



Full Length Article

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

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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 *Phytophthora nicotianae*, hoping to provide a new method for the control of tobacco black shank disease. The results showed that, extract of *Psoralea corylifolia* and *Sophora flavescens* both showed good antifungal effects on *Phytophthora nicotianae*, with mycelial growth inhibition rates of 95.95% and 92.12% at 40 mg/mL and the EC₅₀ values were 1.96 mg/mL and 6.37 mg/mL, respectively. The concentration of *Psoralea corylifolia* extract completely inhibited sporangium production and zoospores release was 1.96 mg/mL and 0.98 mg/mL and the values of *Sophora 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 *Phytophthora 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 *Psoralea corylifolia* led to wall rupture and mycelial adhesion, while extract of *Sophora 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. © 2020 Friends Science Publishers

Keywords: *Phytophthora 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). *Phytophthora 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 *Phytophthora 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). *Phytophthora 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, dexion 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 *Phytophthora nicotianae* (Hu *et al.*, 2008; Cui *et al.*, 2018). Due to the continuous variation of *Phytophthora 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 *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, such as *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 *Alternaria alternate* 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 *Psoralea corylifolia* and *Sophora flavescens* on *Phytophthora nicotianae* are rarely reported. The effects of ethanol extract from *Psoralea corylifolia* and *sophora flavescens* on mycelium growth, sporangium and zoospore release of *Phytophthora 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

The *Phytophthora 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 *Psoralea corylifolia* was collected from hillside with a warm and humid climate in Xishuangbanna, China. Fresh root of *Sophora 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 1 g/mL and kept at 4°C for later use.

In vitro Antifungal Experiment

The mycelium growth rate method was used to determine inhibitory effects of plant extracts on *Phytophthora*

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°C. 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 26°C incubator for 5 days. All treatments and controls were replicated 4 times. The percentage of inhibition was calculated by formula as follows:

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

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 (1/2 EC_{50} , 1 EC_{50} , 2 EC_{50} and 3 EC_{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 *Phytophthora nicotianae* (about 2×2 mm in size) were transferred to 10% V8 fluid medium ethanol extract (including control), and cultured in 26°C 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 26°C 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 4°C for 30 min, then treated in incubator at 26°C 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:

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

Observation of Mycelium Morphology by Electron Microscope

Phytophthora nicotianae was Cultured using the same method as above. An experiment with 2 treatments (extracts of *Psoralea corylifolia* and *Sophora flavescens* with a concentration of EC₅₀) was carried out, control with the same amount of 95% ethanol (CK). After culture in a 26°C 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 > 1.5 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 a volume of 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 *Phytophthora nicotianae* according to Svecova (Svecova *et al.*, 2013). The experiment was repeated twice.

Percentage of inhibition of each compound to *Phytophthora nicotianae* was calculated according to formula (1), the measured value of EC₅₀ was calculated by the toxicity regression equation and the theoretical EC₅₀ was calculated by the following formula:

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

Where y_1 represent theoretical EC₅₀, a and b represent the volume fraction of the two components in the mixture; B and S represent the EC₅₀ of extracts of *Psoralea corylifolia* and *Sophora 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 EC₅₀ and measured EC₅₀.

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 performed by SPSS 22.0.

Results

Inhibitory Effect of 2 Plant Extracts on *Phytophthora nicotianae*

The ethanol extracts of the two plants showed significant inhibitory effects on mycelium growth of *Phytophthora nicotianae* and the inhibition rate increased with the rise of concentration (Fig. 1). Ethanol extract of *Psoralea corylifolia* showed a better Inhibitory effect on *Phytophthora 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 *Sophora flavescens* ethanol extract was similar to *Psoralea corylifolia*. With the decrease of concentration, the antifungal effect of *Sophora flavescens* ethanol extract decreased significantly and the inhibition rate of 1 mg/mL *Sophora 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 *Phytophthora nicotianae*.

According to inhibition rates of five concentrations of extracts of two plants on *Phytophthora 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 of fitting. The EC₅₀ values of ethanol extracts from two plants were calculated according to the toxicity regression equation (Table 1), the EC₅₀ value of ethanol extracts from *Psoralea corylifolia* was 1.96 mg/mL, and the EC₅₀ value of *Sophora flavescens* was 6.37 mg/mL.

Effects of Ethanol Extracts from two Plants on Sporangium Production of *Phytophthora nicotianae*

Ethanol extracts from two plants significantly inhibited the sporangium production of *Phytophthora nicotianae* ($p < 0.01$) and inhibition rate increased with increasing of concentration of extracts (Table 2). Ethanol extracts of *Psoralea corylifolia* show better inhibitory effect on sporangium yield, reaching 96.03% at 1/2EC₅₀ (0.98 mg/mL) and 100% at 1EC₅₀ (1.96 mg/mL).

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

Plant extract	Toxicity regression equation	R ²	EC ₅₀ (mg/mL)
<i>Psoralea corylifolia</i>	y=1.1523x+4.662	0.9065	1.96
<i>Sophora flavescens</i>	y=1.3009x+3.9537	0.8342	6.37

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

<i>Psoralea corylifolia</i> (mg/mL)	Sporangium yield* (spores)	Inhibition rate (%)	<i>Sophora flavescens</i> (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 *Phytophthora nicotianae*

<i>Psoralea corylifolia</i> (mg/mL)	Zoospore density (spores/mL)	Inhibition rate (%)	<i>Sophora flavescens</i> (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

Effects of two Plant Extracts on the Release of Zoospores of *Phytophthora nicotianae*

Ethanol extracts from the two plants inhibited the release of zoospores of *Phytophthora nicotianae* significantly ($p < 0.01$) (Table 3). The ethanol extract of *Psoralea 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 *Sophora flavescens* with a concentration of 6.37 mg/mL (1EC₅₀) inhibited the release of zoospores of *Phytophthora 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 *Phytophthora nicotianae*

Compared with the control group, the morphology of mycelia of *Phytophthora 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 *Phytophthora nicotianae* treated with *Psoralea corylifolia* extract. Mycelium of *Phytophthora nicotianae* treated with ethanol extract of *Sophora flavescens* were enlarged (Fig. 2E) and distorted (Fig. 2F). The results disclosed that ethanol extracts from plants had noticeable destructive effect on the mycelia growth of *Phytophthora nicotianae*.

In vitro Antifungal Effect of Ethanol Extracts Compounds

The optimal compound ratio of *Psoralea corylifolia* and *Sophora flavescens* extract was 9:1(v:v) and the antifungal rate was still 40.72% at the lowest treatment concentration

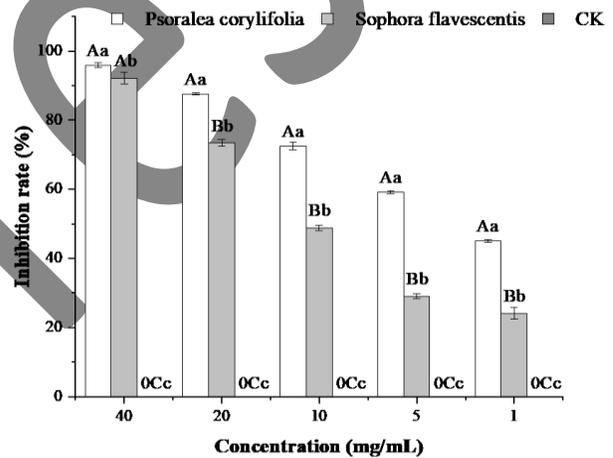


Fig. 1: Inhibitory effect of 2 plant extracts on *Phytophthora nicotianae*

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

(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 EC₅₀ values of each combination were calculated (Table 4). The EC₅₀ value of combination 9:1 was the smallest, followed by combination 1:9. The synergistic ratio (SR) of the five compound combinations of *Psoralea corylifolia* and *Sophora flavescens* 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.

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

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

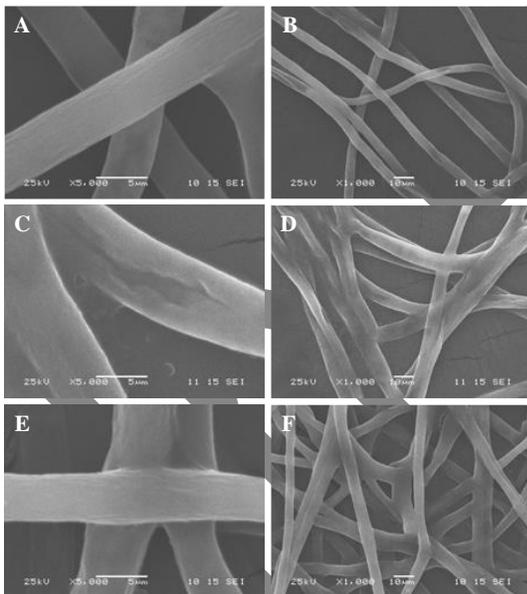


Fig. 2: Scan electron microscopy micrographs of *Phytophthora nicotianae*. Control with 3.8% ethanol, showing healthy mycelia (A and B). The mycelia treated with the *Psoralea corylifolia* extract at a concentration of EC₅₀(1.96 mg/mL) shows mycelial wall rupture (C) and mycelial adhesion (D). The mycelia treated with the *Sophora flavescens* extract at a concentration of EC₅₀(6.37 mg/mL) were enlarged (E) and distorted (F)

Discussion

Though Chemical fungicides are effective methods to control fungal diseases, continuous use of chemical

fungicides will cause environmental and health problems (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 (Shasmita *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 *Phytophthora 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 EC₅₀ 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 active ingredient, activating plant defense through GA and JA signalling pathways.

The present study showed that, ethanol extracts from *Psoralea corylifolia* and *Sophora flavescens* inhibited the growth of *Phytophthora nicotianae* in different degrees. The *Psoralea corylifolia* extracts showed a higher effect in inhibiting mycelial growth of the pathogen compared with *Sophora flavescens* extract, with EC₅₀ 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 *Phytophthora 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 *Psoralea corylifolia* extract concentration of 1.96 mg/mL and 0.98 mg/mL and at the *Sophora flavescens* 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 *Phytophthora 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. *Psoralea corylifolia* extract can destroy the mycelium of *Phytophthora* tobacco at low concentration, and cause mycelium breakage and disruption, which indicates that *Psoralea corylifolia* extract has lethal effect on the mycelium of *Phytophthora nicotianae*. *Sophora 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 *Psoralea corylifolia* and *Sophora flavescens* extracts on *Phytophthora 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.

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References

- Bagy, H.M.M.K., E.A. Hassan, N.A. Nafady and M.F.A. 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* subsp. *atrosepticum* PHY7. *Biol. Contr.*, 134: 103–113
- Boregowda, R.S., N. Murali, A.C. Udayashankar, S.R. Niranjana, O.S. Lund and H.S. Prakash, 2019. Antifungal activity of *Eclipta alba* metabolites against *Sorghum* Pathogens. *Plants (Basel)*, 8: 72–87
- Catto, C., L.D. Vincenti, G. Boronovo, A. Bassoli, S. Marai, F. Villa, F. Cappitelli and 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 and 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, H.S., D. Grulova, B. Baranova, L. Caputo, L.D. Martino, V. Sedlak, I. Camele and V.D. 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 and M. Yang, 2016. Tobacco rotated with rapeseed for soil-borne *Phytophthora* Pathogen biocontrol: mediated by rapeseed root exudates. *Front. Microbiol.*, 7: 894–904
- Gallup, C.A., K.L. McCorkle, K.L. Ivors and D. Shew, 2018. Characterization of the black shank pathogen, *Phytophthora nicotianae*, across north carolina tobacco production areas. *Plant Dis.*, 102: 1108–1114
- Guan, L.J., W.Y. Li, H.Y. Fan, S. Shao, B. Pan and Y.H. Wu, 2007. Screening of plant pesticide and study on antifungal activity of extracts from *Psoralea corylifolia* L. *Agrochemicals*, 46: 860–862
- Hu, J.H., C.X. Hong, E.L. Stromberg and G.W. Moorman, 2008. Mefenoxam sensitivity and fitness analysis of *Phytophthora nicotianae* isolates from nurseries in Virginia, USA. *Plant Pathol.*, 57: 728–736
- Huang, W.C., P.Y. Gu, L.W. Fang, Y.L. Huang, C.F. Lin and C.J. 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 and C. Zhang, 2017. In vitro and in vivo activities of eugenol against tobacco black shank caused by *Phytophthora nicotianae*. *Pestic Biochem. Physiol.*, 142: 148–154
- Li, Y., K. Harris-Shultz, H. Wang, P.A. Wadl and P. Ji, 2017. Population structure and genetic diversity of *Phytophthora nicotianae* from tobacco in georgia. *Plant Dis.*, 101: 1113–1118

- Ma, B.X., X.Q. Ban, J.S. He, B. Huang, H. Zeng, J. Tian, Y. X. Chen and Y.W. 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 and 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 and 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, E.M., B. Liu, J. Ma, L. Shi, M. Jiang, K. Zhang and B. Ge, 2019. Evaluation of *Bacillus velezensis* NKG-2 for bio-control activities against fungal diseases and potential plant growth promotion. *Biol. Contr.*, 134: 23–31
- Ncama, K., A. Mditshwa, S.Z. Tesfay, N.C. Mbili and L.S. 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 and 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 and 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 and 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 and S.P. Voravuthikunchai, 2013. Anti-Rhizoctonia solani activity by *Desmos chinensis* extracts and its mechanism of action. *Crop. Prot.*, 43: 65–71
- Seo, E., E.K. Lee, C.S. Lee, K.H. Chun, M.Y. Lee and H.S. 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
- Seo, E., Y.S. Oh and H.S. Jun, 2016. *Psoralea corylifolia* L. Seed extract attenuates nonalcoholic fatty liver disease in high-fat diet-induced obese mice. *Nutrients*, 8: 83–94
- Serrano-Perez, P., E. Roskopf, A. De Santiago and M.D.C. Rodriguez-Molina, 2017. Anaerobic soil disinfestation reduces survival and infectivity of *Phytophthora nicotianae* chlamydospores in pepper. *Sci. Hortic-Amsterdam*, 215: 38–48
- Shasmita, H. Swain, S.K. Naik and A.K. 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. Contr.*, 133: 123–133
- Songsomboon, K., R. Crawford, J. Crawford, J. Hansen, J. Cummings, N. Mattson, G. Bergstrom and 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. Abeyssekara and A.E. Robertson, 2014. Pathotype and genetic shifts in a population of *Phytophthora sojae* under soybean cultivar rotation. *Plant Dis.*, 98: 614–624
- Sullivan, M.J., T.A. Melton and H.D. 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 and P. Crino, 2013. Antifungal activity of *Vitex agnus-castus* extract against *Pythium ultimum* in tomato. *Crop. Prot.*, 43: 223–230
- Tan, J., Q. Li, L.J. Chen, Y.Y. Zhang, Y. Zhang and L.K. 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 and 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, T.B. Ng and 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 and 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 P.*, 101: 127–145
- Weng, Z.B., Q.Q. Gao, F. Wang, G.H. Zhao, F.Z. Yin, B.C. Cai, Z.P. Chen and W.D. 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, X.P., X.D. Jiang, J.J. Chen and S.S. Zhang, 2013. Control of postharvest grey mould decay of nectarine by tea polyphenol combined with tea saponin. *Lett. Appl. Microbiol.*, 57: 502–509
- Yuan, G.Q., Q.Q. Li, J. Qin, Y.F. Ye and W. Lin, 2012. Isolation of Methyl Gallate from *Toxicodendron sylvestri* and its effect on tomato bacterial wilt. *Plant Dis.*, 96: 1143–1147
- Zhang, C., J. Gao, T. Han, X. Tian and F. Wang, 2017. Integrated control of tobacco black shank by combined use of riboflavin and *Bacillus subtilis* strain Tpb55. *BioContr.*, 62: 835–845
- Zhang, Y.B., D. Luo, L. Yang, W. Cheng, L.J. He, G.K. Kuang, M.M. Li, Y.L. Li and G.C. 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
- Zhou, X., C. Li, L. Liu, J. Zhao, J. Zhang, Z. Cai and 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

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