INHIBITING EFFECT OF 4 TOXIC MUSHROOM STRAINS AND THEIR TOXINS ON *Cytospora chrysosperma* (Pers.) Fr.

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ABSTRACT

Inhibiting effect of 4 toxic mushroom strains (*Amanita virosa* Lamb. ex. Secr., *Amanita muscaria* (L. ex Fr.) Pers. ex Hook., *Lepiota clypeolaria* (Bull. ex Fr.) Quil., *Lactarius vellereus* (Fr.) Fr.) and their toxins on *Cytospora chrysosperma* (Pers.) Fr. were studied. The results showed that the strains *Amanita virosa* and *Lepiota clypeolaria* inhibited growth of *Cytospora chrysosperm*. The strain *Amanita virosa* had a better inhibition effect. In antagonistic experiments, *Cytospora chrysosperma* promoted the growth of *Amanita virosa* .The effect being the highest at 74 h, the inhibition of *Amanita virosa* to *Cytospora chrysosperma* is also highest . *Lactarius vellereus* has growth-promoting effect on *Cytospora chrysosperma*. The percentage of growth inhibition of toxin crude extracts from 4 toxic mushroom strains on *Cytospora chrysosperma* mycelium are all 100 %, and the percentage of spore sprouting-inhibition is between 93.36 % - 97.29 %.

Keywords: Toxic Mushroom Strains, *Amanita virosa*, *Amanita muscaria*, *Lepiota clypeolaria*, *Lactarius vellereus*, *Cytospora chrysosperma*

1. INTRODUCTION

Toxic mushrooms are a kind of large-size fungi which can poison mankind or animals after consumption. Most of toxic mushrooms belong to Basidiomycotina and most of them belong to genus *Amanita* (Hymenomycetes, Amanitaceae), *Inocybe* (Cortinariaceae), *Panaeolus* (Coprinaceae) and Russulaceae. At present, there are more than 190 species of toxic mushrooms in China, they belong to 58 genus, 26 families. One hundred and seventy nine species are Basidiomycotina belonging to 50 genus, 20 families. More than 40 species have extreme and grievous toxicity, about 30 species can cause death of mankind. More than 20 species have slight toxicity but can be eaten after treated, so be called conditional edible mushrooms [1, 2].

Mycotoxins are the secondary metabolite produced by fungi. At present, there are more than 200 mycotoxins. The research works have been concentrated on more than 10 mycotoxins which are more harmful to mankind and toxic to plant pathogen, including aflatoxin, patulin, zearalenone, fumonism and the toxins produced by *Helminthosporium* spp., *Alternaria* spp., *Verticillium* spp. and *Cercospora* spp. [3]. The national and international research on mycotoxin and toxic mushrooms are limited in harmful aspect to mankind.

Along with modern technology development, people find that amanita hemolysin is specific to actin [4, 5]. It was reported that the culture fluids of *Amanita pantherina* and *A. kwangsiensis* have higher disinfection effect on flies, the culture fluids of *Russula laurosi* and *R. emetica* have obvious inhibiting effect to moulds, a kind of clitocybin isolated from *Leucopaxillus giganteus* and *L. candldus* has inhibiting effect to Gram-negative and Gram-positive bacteria. Clitocybin isolated from the fermented broth of *Clitocybe illudens* has inhibiting effect to moulds and antitumour effect, lammterol isolated from the fruiting body of *Lampteromyces japonicus* has inhibiting effect to moulds as well [4, 6, 7]. To sum up, it is practical to control plant pathogens by using the toxin of toxic mushrooms. However, to exploit the toxin as another effective way for biological pesticide, more researches have to be investigated.

Microbes in one community or system show many different interaction, some are neutralism, some are mutualism or positive interaction and some are harmful or negative interaction. Microbes must live together – symbiosis, if there are interactions between them. Symbiosis can be divided into 3 groups: neutral associations, positive associations and negative associations. Negative associations include antagonism, competition, killing and parasitism. Microbes with negative interactions to pathogens can be used for plant disease control [8].

Through the research work, we try to find some toxic mushroom which can be used in plant disease control, and to study the control mechanisms by toxic mushroom and toxin to pathogen.

2. MATERIALS AND METHODS

Microorganisms

Toxic mushroom strains: *Amanita virosa* Lamb. ex. Secr., *Lepiota clypeolaria* (Bull. Ex Fr.) Quil. and *Lactarius vellereus* (Fr.) Fr.) were collected from Maoershan Experiment Forestry Center of Northeast Forestry University , *Amanita muscaria* (L. ex Fr.) Pers. ex Hook. was collected from Keshiketeng of inner Mongolia.

Pathogen strain: *Cytospora chrysosperma* (Pers.) Fr. was collected from popular trunk in Harbin.

Testing of growth-inhibiting effect of toxic mushroom strains to pathogen strain

Diameters of 0.5 cm colonies of toxic mushroom strain and pathogen strain were cut and cultured on PDA at 25 °C. The distance between them was 3cm. Diameter of 0.5 cm colony of single strain cultured at the center of PDA served as control. The colony radius was measured every 12 h interval. In antagonistic culture test, the directional radius of 2 colonies (1 toxic mushroom and 1 pathogen) were used for antagonistic growth determining. The experiments were repeated 5 times.

Inhibition rate = (colony radius of individually cultured—directional radius of colony)/ colony radius of individually cultured

Relative inhibitory effect = Inhibition rate of pathogen/ Inhibition rate of antagonistic fungus

Testing of growth-inhibiting effect of toxin crude extract to pathogen strain

Preparation of toxin crude extract: the culture fluid of toxic mushrooms cultured for 10 days was extracted by microwave extraction, and the toxin crude extract was collected after filtering.

Growth-rate method was used for determining growth-inhibiting effect of 4 toxin crude extracts to pathogen. Diameter of 0.5 cm colony of pathogen strain was cut and cultured on PDA with the addition of 1% toxin crude extract at 25 °C for 72 h, then measuring colony radius. The culture on PDA without toxin crude extract was used as control. The experiments were repeated 5 times.

Percent growth inhibition = [(net production of control - net production of treatment)/ net production of control] × 100%

Testing of sprouting-inhibiting effect of toxin crude extract to pathogen's conidia

(1) Method of mycostatic circle producing: 150 μl of conidial suspension (20~25 spores/visual field) was put on PDA with well-distribution, then filter paper (diameter of 6 mm) which was sterilized and soaked with 20 μl of 10 % toxin crude extract was put at the center, then cultured at 25 °C. The control used sterile water instead of toxin crude extract. The experiment were repeated 4 times. Diameter of mycostatic circle was measured after 72 h.

(2) Pendent drop method: The slide with 1drop of mixed fluid (10 μl of 10 % toxin crude extract and 10μl conidial suspension) (20-25 spores/visual field) was put in moist chamber at 25 °C, checked every 4 h interval. The control used using sterile water instead of toxin crude extract.

3. RESULTS AND DISCUSSION

Growth-inhibiting effect of toxic mushroom strains to pathogen strain

Before, screening of antagonistic fungi, antagonistic fungi was mostly collected from host or soil, and the width of antagonistic line produced in antagonistic culture was considered as the antagonistic effect standard. In nature, the fungi which produce antagonistic line are difficult to coexist in one mycoflora, so the effect of antagonistic fungi screened outdoor is worse than that indoor.

Antagonistic fungi were also inhibited when they inhibited pathogen. The results of antagonistic culture between toxic mushroom strains and pathogen strain are showed in Tables 1- 3.

The results in Table 1 showed that the inhibition rate of *Cytospora chrysosperma* by *Amanita virosa* rose with time increasing, the highest inhibition rate is 74.24 % which is at 74 h during antagonistic culture and not only *Amanita virosa* was inhibited by *Cytospora chrysosperma*, but also promoted the growth. Promoting-effect is highest at 74 h.

The results in Table 2 showed that the relative inhibitory effect of *Lepiota clypeolaria* rose rapidly after 38h and reached 4.19% at 50h

The results in Table 3 shoeds that *Lactarius vellereus* did not grow in any individual or antagonistically cultured with *Cytospora chrysosperma*. *Cytospora chrysosperma* grew faster when cultured with *Lactarius vellereus* than when cultured individually during 26-74 h, indicating that *Lactarius vellereus* has growth-promoting effect on *Cytospora chrysosperma*.

The strains of *Amanita virosa* and *Lepiota clypeolaria* showed definite inhibitory effect on strain of *Cytospora chrysosperma* whereas the strain of *Lactarius vellereus* had not only inhibitory effect, but also promoted the growth of *Cytospora chrysosperma*.

The antagonistic fungi which have high relative inhibiting effect should be selected as biocontrol fungi such as *Amanita virosa* and *Lactarius vellereus*, especially the growth of *Amanita virosa* can be promoted by the pathogen. However the results above should be checked in field trial in future.

Growth-inhibiting effect of toxin crude extract to pathogen strain

The result showed that *Cytospora chrysosperma* colonies on PDA mixed with every 4 toxin crude extracts all did not grow within 74h (Table 4). Four toxin crude extracts completely inhibited the growth of *Cytospora chrysosperma* strain.

Table 1. Antagonistic culture of *Amanita virosa* and *Cytospora chrysosperma*

time	strain	Av vs Cc colony directional radius (cm)	colony radius of individually cultured (cm)	inhibition rate(%)	relative inhibitory effect
26 h	Av	0.30	0.30	0	
	Cc	0.30	0.30	0	0
38 h	Av	0.60	0.55	-9.09	**
	Cc	0.45	0.70	35.71	
50 h	Av	0.80	0.80	0	**
	Cc	0.70	1.60	56.25	
62 h	Av	2.30	1.70	-35.29	**
	Cc	0.73	2.20	66.82	
74 h	Av	3.90	2.00	**-95**	**
	Cc	0.85	3.30	**74.24**	
86 h	Av	3.90	2.30	-69.57	**
	Cc	1.00	3.75	73.33	

Notes: Av=*Amanita virosa*, Cc=*Cytospora chrysosperma*, ** unjustifiable figure

Table 2. Antagonistic culture of *Lepiota clypeolaria* and *Cytospora chrysosperma*

time	strain	Av vs Cc colony directional radius (cm)	colony radius of individually cultured (cm)	inhibition rate(%)	relative inhibitory effect
26 h	Lc	0.30	0.30	0	0
	Cc	0.30	0.30	0	
38 h	Lc	0.45	0.50	10	1
	Cc	0.63	0.70	10	
50 h	Lc	0.58	0.63	7.9	**4.19**
	Cc	1.07	1.60	33.13	
62 h	Lc	0.63	0.74	14.86	3.06
	Cc	1.20	2.20	45.45	
74 h	Lc	0.72	0.86	16.27	3.11
	Cc	1.63	3.30	**50.61**	
86 h	Lc	0.79	0.98	19.39	2.60
	Cc	1.86	3.75	50.4	

Notes: Lc=*Lepiota clypeolaria* Cc=*Cytospora chrysosperma*

Table 3. Antagonistic culture result of *Lactarius vellereus* to *Cytospora chrysosperma*

time	strain	Av vs Cc colony directional radius (cm)	colony radius of individually cultured (cm)	inhibited rate(%)	relative inhibiting effect
26 h	Lv	0.30	0.30	0	0
	Cc	0.30	0.30	0	
38 h	Lv	0.30	0.30	0	–
	Cc	0.87	0.70	-24.28	
50 h	Lv	0.30	0.30	0	–
	Cc	2.03	1.60	-26.88	
62 h	Lv	0.30	0.30	0	–
	Cc	2.77	2.20	-25.91	
74 h	Lv	0.30	0.30	0	–
	Cc	3.43	3.30	-3.03	
86 h	Lv	0.30	0.30	0	–
	Cc	3.53	3.75	5.87	

Notes: v=*Lactarius vellereus* Cc=*Cytospora chrysosperma*

Table 4. Colony diameters of *Cytospora chrysosperma* on PDA with toxin crude extract (cm)

time	colony diameters of *Cytospora chrysosperma* on PDA with different toxin crude extract(cm)				
	Amanita virosa	*Lepiota clypeolaria*	*Lactarius vellereus*	*Amanita muscaria*	control
26 h	0.5	0.5	0.5	0.5	0.60
38 h	0.5	0.5	0.5	0.5	1.40
50 h	0.5	0.5	0.5	0.5	3.20
62 h	0.5	0.5	0.5	0.5	4.40
74 h	0.5	0.5	0.5	0.5	6.60

Sprouting-inhibiting effect of toxin crude extract to pathogen conidia

(1) Method of mycostatic circle producing

The result showed that 4 toxin crude extracts had inhibiting effect on *Cytospora chrysosperma* spore sprouting(Table 5). The effect of extract from *Amanita virosa* was the best (the diameter of mycostatic circle was 2.50 cm). The second best was from *Lepiota clypeolaria*, (the diameter of mycostatic circle was 1.64 cm) and the diameters of mycostatic circle produced by toxin crude extracts from *Lactarius vellereus* and *Amanita muscaria* were 1.00 cm and 0.90 cm. repestively

(2) Pendent drop method

The result of conidia sprouting in 24h was shown in Table 6. All of 4 toxin crude extracts had sprouting-inhibiting effect on *Cytospora chrysosperma* conidia. Percentage of inhibition was 93.96- 97.29% The best effective one was the toxin crude extract from *Amanita virosa*, followed by the extract from *Lepiota clypeolaria*.

Table 5. Diameter of mycostatic circle produced by toxin of mushroom strains (cm)

Strain	Diameter of mycostatic circle(cm)
Amanita virosa	2.50
Lepiota clypeolaria	1.64
Lactarius vellereus	1.00
Amanita muscaria	0.90
Contract	0.60

Table 6. Conidia sprouting of *Cytospora chrysosperma* in different toxin crude extract

strain	number of conidia	number of sprouted	sprouting rate (%)	sprouting inhibiting rate (%)
Amanita virosa	84	2	2.38	97.29
Lepiota clypeolaria	118	6	3.08	96.49
Lactarius vellereus	78	4	3.84	95.63
Amanita	105	6	5.13	93.96
Contract	82	72	87.8	

The results in Table 5 and Table 6 showed that sprouting-inhibiting effect of toxin crude extract to pathogen conidia by methods of mycostatic circle producing and pendent drop were the same. The mushroom toxin extracted from 4 strains above could effectively inhibit conidia sprouting of *Cytospora chrysosperma*.

4. CONCLUSION

The strains of *Amanita virosa* and *Lepiota clypeolaria* showed inhibiting effect on *Cytospora chrysosperma*. The effect of *A. virosa* was higher than *L. clypeolaria*. All toxin crude extracts from *Amanita virosa*, *Lepiota clypeolaria*, *Lactarius vellereus* and *Amanita muscaria* showed very high growth-inhibiting effect and conidia sprouting-inhibiting of *Cytospora chrysosperma*. These 4 strains and their toxins should be used in biocontrol for *Cytospora chrysosperma*, and the ecological safety examination should be done in future.

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