the financial grants from the Education Department of Hunan Province (SCX1840 and CX20190515) and Hunan Agricultural University (XCX18082).

References

Bagy HMMK, EA Hassan, NA Nafady, MFA Dawood (2019). Efficacy of *arbuscular mycorrhizal* fungi and endophytic strain *Epicoccum nigrum ASU11* as biocontrol agents against blackleg disease of potato caused by bacterial strain *Pectobacterium carotovora* subspp. *atrosepticum* PHY7. *Biol Cont* 134:103–113

Boregowda RS, N Murali, AC Udayashankar, SR Niranjana, OS Lund, HS Prakash (2019). Antifungal activity of *Eclipta alba* metabolites against *Sorghum Pathogens*. *Plants-Basel*, 8:72–87

Catto C, LD Vincenti, G Borgonovo, A Bassoli, S Marai, F Villa, F Cappitelli, M Saracchi (2019). Sub-lethal concentrations of *Perilla frutescens* essential oils affect phytopathogenic fungal biofilms. *J Environ Manage* 245:264–272

Cui L, P Gao, J Guo, Y Kang, Y Hu (2018). Mating type and sensitivity of *Phytophthora nicotianae* from tobacco to metalaxyl and dimethomorph in Henan province, China. *J Phytopathol* 166:648–653

Elshafie HS, D Grulova, B Baranova, L Caputo, LD Martino, V Sedlak, I Camele, VD Feo (2019). Antimicrobial activity and chemical composition of essential oil extracted from *Solidago canadensis* L. growing wild in Slovakia. *Molecules* 24:1206–1217

Fang Y, L Zhang, Y Jiao, J Liao, L Luo, S Ji, J Li, K Dai, S Zhu, M Yang (2016). Tobacco rotated with rapeseed for soil-borne *Phytophthora Pathogen* biocontrol: mediated by rapeseed root exudates. *Front Microbio*, 7:894–904

Gallup CA, KL McCorkle, KL Ivors, D Shew (2018). Characterization of the black shank pathogen, *Phytophthora nicotianae*, across north carolina tobacco production areas. *Plant Dis* 102:1108–1114

Guan LJ, WY Li, HY Fan, S Shao, B Pan, YH Wu (2007). Screening of plant pesticide and study on antifungal activity of extracts from *Psoralea corylifalia* L. *Agrochemicals* 46:860–862

Hu JH, CX Hong, EL Stromberg, GW Moorman (2008). Mefenoxam sensitivity and fitness analysis of *Phytophthora nicotianae* isolates from nurseries in Virginia, USA *Plant Pathol* 57:728–736

Huang WC, PY Gu, LW Fang, YL Huang, CF Lin, CJ Liou (2019). *Sophora flavanone* G from *Sophora flavescens* induces apoptosis in triple-negative breast cancer cells. *Phytomedicine* 61:152852

Jing C, J Gou, X Han, Q Wu, C Zhang (2017). *In vitro* and *in vivo* activities of eugenol against tobacco black shank caused by *Phytophthora nicotianae*. *Pest Biochem Physiol* 142:148–154

Li Y, K Harris-Shultz, H Wang, PA Wadl, P Ji (2017). Population structure and genetic diversity of *Phytophthora nicotianae* from tobacco in georgia. *Plant Dis* 101:1113–1118

Ma BX, XQ Ban, JS He, B Huang, H Zeng, J Tian, YX Chen, YW Wang (2016). Antifungal activity of *Ziziphora clinopodioides* Lam. essential oil against *Sclerotinia sclerotiorum* on rapeseed plants, *Brassica campestris* L.). *Crop Prot* 89:289–295

Ma H, Q Huang, W Qu, L Li, M Wang, S Li, F Chu (2018). *In vivo* and *in vitro* anti-inflammatory effects of *Sophora flavescens* residues. *J Ethnopharmacol* 224:497–503

Mulugeta T, K Abreha, H Tekie, B Mulatu, M Yesuf, E Andreasson, E Liljeroth, E Alexandersson (2019). Phosphite protects against potato and tomato late blight in tropical climates and has varying toxicity depending on the *Phytophthora infestans* isolate. *Crop Prot* 121:139–146

Myo EM, B Liu, J Ma, L Shi, M Jiang, K Zhang, B Ge (2019). Evaluation of *Bacillus velezensis NKG-2* for bio-control activities against fungal diseases and potential plant growth promotion. *Biol Cont* 134:23–31

Ncama K, A Mditshwa, SZ Tesfay, NC Mbili, LS Magwaza (2019). Topical procedures adopted in testing and application of plant-based extracts as bio-fungicides in controlling postharvest decay of fresh produce. *Crop Prot* 115:142–151

Ni W, C Li, Y Liu, H Song, L Wang, H Song, Q Wang (2017). Various bioactivity and relationship of structure–activity of matrine analogues. *J Agric Food Chem* 65:2039–2047

Pan J, X Hao, H Yao, K Ge, L Ma, W Ma (2019). Matrine inhibits mycelia growth of *Botryosphaeria dothidea* by affecting membrane permeability. *J For Res* 30:1105–1113

Pane C, F Fratianni, M Parisi, F Nazzaro, M Zaccardelli (2016). Control of alternaria post-harvest infections on cherry tomato fruits by wild pepper phenolic-rich extracts. *Crop Prot* 84:81–87

Plodpai P, S Chuenchitt, V Petcharat, S Chakthong, SP Voravuthikunchai (2013). Anti-Rhizoctonia solani activity by *Desmos chinensis* extracts and its mechanism of action. *Crop Prot* 43:65–71

Seo E, YS Oh, HS Jun (2016). *Psoralea corylifolia* L. seed extract attenuates nonalcoholic fatty liver disease in high-fat diet-induced obese mice. *Nutrients* 8:83–94

Seo E, EK Lee, CS Lee, KH Chun, MY Lee, HS Jun (2014). *Psoralea corylifolia* L. seed extract ameliorates streptozotocin-induced diabetes in mice by inhibition of oxidative stress. *Oxid Med Cell Longev* 2014:1–9

Serrano-Perez P, E Rosskopf, AD Santiago, MDC Rodriguez-Molina (2017). Anaerobic soil disinfestation reduces survival and infectivity of *Phytophthora nicotianae* chlamydospores in pepper. *Sci Hortic* 215:38–48

Songsomboon K, R Crawford, J Crawford, J Hansen, J Cummings, N Mattson, G Bergstrom, D Viands (2019). Recurrent phenotypic selection for resistance to diseases caused by *Bipolaris oryzae* in switchgrass (*Panicum virgatum* L.). *Biomass Bioener* 125:105–113

Stewart S, N Abeysekara, AE Robertson (2014). Pathotype and genetic shifts in a population of *Phytophthora sojae* under soybean cultivar rotation. *Plant Dis* 98:614–624

Sullivan MJ, TA Melton, HD Shew (2005). Managing the race structure of *Phytophthora parasitica* var. *nicotianae* with cultivar Rotation. *Plant Dis* 89:1285–1294

Svecova E, S Proietti, C Caruso, G Colla, P Crino (2013). Antifungal activity of *Vitex agnus-castus* extract against *Pythium ultimum* in tomato. *Crop Prot* 43:223–230

Swain H, SK Naik, AK Mukherjee (2019). Comparative analysis of different biotic and abiotic agents for growth promotion in rice (*Oryza sativa* L.) and their effect on induction of resistance against *Rhizoctonia solani*: A soil borne pathogen. *Biol Cont* 133:123–133

Tan J, Q Li, LJ Chen, YY Zhang, Y Zhang, LK Zhou (2018). Study on the migration of *Phytophthora nicotianae* zoospores in the soil. *Intl J Agric Biol* 20:397–403

Vuko E, G Rusak, V Dunkic, D Kremer, I Kosalec, B Rada, N Bezic (2019). Inhibition of satellite RNA Associated cucumber mosaic virus infection by essential oil of *Micromeria croatica* (Pers.) Schott. *Molecules* 24:1342–1354

Wang C, S Yuan, W Zhang, TB Ng, X Ye (2019). Buckwheat antifungal protein with biocontrol potential to inhibit fungal (*Botrytis cinerea*) infection of cherry tomato. *J Agric Food Chem* 67:6748–6756

Wang R, S Wang, W Pan, Q Li, Z Xia, E Guan, M Zheng, G Pang, Y Yang, Z Yi (2018). Strategy of tobacco plant against black shank and tobacco mosaic virus infection *via* induction of PR-1, PR-4 and PR-5 proteins assisted by medicinal plant extracts. *Physiol Mol Plant Pathol* 101:127–145

Weng ZB, QQ Gao, F Wang, GH Zhao, FZ Yin, BC Cai, ZP Chen, WD Li (2015). Positive skeletal effect of two ingredients of *Psoralea corylifolia* L. on estrogen deficiency-induced osteoporosis and the possible mechanisms of action. *Mol Cell Endocrinol* 417:103–113

Yang XP, XD Jiang, JJ Chen, SS Zhang (2013). Control of postharvest grey mould decay of nectarine by tea polyphenol combined with tea saponin. *Lett Appl Microbiol* 57:502–509

Yuan GQ, QQ Li, J Qin, YF Ye, W Lin (2012). Isolation of Methyl Gallate from *Toxicodendron sylvestre* and its effect on tomato bacterial wilt. *Plant Dis* 96:1143–1147

Zhang C, J Gao, T Han, X Tian, F Wang (2017). Integrated control of tobacco black shank by combined use of riboflavin and *Bacillus subtilis* strain Tpb55. *BioControl* 62:835–845

Zhou X, C Li, L Liu, J Zhao, J Zhang, Z Cai, X Huang (2019). Control of *Fusarium wilt* of lisianthus by reassembling the microbial community in infested soil through reductive soil disinfestation. *Microbiol Res* 220:1–11

Zhang YB, D Luo, L Yang, W Cheng, LJ He, GK Kuang, MM Li, YL Li, GC Wang (2018). Matrine-type alkaloids from the roots of *Sophora flavescens* and their antiviral activities against the *Hepatitis B Virus*. *J Nat Prod* 81:2259–2265