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ORIGINAL ARTICLE

Extraction of Chelerythrine and its Effects on Pathogenic Fungus Spore Germination

Qinghui Wei, Min Zhao, Xiaoyan Li

Northeast Forestry University, Harbin 150040, China

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ABSTRACT

Background: Chemical fungicides are widely used to control crop diseases, but these chemicals have adverse effects. They destroy the ecological environment and even have toxic effects on human beings. In this context, the development of botanical pesticides is relevant. One potential botanical pesticide is chelerythrine, a main alkaloid of *Chelidonium majus* L., which has high antitumor, fungistasis, and antiphlogosis bioactivity. **Objective:** This study was designed to present an ultrasonic extraction method for chelerythrine and spore germination experiments to inhibit pathogenic fungi. Fungistasis of chelerythrine is now centralized in basic microbiology experiments, such as observing bacteriostatic rings. This study investigates chelerythrine based on pathogenic fungal spore germination and the influence of germ tube elongation. **Materials and Methods:** Samples of *C. majus* L., which were wild used in this experiment, were picked from Harbin experimental forest farm of Northeast Forestry University. An L_9 (3^4) orthogonal experiment was designed to optimize the ultrasonic extraction method. All the plant pathogenic fungus strains used in the experiment were preservation strains of Northeast Forestry University Microbial preservation center. Pathogenic fungi were cultivated by joining chelerythrine with and observed germ tube growth and spore germination. **Results:** The optimum ultrasonication extraction process for chelerythrine has a liquid/solid ratio of 1:8, 35 min of extraction time, 85% of ultrasonic frequency, and 75% of ethanol concentration. When the concentration of chelerythrine was 1.7×10^{-2} mg/ml, the inhibition rates of *Septoria microspora* Speg. spores and *Curvularia lunata* were 96.67% and 84.94%, respectively. Moreover, when the concentration of chelerythrine was 1.7×10^{-6} mg/ml, the inhibition rates of *S. microspora* spores and *C. lunata* were 47.64% and 12.05%, respectively. **Conclusion:** Fungistasis activity reached a high level with 1.7×10^{-6} mg/ml of chelerythrine. Chelerythrine has the characteristics of less dosage and obvious fungistasis and has a good prospect for botanical fungicide development.

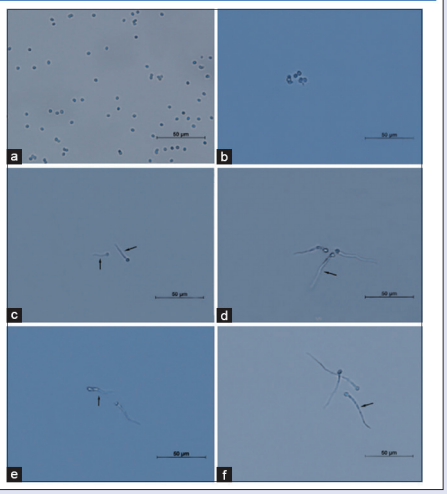
Key words: Chelerythrine, spore germination, spore inhibition rate, ultrasonic extraction

SUMMARY

- *S. microspora* spores in chelerythrine concentration of 1.7×10^{-6} mg/ml have an inhibition rate of 47.64%. For chelerythrine concentrations 1.7×10^{-5}, 1.7×10^{-4}, and 1.7×10^{-3} mg/ml, the inhibition rate is 70%, 80%, and 90%, respectively. When the concentration of chelerythrine was original 1.7×10^{-2} mg/ml, the inhibition rate was 96.67%. As shown in the diagram, the germinal tubes of *S. microspora* spores were shorter than 50 μm with 1.7×10^{-6}, 1.7×10^{-5}, and 1.7×10^{-4} mg/ml concentrations of chelerythrine. However, the germinal tubes of spores without chelerythrine could reach 80 μm. With 1.7 $\times 10^{-3}$ mg/ml liquid concentration, the germination was severely inhibited; the germination under concentrate chelerythrine was limited. The inhibitory effect of chelerythrine was greatest in *S. microspora*.

Table 4: Effect of chelerythrine concentration on *Septoria microspora* Speg. spore germination

Extract concentration	Original	10^{-1}	10^{-2}	10^{-3}	10^{-4}
Germination inhibition rate (%)	96.67	91.27	83.38	72.29	47.64
Germ tube length (μm)	Almost no germination	10	20	40	48



Abbreviations used: *C. majus* L.: *Chelidonium majus* L.; *Sphaerulina juglandis*: *S. juglandis; Septoria microspora* Speg.: *S. microspora*; *Fusarium oxysporum* f. sp. *Lycopersici*: *F. oxysporum* f. sp. *lycopersici;* F. oxysporum f. cucumerinum: *F. oxysporum f. cucumerinum; Curvularia lunata*: *C. lunata.*

Correspondence:

Prof. Min Zhao,
College of Life Sciences,
Northeast Forestry University,
Hexing Road No. 26, Harbin 150040, China.
E-mail: 82191513@163.com

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INTRODUCTION

Chelidonium majus L. belongs to the poppy plant family of genus celandine and is also known as Tuhuanglian or Duanchang Herb. *C. majus* L. is bitter in taste and cold natured.[1,2] This herb is poisonous and its main active components are alkaloids such as chelerythrine, sanguinarine, berberine, and coptisine.[3,4] The herb can be an analgesic, antitussive, diuretic, or detoxification medicine that is mainly used to treat abdominal pain, enteritis, chronic bronchitis, and whooping cough.[5,6] *C. majus* L.

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is recorded in Herbs for Relief of Famines and Chinese Pharmacopoeia published in 1977. Alkaloids are also the main active ingredient of *Papaveraceae chelidonium*. In a previous study, chelerythrine was reported as a potent and specific inhibitor of tumor, fungi, and pests.[7,8] Chelerythrine is now centralized in medical science and basic biology, fungistasis of chelerythrine is now centralized in basic microbiology experiments, such as observing bacteriostatic rings. This paper investigates chelerythrine based on pathogenic fungal spore germination and the influence of germ tube elongation.[9,10] Thus, developing chelerythrine as a new type of botanical fungicide has good potential.

For the past few years, ultrasonic extraction has become a popular method of obtaining medicinal plant chemical components.[11,12] Microflow can generate tangential force to plant powder through ultrasonic vibration and can enable the solvent to permeate cells and accelerate active ingredients into the solvent in the plant, thereby improving the extraction rate. At the same time, the activity of extracted substances is not reduced.[13,14] Thus, this study used the ultrasonic extraction of chelerythrine for shorter duration and increased extraction rate.

Fungus is the main pathogen of plants, and spores are produced directly by hyphal differentiation characterized by asexual reproduction.[15,16] These spores usually grow under poor conditions, such as insufficient nutrients.[17-21] In this paper, we present the optimum extraction method of chelerythrine and explore its inhibitory effect on pathogenic fungi.

MATERIALS AND METHODS

Samples of *C. majus L.* used in this experiment were picked from Harbin experimental forest farm which were wild of Northeast Forestry University. All the pathogenic fungus strains used were preservation strains of Northeast Forestry University Microbial preservation center, these strains were *Sphaerulina juglandis* W1002, *Septoria microspora* Speg. Z0409, *Fusarium oxysporum* f. sp. *lycopersici* Z0413, *F. oxysporum* f. *cucumerinum* Z0418, and *Curvularia lunata* Jqh-100**.** A standard sample of chelerythrine (purity of ≥97%) was purchased from Tongtian Biotechnology Corp. Ethanol (concentrated 95%, analytically pure) and petroleum ether were bought from Tianjin Guangfu Technology Development Co. Ltd.

Chelerythrine was dissolved in 95% concentrated ethanol to draw the standard curve. With the concentration of 95% ethanol, the chelerythrine standard sample was confected to the following series of concentration solutions: 0.0005, 0.0010, 0.0015, 0.0020, and 0.0025 g/L. The chelerythrine solutions were scanned under ultraviolet-visible light with 200–500 nm wavelength. Results showed a 282 nm characteristic absorption peak wave. A standard curve was drawn with abscissa as the concentration and ordinate as the absorption value [Figure 1].

Extraction method of single-factor experiment

Liquid/solid ratio (1:5, 1:6,1:7, 1:8, 1:9, 1:10, 1:11, 1:12, 1:13, and 1:14), extraction time (20, 25, 30, 35, 40, and 45 min), ultrasonic frequency (55%, 65%, 75%, 85%, and 95%), and ethanol concentration (35%, 45%, 55%, 65%, 75%, 85%, and 95%) were selected as influential factors to study the effect of a single factor on the extraction.

In the experiment, 5 g dried powder of *C. majus L.* was added to the different concentrations of ethanol solvent. The mixture was placed in an ultrasonic cell crusher and extracted for different time periods. Extracting solution was filtered by 50 mL petroleum ether to remove chlorophyll and was subsequently centrifuged at 5000 rpm for 10 min to obtain the supernatants. The supernatants were then pumped through a Buchner funnel, and concentrated filtration fluid was processed using a rotary evaporator to obtain chelerythrine. Chelerythrine was washed with 10 mL 95% ethanol. Then, chelerythrine was diluted 100 times and measured using an ultraviolet spectrophotometer. The extraction rate was calculated by regression equation for the standard curve.

Extraction method of orthogonal experiment

According to the results of single factor experiment, an L_9 (3^4) orthogonal experiment was designed to optimize the extraction method as shown in Table 1. Chelerythrine was obtained by the optimum extraction method and placed in a 4°C refrigerator.

Reagent preparation and spore suspension

Pathogenic fungi were cultured on the potato dextrose agar (PDA) solid media and fungus hypha split into spores. Then the spores were gently washed three times with sterile water in PDA solid media. The spore suspension was placed in sterilized 50 ml triangle bottles filled with many 2–3 mm glass balls for dispersing spores. The bottles were then sealed with absorbent cotton and centrifuged two times at 5000 rpm for 5 min to remove the supernatants. All experiments were performed in triplicate and repeated at least once.

A series of concentration of chelerythrine was confected: 1.7×10^{-2} (original concentration), 1.7×10^{-3}, 1.7×10^{-4}, 1.7×10^{-5}, and 1.7×10^{-6} mg/ml, and each treatment of fungal spores was set up control check (CK) which was no chelerythrine. The 5 uL spore suspension with different concentrations was incubated at 28°C, whereas sterile water was added to the contrast. Spore germination was observed under an inverted microscope with ×40 lens.

Determining the optimum time for spore germination

The spore germination was generally calculated from sprouts, and the germination percentage was subsequently obtained. However, the spores did not germinate at the same time. The calculation results were affected by checking time; late checking time results in differences in processes. Therefore, the effects of various periods should be compared when the spore germination reaches a certain percentage.[22]

Spore germination was observed every 4 h of alternating light and dark. The optimal time is achieved when the CK spore germination rate reaches 90%.[22] In the spore germination, the spore germ tube length should be longer than the short spore radius:

Germination rate of spores (%) = Spore germination number × 100%/spore number

Inhibiting effect of chelerythrine on fungal spore

The spores were observed in more than three random perspectives, and the total number of spores was checked to obtain the inhibition rate:

Inhibition of germination (%) = (germination rate of check sample – post-treatment spore germination rate) × 100%/germination rate of check sample

Table 1: Orthogonal experimental design of roots

Level	Factors			
	Liquid/solid ratio (g/mL)	Extraction time (min)	Ultrasonic frequency (%)	Ethanol concentration (%)
1	1:8	25	65	65
2	1:10	30	75	75
3	1:12	35	85	85

RESULTS

Standard curve of chelerythrine

Characteristic wavelength of chelerythrine was 282 nm and the regression equation for the standard curve was:

$y = 104x + 0.030$, $R^2 = 0.996$.

Influence of single-factor conditions to chelerythrine extraction

Chelerythrine extraction rate and liquid/solid ratio were parabolic in shape; the maximum is at 1:10 and the extraction yield decreases. Similarly, the extraction rate was normally distributed as the time changes. A peak was observed between 25 and 35 min, and the extraction rate was gradually reduced. Increased ultrasonic frequency also increases and subsequently decreases the extraction rate. A peak was observed between 65% and 75% of ultrasonic frequency. As the ethanol concentration increases, the peak of extraction rate was observed between 65% and 85%. Thereafter, the concentration decreased. However, the extraction rate increased from 90% ethanol concentration. Based on the economic costs and other factors, the first peak was adopted. All data are shown in Figure 2.

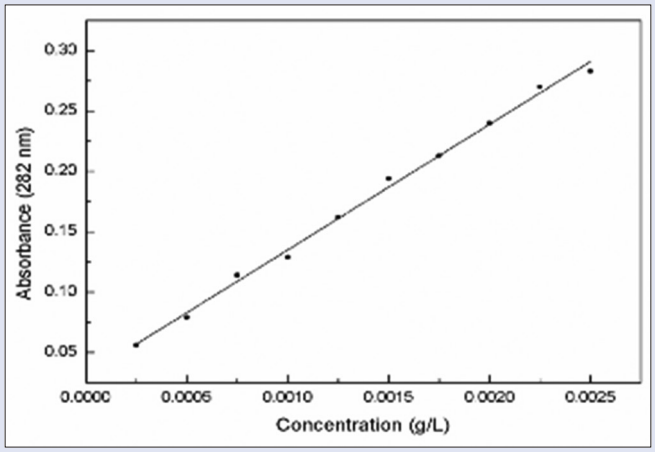

Figure 1: Standard curve of chelerythrine

$L_9(3^4)$ analysis of orthogonal

Ultrasonic extraction time has the greatest influence on extraction yield, followed by ultrasonic frequency [Table 2]. In addition, ethanol concentration and liquid/solid ratio have a minimal effect. Effect trends of the four factors were the same as those of single-factor experiments. The confirmed optimum extraction process of chelerythrine is the liquid/solid ratio of 1:8, 35 min of extraction time, 85% of ultrasonic frequency, and 75% of ethanol concentration.

Optimum time of spore germination

Five kinds of fungal spores were observed every 4 h. The spore growth curve is shown in Figure 3.

S. juglandis spores developed after 4 h with a germination rate of 8%. The growth curve rapidly increased, and a 50% germination rate was achieved at 16 h. The peak was reached at 20 h. Both germination of spores and germination rate were reduced.

S. microspora. spores have a germination rate of 22% that started from 4 h. The growth curve rapidly rose with the first peak observed at 9 h and the germination rate was 90%. Thereafter, the growth curve slowly rose, reaching its highest point at 16 h with a germination rate of 95%. The growth curve then slowly declined. The highest germination rate of *S. microspora* spores was at 16 h.

The growth curve of *F. oxysporum* f. sp. *lycopersici* spores showed that around 4–8 h, the germination rate increased from 8% to 48%. At 8–20 h, the growth curve rose slowly, reaching the peak and germination rate of

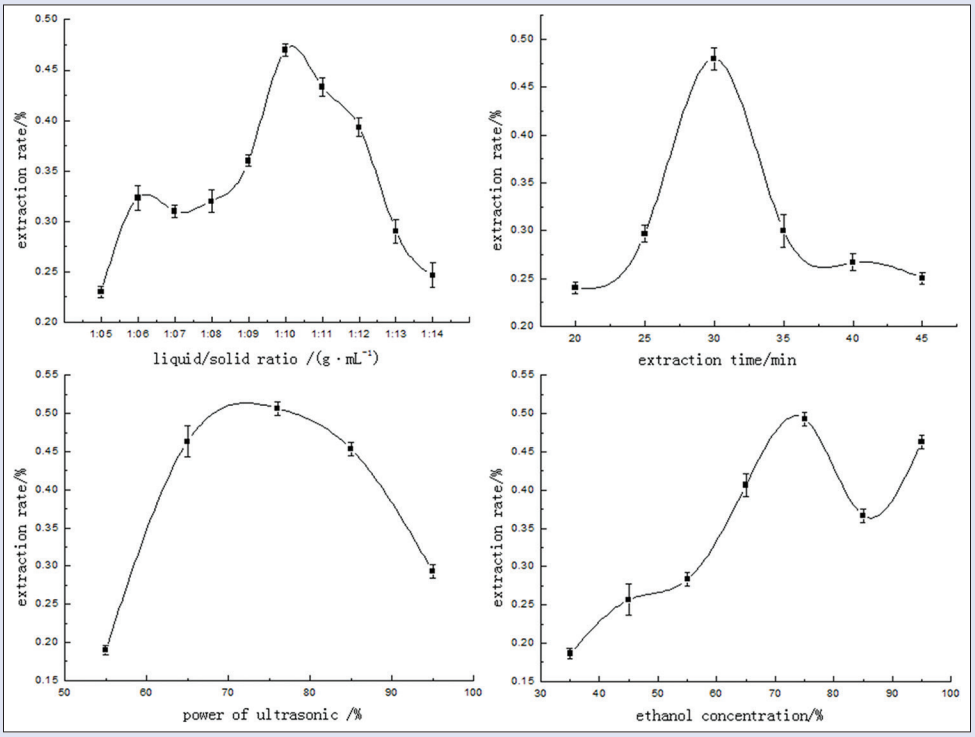

Figure 2: Effect of single factor on chelerythrine extraction efficiency

Table 2: Analysis of orthogonal test

Factors	Liquid/solid ratio	Extraction time	Ultrasonic frequency	Ethanol concentration	Results
1	1	1	1	1	0.36
2	1	2	2	2	0.45
3	1	3	3	3	0.52
4	2	1	2	3	0.34
5	2	2	3	1	0.45
6	2	3	1	2	0.48
7	3	1	3	2	0.43
8	3	2	1	3	0.41
9	3	3	2	1	0.39
Mean 1	0.443	0.377	0.417	0.400	
Mean 2	0.423	0.437	0.393	0.453	
Mean 3	0.410	0.463	0.467	0.423	
Range	0.033	0.086	0.074	0.053	

80% at 20 h. The growth curve then slowly declined. Thus, the greatest spore germination rate was observed at 20 h.

F. oxysporum f. *cucumerinum* spore growth curve was approximately the shape of a parabola. After 4 h, the spore germination rate increased from 3%. The maximum germination rate of 58% was achieved at 16 h. Then, the germination rate declined.

C. lunata spore germination rate of 2% at 4 h increased to 90% at 24 h. The optimum time to observe *C. lunata* spore growth was at 24 h.

Chelerythrine effects on spore germination

Fungal spores were observed at their optimum germination time with different chelerythrine concentrations. *S. juglandis* spores in chelerythrine concentration of 1.7×10^{-6} mg/ml have an inhibition rate of 18.12%. The inhibition rate increased to 34.09% when the concentration was 1.7×10^{-5} mg/ml. The spore germination inhibition rate increases with the increase of solution concentration. When the concentration of chelerythrine was original 1.7×10^{-2} mg/ml, inhibition rate could reach 87.89% [Table 3].

S. microspora spores in chelerythrine concentration of 1.7×10^{-6} mg/ml have an inhibition rate of 47.64%. For chelerythrine concentrations 1.7×10^{-5}, 1.7×10^{-4}, and 1.7×10^{-3} mg/ml, the inhibition rate is 70%, 80%, and 90%, respectively. When the concentration of chelerythrine was original 1.7×10^{-2} mg/ml, the inhibition rate was 96.67% [Table 4].

The germination inhibition performance of *F. oxysporum* f. sp. *lycopersici* spores with chelerythrine was also strong. The spore inhibition rate reaches 19.72% with chelerythrine concentration of 1.7×10^{-6} mg/ml. As the solution concentration increases, so does the spore germination inhibition rate. The germination inhibition rate of the spore could reach 78.92% with chelerythrine concentration of 1.7×10^{-3} mg/ml. When the concentration of chelerythrine was original 1.7×10^{-2} mg/ml, the inhibition rate could reach 94.41% [Table 5].

The germination inhibition rate of *F. oxysporum* f. *cucumerinum* spores was significantly affected by the chelerythrine concentration. The solution concentration 1.7×10^{-6} mg/ml and 1.7×10^{-4} mg/ml have inhibition rates of 37% and 60%, respectively. The inhibition rate reaches more than 90% with the original chelerythrine concentration 1.7×10^{-2} mg/ml [Table 6].

C. lunata spores with low concentration of chelerythrine liquid have low germination inhibition (<20%). The 1.7×10^{-5} mg/ml liquid concentration has an inhibition rate of 17%. As the solution concentration increases, so does the inhibition rate. The solution concentration of 1.7×10^{-3} mg/ml has an inhibition rate of less than 40%. When the concentration of chelerythrine was original 1.7×10^{-2} mg/ml, the inhibition rate is <85% [Table 7].

Table 3: Effect of chelerythrine concentration on *Sphaerulina juglandis* spore germination

Extract concentration	Original	10^{-1}	10^{-2}	10^{-3}	10^{-4}
Germination inhibition rate (%)	87.89	69.53	45.85	34.09	18.12
Germ tube length (μm)	Flocculent	5	20	45	50

Table 4: Effect of chelerythrine concentration on *Septoria microspora* Speg. spore germination

Extract concentration	Original	10^{-1}	10^{-2}	10^{-3}	10^{-4}
Germination inhibition rate (%)	96.67	91.27	83.38	72.29	47.64
Germ tube length (μm)	Almost no germination	10	20	40	48

Table 5: Effect of chelerythrine concentration on *Fusarium oxysporum* f. sp. *lycopersici* spore germination

Extract concentration	Original	10^{-1}	10^{-2}	10^{-3}	10^{-4}
Germination inhibition rate (%)	94.41	78.92	59.85	34.80	19.72
Germ tube length (μm)	Ungerminated	10	15	20	50

Table 6: Effect of chelerythrine concentration on *Fusarium oxysporum* f. *cucumerinum* spore germination

Extract concentration	Original	10^{-1}	10^{-2}	10^{-3}	10^{-4}
Germination inhibition rate (%)	90.35	76.95	57.66	48.19	37.01
Germ tube length (μm)	Flocculent	20	30	55	100

Table 7: Effect of chelerythrine concentration on *Curvularia lunata* spore germination

Extract concentration	Original	10^{-1}	10^{-2}	10^{-3}	10^{-4}
Germination inhibition rate (%)	84.94	39.19	23.50	17.01	12.05
Germ tube length (μm)	2	10	15	25	60

Micrographs of chelerythrine effect on spores

S. juglandis spores were cultivated and their growth was observed under a × 40 microscope. Figures 4a-e show the 1.7×10^{-2}, 1.7×10^{-3}, 1.7×10^{-4}, 1.7×10^{-5}, and 1.7×10^{-6} mg/ml solution concentrations, respectively. Figure 4f is for CK.

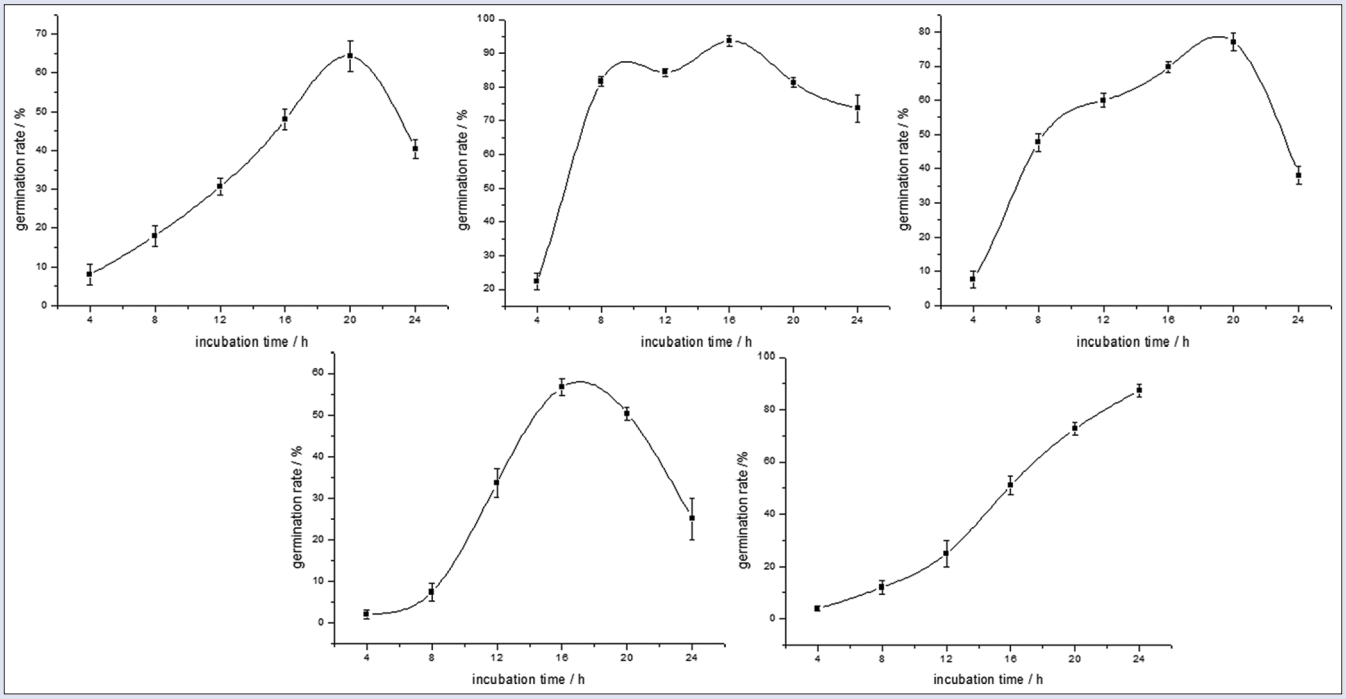

Figure 3: Growth curves of five kinds of fungal spore

As shown in the diagram, the germinal tubes of *S. juglandis* spores were 100 μm long without chelerythrine and shorter than 50 μm with chelerythrine. The germinal tubes were flocculent in the original concentration.

S. microspora spores were cultivated and their growth was observed under a ×40 microscope. Figures 5a-e show the 1.7×10^{-2}, 1.7×10^{-3}, 1.7×10^{-4}, 1.7×10^{-5}, and 1.7×10^{-6} mg/ml solution concentrations, respectively. Figure 5f is for CK.

As shown in the diagram, the germinal tubes of *S. microspora* spores were shorter than 50 μm with 1.7×10^{-6}, 1.7×10^{-5}, and 1.7×10^{-4} mg/ml concentrations of chelerythrine. However, the germinal tubes of spores without chelerythrine could reach 80 μm. With 1.7×10^{-3} mg/ml liquid concentration, the germination was severely inhibited; the germination under concentrate chelerythrine was limited. The inhibitory effect of chelerythrine was greatest in *S. microspora*.

F. oxysporum f. sp. *lycopersici* spores were cultivated and their growth was observed under a × 40 microscope. Figures 6a-e show 1.7×10^{-2}, 1.7×10^{-3}, 1.7×10^{-4}, 1.7×10^{-5}, and 1.7×10^{-6} mg/ml solution concentrations, respectively. Figure 6f is for CK.

As shown in the diagram, the germinal tubes of *F. oxysporum* f. sp. *lycopersici* spores reached 60 μm long without chelerythrine. However, the germinal tubes of spores were shorter with chelerythrine. Germination was limited with the original concentration.

F. oxysporum f. *cucumerinum* spores were cultivated and their growth was observed under a ×40 microscope. Figures 7a-e show 1.7×10^{-2}, 1.7×10^{-3}, 1.7×10^{-4}, 1.7×10^{-5}, and 1.7×10^{-6} mg/ml solution concentrations, respectively. Figure 7f is for CK.

As shown in the diagram, the germinal tubes of *F. oxysporum* f. *cucumerinum* spores reach 150 μm long with CK. However, the germinal tubes of spores were shorter with 1.7×10^{-6} mg/ml chelerythrine; the longest was 100 μm. Shorter germinal tubes were gradually formed as the solution concentration increased. Germination was minimal with original concentration. The germination inhibitory effect of chelerythrine was evident.

C. lunata spores were cultivated and their growth was observed under a ×40 microscope. Figures 8a-e show 1.7×10^{-2}, 1.7×10^{-3}, 1.7×10^{-4}, 1.7×10^{-5}, and 1.7×10^{-6} mg/ml solution concentrations, respectively. Figure 8f is for CK.

As shown in the diagrams, the germ tubes of *C. lunata* spores in the CK group were evidently longer than those in the liquid concentration. Worst inhibition was observed.

DISCUSSION

The characteristic wavelength of chelerythrine was 282 nm and the regression equation of the standard curve was $y = 104x + 0.030$, $R^2 = 0.996$. Combined with single-factor and orthogonal experiments, the confirmed optimum extraction process had a liquid/solid ratio of 1:8, 35 min of extraction time, 85% of ultrasonic frequency, and 75% concentrated ethanol.

This experiment observed the cultivation of pathogen spores. The optimum spore germination time is as follows: 16 h for *S. microspora*, and *F. oxysporum* f. *cucumerinum;* 20 h for *S. juglandis* and *F. oxysporum* f. sp. *lycopersici*; and 24 h for *C. lunata*.

With chelerythrine concentrations of 1.7×10^{-2}, 1.7×10^{-3}, 1.7×10^{-4}, 1.7×10^{-5}, and 1.7×10^{-6} mg/ml on five kinds of spores, the spores were compared with the CK group and their spore germination was observed. The spore germ tubes of CK were evidently longer than those in treated chelerythrine.

The inhibition rate of the spore germination was obtained from the rate of spore germination. The result revealed that with chelerythrine 1.7×10^{-6} mg/ml, the inhibition rates are as follows: 47.64% for *S. microspora*, 37.01% for *F. oxysporum* f. *cucumerinum*; 19.72% for *F. oxysporum* f. sp. *lycopersici;* 18.12% for *S. juglandis*; and 12.05% for *C. lunata*. With the original concentration of 1.7×10^{-2} mg/ml, the inhibition rate of *S. microspora* spores was the strongest (up to 96.67%), whereas the inhibition rate of *C. lunata* spores was the weakest (84.94%).

Figure 4: Electron microscope pictures of *Sphaerulina juglandis* spore germination. (a-e) The 1.7×10^{-2} mg/ml, 1.7×10^{-3} mg/ml, 1.7×10^{-4} mg/ml, 1.7×10^{-5} mg/ml, and 1.7×10^{-6} mg/ml solution concentrations, respectively. (f) Control check

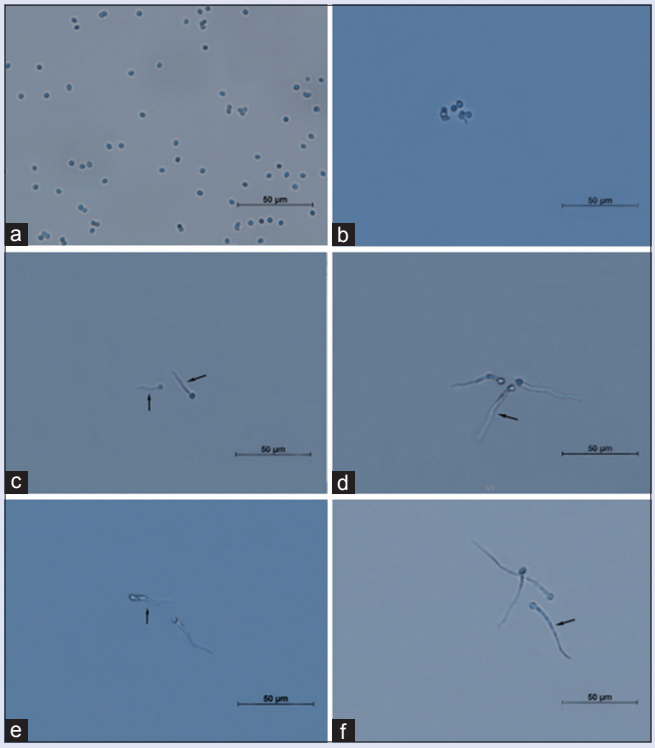

Figure 5: Electron microscope pictures of *Septoria microspora* Speg. spore germination. (a-e) The 1.7×10^{-2} mg/ml, 1.7×10^{-3} mg/ml, 1.7×10^{-4} mg/ml, 1.7×10^{-5} mg/ml, and 1.7×10^{-6} mg/ml solution concentrations, respectively. (f) Control check

Figure 6: Electron microscope pictures of *Fusarium oxysporum* f. sp. *lycopersici* spore germination. (a-e) 1.7×10^{-2} mg/ml, 1.7×10^{-3} mg/ml, 1.7×10^{-4} mg/ml, 1.7×10^{-5} mg/ml, and 1.7×10^{-6} mg/ml solution concentrations, respectively. (f) Control check

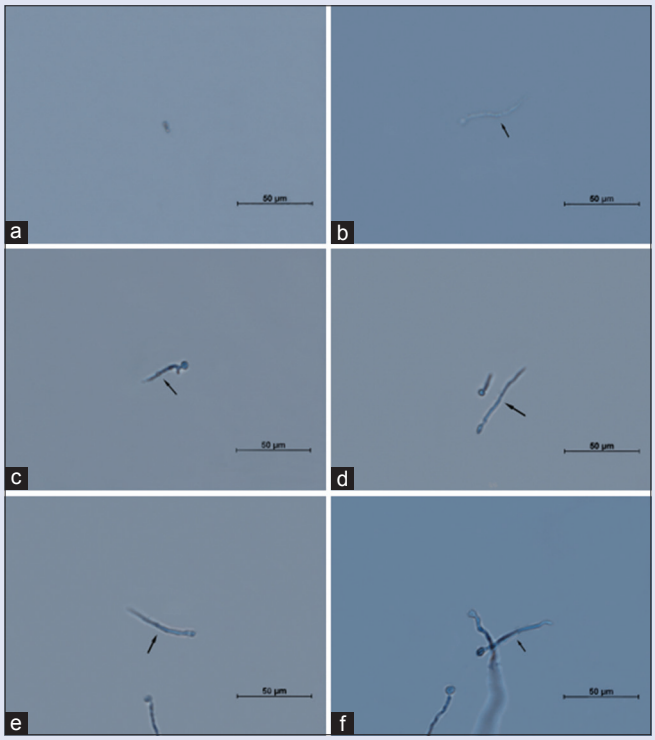

Figure 7: Electron microscope pictures of *Fusarium oxysporum* f. *cucumerinum* spore germination. (a-e) 1.7×10^{-2} mg/ml, 1.7×10^{-3} mg/ml, 1.7×10^{-4} mg/ml, 1.7×10^{-5} mg/ml, and 1.7×10^{-6} mg/ml solution concentrations, respectively. (f) Control check

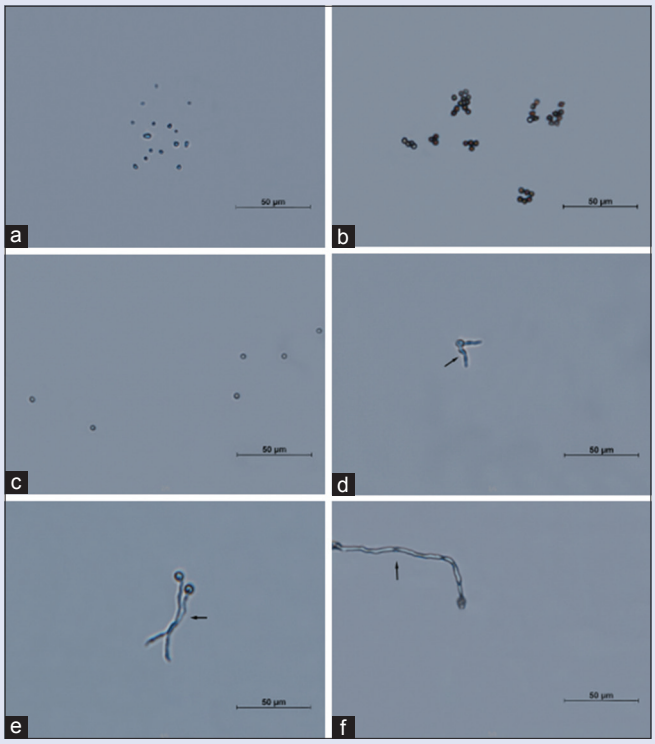

Figure 8: Electron microscope pictures of *Curvularia lunata* spore germination. (a-e) show 1.7×10^{-2} mg/ml, 1.7×10^{-3} mg/ml, 1.7×10^{-4} mg/ml, 1.7×10^{-5} mg/ml, and 1.7×10^{-6} mg/ml solution concentrations, respectively. (f) Control check

CONCLUSION

This paper presents ultrasonic extraction as a simple operation with short extraction time, high extraction efficiency, no heating and solvent consumption, and relatively low cost. The optimum ultrasonication extraction process for chelerythrine has a liquid/solid ratio of 1:8, 35 min of extraction time, 85% of ultrasonic frequency, and 75% of ethanol concentration. Mass production of chelerythrine is of great significant. CK spore germ tubes were significantly longer than those treated with chelerythrine. With 1.7×10^{-6} mg/ml of chelerythrine, fungistasis activity reached a high level. Thus, chelerythrine has the characteristics of less dosage and obvious fungistasis. Further work is required to elucidate the inhibitory effect of chelerythrine under field condition, several members of our group are working on this subject, including fungi generation time. Nevertheless, we believe that the present findings are not only useful in the laboratory but also provide new perspectives for the development of botanical fungicides.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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