Evaluation of Ketomium-mycofungicide on Siberian isolates of phytopathogenic fungi

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The fungicidal effects of the biological preparation Ketomium® comprising a *Chaetomium* spore suspension were evaluated for its effect on Siberian isolates of the phytopathogenic fungi *Botrytis cinerea*, *Didymella applanata*, *Fusarium oxysporum* and *Rhizoctonia solani*. Ketomium-mycofungicide inhibited the growth of the taxa *in vitro*; however, the degree of the inhibition depended on the fungal pathogen and the concentration of preparation used. *In vitro*, Ketomium-mycofungicide was most efficient at suppressing *Didymella applanata* which causes Raspberry Spur Blight. Field-testing of Ketomium-mycofungicide on potato yields was carried out in a potato plantation. Ketotomium-mycofungicide led to significant suppression of potato disease caused by *Rhizoctonia solani*. Introduction of Ketomium-mycofungicide into the soil during planting resulted in a significant potato tuber yield increase. This preparation of Ketomium-mycofungicide showed promise as a new means of biological control of plant diseases in Siberian conditions. Ketomium-mycofungicide appears to be a promising preparation with activity following a rather long period of storage and acts as a new broad-spectrum mycofungicide.

Key words: biological control, Ketomium-mycofungicide, phytopathogenic fungi, Siberian isolates

Introduction

Siberian isolates of phytopathogenic fungi are adapted to severe climatic conditions. Therefore, it is interesting to study the effect of biological preparations based on southern isolates of biocontrol agents for plant disease suppression. The example of such a preparation is Ketomium®-mycofungicide

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(Soytong *et al*., 2001) which comprises a spore mixture of species of *Chaetomium*. This preparation has not previously been used as a biocontrol agent in Siberia.

There has been previous research to control plant diseases using promising microbial antagonists such as *Chaetomium* spp. For example, *C. globosum* and *C. cochlioides* can inhibit the growth of *Fusarium* sp. and *Helminthosporium* sp. (Tveit and Moore, 1954). Seed dressing with *C. globosum* can prevent seedling blight of corn caused by *Fusarium roseum* f. sp. *cerealis* 'graminearum' (Chang and Kommedahl, 1968). It was also stated that spraying the ascospore suspension of *C. globosum* to apple trees can reduce apple scab caused by *Venturia inequalis* (Heye and Andrews, 1983; Cullen and Andrews, 1984; Boudreau and Andrews, 1987). *Chaetomium globosum* can also reduce the pathogenic inoculum of *Botrytis cinerea* on lily leaves in the field (Kohl *et al*., 1995). *Chaetomium cupreum* is also an interesting specie as it has been reported to control soybean plant pathogens e.g. *Phomopsis* and *Colletotrichum* spp. (Manandhar *et al*., 1986).

In Thailand, strains of *C. cupreum* and *C. globosum* can reduce leaf spot disease of corn caused by *Curvularia lunata*, rice blast caused by *Pyricularia oryzae* and sheath blight of rice caused by *Rhizoctonia oryzae* (Soytong, 1989, 1992). These strains can also reduce tomato wilt caused by *Fusarium oxysporum* f.sp. *lycopersici* in the greenhouse and field (Soytong, 1990) and reduce basal rot of corn caused by *Sclerotium rolfsi* (Soytong, 1991). Twenty-two strains of *C. cupreum* and *C. globosum* were formulated in the form of biopellets and biopowder as a new broad spectrum mycofungicide for plant disease control (Soytong and Soytong, 1997). Chaetomium-mycofungicide completely prevented root rot caused by *Phytophthora* spp., e.g. of black pepper (Sodsaard and Soytong, 1999) and tangerine (Soytong *et al*., 1999).

The aim of the work presented here was to evaluate the effects of biological preparations of the genus *Chaetomium* on the soil-borne and air-borne phytopathogens, *Rhizoctonia solani*, *Fusarium oxysporum*, *Didymella applanata* and *Botrytis cinerea* due to the wide distribution of diseases caused by these phytopathogens in Siberia.

Materials and methods

Pure cultures of Siberian isolates of *Botrytis cinerea*, *Didymella applanata*, *Fusarium oxysporum* and *Rhizoctonia solani* and the Ketomium-mycofungicide preparation were used in this study. The biological preparation Ketomium-mycofungicide (Neoworld Company, Thailand), containing 22

strains of the genus *Chaetomium* (*C. cupreum* and *C. globosum*) were provided by Biocontrol Research Unit, KMITL, Bangkok, Thailand.

The effects of the preparation of Ketomium-mycofungicide on the pure culture of phytopathogenic fungi were evaluated by the agar block technique (Bilai, 1982; Sokolova, 1995) recording growth inhibition of fungal colonies. Suspensions of the preparation were added to the nutrient medium at 36-37ºC. Czapek's medium was used for *Didymella applanata*, and potato agar for the other phytopathogens. Preparations of Ketomium-mycofungicide at 0.1, 0.2, 0.4, 0.8 and 1% were evaluated fro their effect on the soil-borne pathogens and 0.1 and 0.5% for evaluation of the effect on air-borne pathogens *in vitro*. Medium supplemented with the biological agent was poured into Petri dishes and allowed to cool. A 10 mm diameter agar block cut from the periphery of the growing culture of the phytopathogen and was placed into the center. Each strain was replicated five times. The activity of the preparation was assessed according to the size of fungal colonies and compared with the control (the control was medium lacking the biological preparation under study).

Field testing of Ketomium-mycofungicide was carried out on the cultivar Nevsky of the potato plantation near Novosibirsk. Experimental plots were 25 m^2. Randomized Complete Block Design was used to assign treatments to four replicates. Yields were determined by weighing all harvested tubers from each plant. Ketomium-mycofungicide, 1g per plant, was placed into the soil during planting. The control plots were left untreated. A scale for damage estimation of sprouts, stolons and tubers produced by potatoes were used (Frank *et al*., 1976).

The experimental data were processed statistically by dispersion analysis using the program ANOVA. Treatment means were compared with the least significant difference (LSD).

Results and discussion

Taking into account, the mode of application of the preparation (introduction in soil), we estimate the influence of Ketomium-mycofungicide on soil-borne pathogens (*Fusarium oxysporum* and *Rhizoctonia solani*) in more detail. The results of experiments using pure cultures of *Fusarium oxysporum* and *Rhizoctonia solani* are presented in Table 1.

Maximal inhibitory effect was observed on day 3 for *Rhizoctonia solani* and on day 7 for *Fusarium oxysporum.* The inhibitory effect of Ketomium-mycofungicide increased with the concentration growth. This effect is due not only to antibiotics produced by *Chaetomium* but also to hyperparasitism that we observed in Petri dishes.

Table 1. The influence of Ketomium-mycofungicide on growth of phytopathogenic fungi (diameter of Petri dish is 9 mm).

Concentration of formulation, %	Diameter of colony, cm		
	3 days	5 days	7 days
Rhizoctonia solani			
0 (control)	8.2	9	9
0.1	6.2	8.8	7.9
0.2	3.6	5.5	5.7
0.4	4	4.7	4.9
0.8	2.1	3	3
1.0	1	2.5	3
LSD 05	**0.4**	**0.7**	**1.3**
Fusarium oxysporum			
0 (control)	4.9	7	7.9
0.1	3.5	4.5	4.9
0.2	3.3	4.8	4.8
0.4	4.4	4.6	4.4
0.8	2.6	3.2	3.1
1	2	2.2	3.2
LSD 05	**1.4**	**1.5**	**0.9**

The most common crop infected by *Rhizoctonia solani* in Siberia is potato. Therefore, we tested the preparation in field in a potato plantation. Ketomium-mycofungicide reduced disease severity compared with the control.

The number of dead sprouts and damaged stolons decreased significantly (Table 2). Ketomium-mycofungicide reduced the number of fall-off stolons that was very important for yield development.

Table 2. The influence of Ketomium-mycofungicide on potato disease caused by *Rhizoctonia solani* in 2001.

Treatment	Death of sprouts, %	Disease severity, %	Damaged stolons, %	Fall-off stolons, %
Control	7.4	20.2	7	5.5
Ketomium	2.8	14.2	2.3	1.9

The treated plants were much more resistant to *Rhizoctonia solani* than the untreated ones. The number of healthy tubers produced by the plants treated with Ketomium-mycofungicide increased. There were no tubers damaged by sclerotia, while sclerotia covered more than 50% of the surface of the control tubers. Such tubers were not suitable for storage.

Table 3. The influence of Ketomium-mycofungicide on growth of phytopathogenic fungi (diameter of Petri dish is 9 mm) in 2002 (after 1 year of storage).

Concentration of formulation, %	Diameter of colony, cm		
	3 days	5 days	7 days
Rhizoctonia solani			
0	9	9	9
0.1	7.8	9	9
0.2	7.4	9	9
0.4	5.8	9	9
0.8	1.6	4.7	4.3
1	1	1.5	1.7
LSD 05	**0.9**	**0.8**	**0.6**
Fusarium oxysporum			
0	3.1	4.7	5.7
0.1	3	2.6	3
0.2	2.4	3.9	4.6
0.4	2.6	3.3	3.2
0.8	2.5	3.3	2.9
1	2.8	3.2	3.1
LSD 05	**0.7**	**1.5**	**0.9**

The introduction of Ketomium-mycofungicide in soil during planting provided a potato yield of 97.5 kg per 100 bushes compared with 79.6 kg per 100 bushes in the control (LSD = 15.8). It is interesting to note that Ketomium-mycofungicide maintained its activity for one year of storage after first experiments on pure cultures (Table 3). Later, propagules of *Chaetomium* taxa were inactivated, however, the high concentration (1%) of the preparation were able to suppress the growth of phytopathogenic colonies (2-5 fold) after 2 years of storage, possibly, due to antibiotics contained in this preparation.

Evaluation of the effects of Ketomium-mycofungicide on the phytopathogenic fungi, *Didymella applanata* and *Botrytis cinerea* is shown in Table 4. Surprisingly, *Didymella applanata* appeared to be the most susceptible to *Chaetomium*. Ketomium-mycofungicide application (0.1%) caused an insignificant decrease in the size of the *D. applanata* colony on day 3 and a 1.5-fold decrease on day 7. This preparation at a concentration of 0.5% inhibited the growth of this phytopathogen to a greater degree. The fungistatic effect increased with time: the colony size decreased 2.3-fold compared to the control on day 3 and almost 5-fold on day 7. This effect may result from released antibiotics and enzymes produced by *Chaetomium* (Fokkema, 1993).

Table 4. Effects of Ketomium preparation on the growth of *Didymella applanata* and *Botrytis cinerea*.

Concentration of formulation, %	Colony size, cm			
	D. applanata		*B. cinerea*	
	3 days	7 days	3 days	7 days
0	3	7.4	5	9.3
0.1	2.8	4.8	3.5	7.2
0.5	1.3	1.5	2	2.5
LSD 05	**0.3**		**0.6**	

The effect of Ketomium-mycofungicide at a concentration of 0.1% on the growth of the *Botrytis cinerea* was insignificant over the observation period. The size of the *B. cinerea* colonies decreased 2.5-fold on day 3 and remained the same over the observation period at high concentrations. In addition the phytopathogen did not sporulate and its growth virtually halted on day 3. The effect of Ketomium-mycofungicide on *Didymella applanata was similar to Botrytis cinerea*. The only difference was in the abundant sporulation of *Chaetomium* and manifestation of hyperparasitism (inhibition of colony growth, a decrease in the thickness of hyphae of the *B. cinerea* and their utilization as a nutrition source). These data demonstrate that biological preparations of a bacterial and fungal nature can be used against pathogens causing raspberry diseases. Application of biological preparations containing *Chaetomium* spores is promising when diseases caused by the phytopathogenic fungi *Didymella applanata* and *Botrytis cinerea* are developing on raspberry simultaneously.

Thus, for the first time we show the possibility of applying *Chaetomium* biocontrol for disease control in the Siberian region of Russia. Ketomium-mycofungicide appears to be a promising preparation maintaining activity over a relatively long storage period and acts as a new broad spectrum mycofungicide (Soytong *et al*., 2001).

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