Antifungal activities of matrine and oxymatrine and their synergetic effects with chlorthalonil

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Abstract: The EC_{50} values of matrine and oxymatrine against five forest pathogenic fungi (*Fusarium oxysporum*, *Valsa pini*, *Cladosporium oxysporum*, *Sphaeropsis sapinea*, *Marssonina brunnea*) were examined by bioassay methods. The results demonstrated that matrine and oxymatrine had strong inhibitory activities to the conidium germination of the tested fungi. The EC_{50} values of matrine for inhibiting the conidium germination of *Marssonina brunnea*, *Cladosporium oxysporum*, *Sphaeropsis sapinea* were 123 μg·mL^{-1}, 272 μg·mL^{-1}, 1133 μg·mL^{-1}, respectively, and the EC_{50} values of oxymatrine for inhibiting the conidium germination of *Fusarium oxysporum*, *Sphaeropsis sapinea* were 532 μg·mL^{-1}, 601 μg·mL^{-1}, respectively. The hyphal growth of the fungi was also significantly inhibited by matrine and oxymatrine. The EC_{50} values of matrine inhibiting the conidium germination of *Sphaeropsis sapinea*, *Valsa pini*, *Fusarium oxysporum* were 428 μg·mL^{-1}, 535 μg·mL^{-1}, 592 μg·mL^{-1}, respectively. The EC_{50} values of oxymatrine inhibiting the conidium germination of *Valsa pini*, *Fusarium oxysporum* were 323, 618 μg·mL^{-1}, respectively. In the synergetic tests the EC_{50} values of the mixtures of thiophanate methyl (or chlorthalonil) and matrine (or oxymatrine) were lower than 34 μg·mL^{-1} while their co-toxicity coefficients were significantly higher than 100. It indicated that the mixture of the alkaloids and the chemical had potential practical utilization in controlling certain forest fungal diseases.

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Introduction

There has been a tendency towards the utilization of alternatives for controlling pest and disease. Natural products including plant extracts might substitute for pesticides and contribute to the development of new agents (Addor 1995). Matrine and oxymatrine are the major quinolizidine alkaloids in *Radix Sophorae flavescentis*, *Radix Sophorae Subprostratae* and *Sophora alopecurvides*. They exhibited sedative, depressant, analgesic, hypothermic, anti-tumor, antipyretic and cardiotonic activities (Kinghorn *et al*. 1985). Quinolizidine alkaloids are important due to some of them possess potentially useful pharmacological activity (Wink *et al*. 1987; Zhao 1998, 1999b). Moreover, quinolizidine alkaloids were also reported to have fungicidal effects (Wippch *et al*. 1985; Zhao 1999a; Wei *et al*. 2001). In most cases, the crude extracts were only used to control insects and pathogens in crop and forest protection. This paper reports the antifungal activities of matrine and oxymatrine against several important forest pathogens and the synergetic effects with certain synthetical fungicides.

Materials and methods

Test fungi

Fusarium oxysporum, *Valsa pini* and *Cladosporium oxysporum* were obtained from Department of Forest Entomology and Pathology, Southwest Forest Institute, P. R. China. *Sphaeropsis sapinea* and *Marssonina brunnea* were gotten from Department of Forest Entomology and Pathology, Nanjing Forestry University, P. R. China.

Test reagents

Matrine and oxymatrine were gained from Entomology Lab., Department of Forest Entomology and Pathology, Nanjing Forestry University, P. R. China. Chlorthalonil (75%) was acquired from Limin Chemical Corp., P. R. China, and Thiophanate methyl (70%) was obtained from Zhenjiang Pesticide Plant, P. R. China.

Bioassay

Conidium germination

Marssonnina brunnea, *Cladosporium oxysporum*, *Fusarium oxysporum* and *Sphaeropsis sapinea* were cultured on potato dextrose agar (PDA). Fungal spores were harvested from 7-day-old agar cultures by washing with sterile water. After filtering the suspensions through two layers of cheesecloth, the spores were centrifuged and then suspended in sterile water at a concentration of 2×10^5 spores per millilitre. Five concentrations of matrine or oxymatrine (25 μg·mL^{-1}, 250 μg·mL^{-1}, 500 μg·mL^{-1}, 1000 μg·mL^{-1}, 2500 μg·mL^{-1}) and one control (sterile water) were used to test the effect of the four forest pathogenic fungi on spore germination. Aliquots (1 mL) from each concentration were mixed with the fungal spores' suspension and placed on separate glass slides in triplicate. Slides containing the spores were incubated in a moist chamber at 25 °C for 24 h (*Marssonnina brunnea* for 8 h). Spore germination in each slide was observed under microscope. Conidia were considered as germination if they had a germ tube at least twice length of the spore. Percentage of germinated spore was calculated in about 200 spores.

Hyphal growth

Different concentrations of the reagents were added to the warm PDA (50°C) and the volume ratio of the reagent to the PDA was 1:9. PDA plates were prepared by using 9-cm Petri

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dishes containing the mixture. A Φ4-mm disc of 1-week-old cultured mycelial mat of tree species was cut and placed on the medium containing the reagents in the plates with the mycelial surface upside down. The plates were incubated at 25°C in the dark. After 3 days, diameter of hyphae growth was measured. Mean growth were calculated from replicates of each species.

Statistics

Inhibition

Conidium germination and hyphal growth inhibition of the treatments against the control were calculated in percentage by the equation as follows:

$$I_R=[(C-T)/C]\times 100 \quad (1)$$

where, I_R is the inhibition rate of conidium germination or hyphal growth, C the number of spore germination or diameter of hyphal growth of control, and T the number of spore germination or diameter of hyphal growth of treated species. A *t*-test was also computed.

Co-Toxicity coefficient (Zhang 1994)

Co-toxicity coefficients *(*C_{TC}) of admixture (M) were calculated by the following equation

$$C_{TC}= (A_{TI,M} / T_{TI,M})\times 100 \quad (2)$$

where, $A_{TI,M}= (E_{C50,A}/ E_{C50,M})\times 100$ is the real toxicity index of admixture and $T_{TI,M}= (T_{IA}\times A\%+T_{IB}\times B\%)$ is the theoretical toxicity index of the admixture. $T_{IA}=100$ is the toxicity index of A in the admixture and $T_{IB} = (E_{C50,A}/ E_{C50,B})\times 100$ is the toxicity index of B in the admixture. The admixture would be synergetic, if C_{TC} was more than 200; it would be antagonistic if C_{TC} was less than 100 or the rest in between 100 and 200 was additive.

Results

Most quinolizidine alkaloids and the synthetic germicides showed a considerable extent of inhibition. The spore germination test for each of the test fungi was shown in Table 1. The least susceptible fungi were *Fusarium oxysporum*, as compared to the other tested fungal strains. It was noticeable that oxymatrine had the strongest inhibitory effects on *Fusarium oxysporum* with the EC_{50} values of 26 μg ·mL^{-1}. It was observed that the germinated spores treated with low concentration of matrine produced short germ tubes as compared to the control.

As shown in Table 2, both quinolizidine alkaloids and synthetical pestisides could inhibit the hyphal growth at the EC_{50} value ranging from 150 μg·mL^{-1} to 600 μg·mL^{-1}. It seemed that there were no significant differences between the two categories of the reagents.

Table 1. The EC_{50} values of matrine and oxymatrine for inhibiting conidium germination of the fungi tested

Substance	Pathogen	Regression equation	Correlation coefficient	EC_{50} (μg·mL^{-1})
Matrine	*Marssonina brunnea*	*Y*=2.02+1.43*X*	0.99	123
	Cladosporium oxysporum	*Y*=1.44+1.46*X*	0.89	272
	Fusarium oxysporum	*Y*=1.13+1.27*X*	0.86	1133
	Sphaeropsis sapinea	*Y*=2.37+0.230*X*	0.99	--
Oxymatrine	*Fusarium oxysporum*	*Y*=3.29+1.21*X*	0.98	26
	Sphaeropsis sapinea	*Y*=-0.969+2.19*X*	0.91	532
	Cladosporium oxysporum	*Y*=2.60+0.870*X*	0.96	573
	Marssonina brunnea	*Y*=2.59+0.867*X*	0.99	601
Chlorthalonil	*Sphaeropsis sapinea*	*Y*=5.47+0.767*X*	0.99	0.24
	Marssonina brunnea	*Y*=4.62+0.754*X*	0.99	3
	Cladosporium oxysporum	*Y*=2.45+2.00*X*	0.99	19
	Fusarium oxysporum	*Y*=-3.60+4.39*X*	0.92	91
Thiophanate methyl	*Marssonina brunnea*	*Y*=4.62+0.615*X*	0.96	4
	Cladosporium oxysporum	*Y*=0.736+2.61*X*	0.93	43
	Fusarium oxysporum	*Y*=-1.10+2.49*X*	0.96	283
	Sphaeropsis sapinea	*Y*=1.67+0.791*X*	0.97	16212

Notes: "--"stands for EC_{50} too high.

Table 2. The EC_{50} values of matrine and oxymatrine for Inhibiting hyphal growth of the fungi tested

Substance	Pathogen	Regression equation	Correlation coefficient	EC_{50} (μg·mL^{-1})
Matrine	*Valsa pini*	*Y*=1.38+1.33*X*	0.95	535
	Fusarium oxysporum	*Y*=0.869+1.49*X*	0.96	592
Oxymatrine	*Valsa pini*	*Y*=-0.709+2.28*X*	0.99	323
	Fusarium oxysporum	*Y*=1.48+1.29*X*	0.97	528
Chlorthalonil	*Valsa pini*	*Y*=2.66+1.12*X*	0.92	123
	Fusarium oxysporum	*Y*=-1.51+2.99*X*	0.99	151
Thiophanate methyl	*Fusarium oxysporum*	*Y*=-0.662+2.30*X*	0.99	287
	Valsa pini	*Y*=0.843+1.67*X*	0.99	310

In synergetic test, four kinds of mixtures, such as chlorthalonil/matrine (m/m=5:1), chlorthalonil/oxymatrine (m/m=5:1), thiophanate methyl/matrine (m/m=5:1), thiophanate methyl/oxymatrine (m/m=5:1) were used in experiments. According to the co-toxicity coefficients, the results (Table 3) showed that each mixture had either additive or synergetic ef-

fects. Synergetic effects of the two categories of the reagents on *Fusarium oxysporum* were stronger than that of *Valsa pini*.

Table 3. Synergetic effects with certain synthetical fungicides

Substance (m:m=5:1)	Pathogen	Regression equation	Correlation coefficient	EC_{50} (μg·mL^{-1})	Co-toxicity coefficient	Co-toxicity
Chlorthalonil:matrine	*Valsa pini*	Y=4.11+0.606X	0.99	30	470	Significantly synergetic
	Fusarium oxysporum	Y=-0.154+2.46X	0.99	175	99	Additive
Chlorthalonil:oxymatrine	*Valsa pini*	Y=4.59+0.267X	0.99	34	403	Significantly synergetic
	Fusarium oxysporum	Y=-0.284+2.62X	0.99	104	165	Additive
Thiophanate methyl:matrine	*Valsa pini*	Y=2.62+1.75X	0.93	23	1449	Significantly synergetic
	Fusarium oxysporum	Y=-0.166+2.39X	0.99	145	217	Synergetic
Thiophanate methyl:oxymatrine	*Valsa pini*	Y=2.31+1.83X	0.94	30	1040	Significantly synergetic
	Fusarium oxysporum	Y=-0.531+2.65X	0.99	124	251	Synergetic

Discussion

The matrine and oxymatrine had significant differences in antifungal activity for the tested fungal species. The effective concentration of oxymatrine to inhibit *Fusarium oxysporum* was lower than that of the synthetic pesticides. However, for matrine, the concentrations were much higher than corresponding ones of the synthetic pesticides. The pathogen (*Fusarium oxysporum*) was likely more sensitive to the molecular structure change of N in matrine than the other tested fungi. It was also observed that spores of *Sphaeropsis sapinea* treated with matrine could germinate, but the germ tubes were shorter, weaker and had more branches. The antifungal mechanisms of matrine and oxymatrine need to be further researched in the future.

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