Control stem white rot of rapeseed caused by *Sclerotinia sclerotiorum* with *Coniothyrium minitans*, focused on application time

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Abstract

Stem white rot of rapeseed caused by *Sclerotinia sclerotiorum* in China is a major obstacle for further improvement of seed yield. *Coniothyrium minitans,* a mycoparasite of *S. sclerotiorum*, was introduced to control this disease in field. To find out the optimal delivery time, conidia of *C. minitans* were aerially delivered onto plants at 5 % bloom, 10 % bloom and 50 % bloom at Xishui county, Tianmen county and Wuxue county of Hubei Province from 2004 to 2006. The efficiency of *C. minitans* on control stem white rot was evaluated *via* yield improvement and reduction of disease severity. Among 36 valid *C. minitans* treatments, 30 treatments showed that the seed yields were improved with a range of 6.0 % to 27.3 %. *C. minitans* delivered at 5 % bloom and 10 % bloom gave higher efficiency than at 50 %; and applied twice (one at 5 % bloom and the other at 10 % bloom) gave the highest efficiency. The results also showed that *C. minitans* could reduce the disease severity when applied at 5 % bloom and 10 % bloom, however, the disease severity was not related to the loss of seed yield. In all experiments, dimethachlon was used as control, and results showed that this fungicide gave better efficiency than *C. minitans* did. This is the first time we evaluated the efficiency of *C. minitans via* seed yield of rapeseed. The potential of aerial application and the factors which may affect the efficiency of *C. minitans* against stem white rot were also discussed.

Key words: *Sclerotinia sclerotiorum*, Coniothyrium minitans, *Brassica napus*, Stem white rot, Biological control

Introduction

Sclerotinia sclerotiorum is a plant pathogenic fungus inhabiting soil ubiquitously in many parts of the world. In China, this pathogen causes stem white rot on rapeseed (*Brassica napus*), and leads to serious losses every year in the middle and low drainage areas of Yangtse River, even in rice- rapeseed rotated field, this disease may also cause serious loss. In each year, the loss caused by stem white rot is about 10-70 % in China (Zhang, 2003; Deng and Tang, 2006). Work has been focused on resistance breeding with traditional and transgenic methods to reduce the utilization of chemical fungicides. However, unlike biotropic plant pathogens, *S. sclerotiorum* has met few host plant with high resistance, currently only partial resistance has been detected in some tested plant material or varieties (Zhao et al, 2006). Genes targeting the degradation of oxalic acid, the most important toxin secreted by *S. sclerotiorum*, were transformed into other plants to enhance the resistance against this pathogen (Cober et al, 2003; Dias et al, 2006; Guo et al, 2006), but no transgenic plants were used practically in field so far.

Coniothyrium minitans Campbell was first isolated from a sclerotium of *S. sclerotiorum* (Campbell, 1947) and its potential as a biocontrol agent of *Sclerotinia* spp was appreciated (Whipps and Gerlagh, 1992). Since then, the biology and ecology of *C. minitans*, especially on biocontrol of *Sclerotinia* diseases and the technique for scale production was studied perfectly (Gerlagh et al, 1999; De Vrije et al, 2001), and leads to the successful development of commercial biocontrol agents based on *C. minitans*, such as KONI and Contans®. *C. minitans* was applied into soil to rot and inhibit the germination of sclerotia and subsequently reduce the inocula of *Sclerotinia* diseases (Jones et al, 2003; Jones et al, 2004). As a biocontrol agent, *C. minitans* was used successfully to control *Sclerotinia* diseases of vegetable crops (such as lettuce) in greenhouse (Jones et al, 2004; Rabeendran et al, 2006).

The number of inocula (sclerotia) in soil is important factor for the epidemic of *Sclerotinia* diseases which initially infected by the infectious hyphae germinating from sclerotia. However, it may be the secondary important factor for the epidemic of stem white rot of rapeseed, which is initially infected by aerial-borne ascospores. In China, the stem white rot in rice-rapeseed rotation fields is still very serious. The main reason is that there are many small dry-land fields scattering in rice-rapeseed rotation fields. Ascospores released from sclerotia in the soil of dry land may be an important source for the primary inocula of stem white rot of rapeseed planted in rice-rapeseed rotation field. This means that application of *C. minitans* into soil to rot the sclerotia may lead to little effect on control stem white rot. As a parasite of *Sclerotinia* spp., *C. minitans* can parasitize and rot sclerotium, but also could parasitize and destroy host's hyphae (Huang, 1980). Aerial application of *C. minitans* was mainly focused on the parasitization of newly formed sclerotia on lesions (Huang et al, 2000;

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Gerlagh et al, 2003), while the interaction between *C. minitans* and *S. sclerotiorum* on petals was attractive, and *C. minitans* could inhibit the germination of ascospores, and block secondary infection initiated by infected petals (Li et al, 2003), and most recently, Li et al (2006) reported that *C. minitans* may reduce severity of stem white rot when aerial applied on rapeseed plant during bloom season.

To improve the efficiency of *C. minitans*, we conducted a three year field experiment to obtain the best delivery time for aerial application. In this paper, we reported the optimal time for *C. minitans* delivery and a new method for evaluation this biocontrol efficiency.

Materials and methods

Strain and maintenances: C. minitans strain ZS-1 (ACCAM 041057), a strain could produce large amount of conidia in shaken liquid medium (Cheng et al, 2003) was used for this study. Strain ZS-1 was grown on PDA plate at 20-22 C and maintained in PDA slant at 4-10 C routinely.

Production of a batch of conidia: A solid medium (OSM) containing 90 % of oatmeal and 10 % of sclerotia meal was used. To make OSM, both oatmeal and sclerotia meal were submerged into tap water overnight to absorb enough water, and then the meal was passed through two layers of cheesecloth to remove surplus water. The meal was packaged into 250 ml-flask, 100 gram for one flask, and autoclaved at 121 C for 1 h. Conidia produced in PDA slants were collected with sterilized distill water, and adjusted to a concentration of 1×107 spores/ml, spore suspension was inoculated into OSM, one milliliter for one flask. The inoculated flasks were placed into an incubator at 20 C for one month. To harvest conidia, a certain volume of water was added into *C. minitans* grown OSM, and blended with a blender. The homogenate was diluted with water and passed through two layers of cheesecloth to remove debris. The concentration of conidia suspension was adjusted to 1×106 with a haemacytometer before use.

Design of field experiments: Field experiments were set at three counties (Xishui County, Wuxue County and Tianmen County) in Hubei Province in the year of 2004, 2005 and 2006. For each county, two types of field were chosen, one was dry land where rapeseed was rotated with other dry crops, and the other was a rice–rapeseed rotation field (rice field), usually white stem rot occurs more severer in dry land than that in rice field. In 2004, in each field, conidia (1×106) were sprayed onto rapeseed plants for one time at 10 % bloom and 50 % bloom once or twice. To compare with fungicide, dimethachlon (Sipeisi 40 % powder, Lucheng Chemical Pesticides Co., Wenzhou, P R China) was instead of *C. minitans* to spray onto plants at 10 % bloom and 50 % bloom once or twice, the powder was diluted for 1200 folds before use. Both the delivery time and dosage for dimethachlon were recommended by the company. In each treatment, three replicates were conducted, the area of each plot was 26.7 m2, and plots were separated by two lines of rapeseed plants at least. Three plots which were not treated with *C. minitans* or dimethachlon were looked as control. Plants were managed as usual but without using any additional fungicides. In 2005 and 2006, Conidia of *C. minitans* were delivered onto plants at 5 % bloom and 10 % bloom once or twice, while, the time and dosage for dimethachlon treatments were not changed. To make a just evaluation for rapeseed seed yield, the area of each plot was half discounted, namely the plot size was 13.34 m2. The management of experiment fields were also not changed comparing to 2004.

Disease survey: In the harvest stage, plants in each plot were collected and the lesions caused by *S. sclerotiorum* were checked. Plants with one or more lesions were looked as diseased plant, and the disease incidence in each plot was calculated. The severity of diseased plants were divided into five degrees as described by Luo and Zhou (1994) with a small modification, namely: level 0, plants without any lesion; level 1, small lesions can be seen on stem or branches, the number of damaged pods in the whole diseased plant is less than 25 %; Level 2, 1/3 to 2/3 branches were infected or large lesion produced on main stem, the number of damaged pods take up 25 % to 50 % of whole pods of the diseased plant; level 3, more than 2/3 branches were infected or the large lesion cyclized the main stem of diseased plant, and 50 % to 75% of pods are damaged; level 4, the whole plant is killed, and more than 75 % pods are damaged. Damaged pods might be directly killed by pathogen or be due to the destruction of stems or branches. The disease indexes (ID) were calculated with the formula:

$$ID= \sum_{i=1}^{k} \sum (Gi\times Ni) \ / (Gk\times N) \times 100$$

Gi= the degree of disease severity, Ni= the number of diseased plants with certain degree, Gk, the highest degress, and N, the number of surveyed plants.

Seed yield: All plants in each plot were harvested, and seeds from each plot were collected and weighted individually. To evaluate the effect of yield improvement (YI), the formula below was used, YI= (Yt－Yc) / Yc×100, here, Yt and Yc represented the average yield in each treatment and control respectively. When YI was larger than 6 %, the treatments against stem rot were looked as valuable.

Statistical analysis: All data obtained were subjected to statistical analysis with ANNOV programme. To compare the efficiency on control stem rot among different treatments, disease index (ID) of each treatment and control were anti-triangle transferred before analysis.

Result and Discussion

C. minitans could control stem white rot with acceptable efficiency: From the three years data we obtained, *C. minitans* could be used to control stem white rot with acceptable efficiency. Totally, there were 54 treatments with *C. minitans* in three selected counties among three years, 30 treatments could enhance the seed yield with a range of 6.0 % to 27.3 %, and 24 treatments were less than 6.0 % (Table 2, 4 and 6). While experiments at Tianmen county and Wuxue county in 2004 were invalid because of the low disease incidence in all plots (Table 1), the experiment at Xishui county in 2006 was also negatively affected by raining, it was a moderate rain after inoculation of *C. minitans* in dry land field and rice field at 3 h and 6 h later, respectively. In these three experiments, the seed yields between treatments (including dimethachlon treatment) and control were not significantly different (Table 2 and Table 6). Removing the invalid 18 treatments, 83.3 % *C. minitans* treatments showed positive results.

In 2004, the seed yield improvement was not good when treated with *C. minitans* one time at 50 % bloom (Table 2). The result was different from dimethachlon treatment, which is required to deliver to field at 50 % bloom to achieve the highest efficiency. The possible reason for the difference between *C. minitans* treatments and dimethachlon treatments is that *C. minitans* need more time to destroy its host (*S. sclerotiorum*), while dimethachlon is an absorbable chemical and just need a short time to damage its targets.

In 2005 and 2006, *C. minitans* was delivered as early as 5 % bloom, which is about one week before 10 % bloom based on the local temperature. Experiments in Tianmen county and Wuxue county showed that *C. minitans* could improve seed yield (YI > 6 %) when treated at 5 % bloom and 10 % bloom, more or less, treated at 5 % bloom was better than treated at 10 % bloom (Table 4 and 5). But the result of 2005 in Xushui county was an exception, it showed that inoculated *C. minitans* at 5 % bloom obtained little yield improvement (Table 4). In most experiment fields, treated with *C. minitans* twice could obtain high seed yield improvement. The highest YI was 27.3 % which was achieved in the dry land experiment in 2004 at Xishui county (Table 2), while the dry land experiment of 2006 at Wuxue county was an exception, which YI was less than 6 %.

Most dimethachlon treatments could improve seed yield in the three counties, and the efficiency was better than *C. minitans* achieved when both of them were applied only once. However, when both *C. minitans* and dimethachlon applied twice, the seed yields were not significantly different in some experiments, such as in rice field at Wuxue county in 2005 and in both dry land and rice field at Tianmen county in 2005, and in rice field at Tianmen county in 2006.

C. minitans reduce the disease severity of stem white rot: C. minitans could reduce the disease severity of stem white rot. The range of disease reducing ability of *C. minitans* was 0-53.78 %, the highest effect of *C. minitans* on reducing the disease severity was achieved in the rice field experiment at Xishui county in 2004 (Table 1), while several experiments did not show any significantly difference from control. Dimethachlon could control the disease severity well, the range was 0-96.0 %, the highest effect of dimethachlon was obtained in dry land experiment at Xishui county in 2006 (Table 5). It seemed that experiments in dry land at Tianmen county in 2005 was failed, there was not any effect on reducing the disease severity with *C. minitans* and dimethachlon (Table 3).

The paradox of these results we obtained was that there was not tightly relationship between seed yield and disease severity. As showed in Table 5 and Table 6, in the dry land at Xishui in 2006 experiments, dimethachlon treatments reduced the disease index with 39 %, 94.33 % and 96 %, respectively, and *C. minitans* treatments reduced the disease index with 30.61 %, 35.96 % and 43.90 %, however, the seed yields between treatments and control were not significantly different (Table 6). On the other hand, in some experiments, the disease indexes between treatments and control were not significant different, but the seed yields were significantly improved. There are two facts to be considered, i) the disease severity of stem white rot was divided into five degrees, but the degree was not correlated with the reduction of seed yield of rapeseed, for example, a plant which was killed by *S. sclerotiorum*, was regarded as level 4, however, early killed plant may lead to "take-all" loss, but later killed plants only slightly affect the thousand seed weight; ii) *C. minitans* could inhibit the extension of lesions (Li et al, 2006), and subsequently enhance the survive of infected plants for longer time. So the fair method to evaluate the efficiency of *C. minitans* is weighting the seed yield.

Table 1 The severities of rapeseed stem white rot caused by *S. sclerotiorum* in *C. minitans* and dimethachlon treated plots in 2004

Treatment[1]	Xishui County				Wuxue County				Tianmen County			
	Dry land (%)[2]	Disease index reduced (%)	Rice field (%)	Disease index reduced (%)	Dry land (%)	Disease index reduced (%)	Rice field (%)	Disease index reduced (%)	Dry land (%)	Disease index reduced (%)	Rice field (%)	Disease index reduced (%)
Control	46.92 ^{A}		22.91 ^{A}		16.93 ^{A}		2.60 ^{A}		13.00 ^{A}		4.83 ^{A}	
CM 10% bloom	35.77 $_{AB}$	23.76	11.74 ^{C}	48.76	12.4 ^{AB}	26.76	2.50 ^{A}	-[3]	7.67 ^{A}	-	5.50 ^{A}	-
CM 50% bloom	42.76 $_{AB}$	8.87-	15.38 ^{B}	32.87	11.93 ^{AB}	29.53	1.7 ^{A}	-	7.17 ^{A}	-	3.83 ^{A}	-
CM10% bloom and 50% bloom	32.29 $_{BC}$	31.18	10.59 $_{CD}$	53.78	11.07 ^{AB}	34.61	2.13 ^{A}	-	10.50 ^{A}	-	5.00 ^{A}	-
Dimethachlon 10% bloom	24.06 $_{CD}$	48.72	15.02 ^{B}	34.44	9.10 ^{BC}	46.25	1.97 ^{A}	-	8.67 ^{A}	-	3.50 ^{A}	-
Dimethachlon50% bloom	20.66 ^{D}	55.97	11.63 $_{CD}$	49.24	7.47 ^{BC}	55.88	2.30 ^{A}	-	5.00 ^{A}	-	3.33 ^{A}	-
Dimethachlon 10% bloom and 50% bloom	10.70 ^{E}	77.19	9.13 ^{D}	60.15	4.27 ^{C}	74.78	1.40 ^{A}	-	6.67 ^{A}	-	2.33 ^{A}	-
L.S.D	6.972		2.43		5.556		3.631		8.425		6.488	
P=0.05												

Note: 1, CM in columns of tables meant that plots were treated with *Coniothyrium minitans*; 2, The same character in columns in tables meant that the anti-angle transferred disease indexes were not significantly different; dash meant that the disease severity was not significantly different among all treatments, and these experiments were looked as invalid experiments.

However, the evaluation for the efficiency of *C. minitans* against stem white rot is a complicated work. The plot size may influence the judgment, for example, the stem white rot in field is not homogeneous distributed as typical aerial borne diseases do, if the plot size is small, the disease incidences among plots may be varied wildly; on the other hand, if the plot size is too large, the difference of seed yield among plots may be affected by the soil fertility and other factors rather than by disease.

Table 2 The seed yield of rapeseed in *C. minitans* and dimethachlon treated plots in 2004.

Treatment	Xishui County				Wuxue County				Tianmen County			
	Dry land (kg)	Yield improved (%)	Rice field (kg)	Yield improved (%)	Dry land (kg)	Yield improved (%)	Rice field (kg)	Yield improved (%)	Dry land (kg)	Yield improved (%)	Rice field (kg)	Yield improved (%)
Control	3.19^{E}		4.46^{D}		7.43^{A}		9.48^{A}		8.33^{B}		7.77^{A}	
CM 10% bloom	3.85^{DE}	20.7	5.20^{ABC}	16.6	6.81^{A}	-[1]	8.56^{A}	-	8.57^{AB}	-	7.9^{A}	-
CM 50% bloom	3.43^{DE}	7.5	4.88^{C}	9.4	7.99^{A}	-	8.67^{A}	-	8.6^{A}	-	7.17^{A}	-
CM10% bloom and 50% bloom	4.06^{CD}	27.3	5.24^{AB}	17.5	7.18^{A}	-	9.59^{A}	-	8.37^{AB}	-	7.70^{A}	-
Dimethachlon 10% bloom	4.56^{BC}	42.9	4.93^{BC}	10.5	7.49^{A}	-	9.88^{A}	-	8.53^{AB}	-	7.6^{A}	-
Dimethachlon50 % bloom	4.76^{AB}	49.2	5.20^{ABC}	16.6	7.84^{A}	-	10.18^{A}	-	8.53^{AB}	-	7.97^{A}	-
Dimethachlon 10% bloom and 50% bloom	5.32^{A}	66.8	5.51^{A}	23.5	6.72^{A}	-	9.62^{A}	-	8.43^{AB}	-	7.9^{A}	-
L.S.D	0.639		0.356		1.480		2.632		0.256		1.206	
P=0.05												

Note: 1, dash means that the seed yield was not significantly different among all treatments, and these experiments were looked as invalid experiments.

Factors influence the efficiency of C. minitans: The disease incidence and severity of stem white rot could influence the efficiency of *C. minitans*. When the disease was very severe (disease index was larger than 40 %), *C. minitans* failed to improve the seed yield highly as dimethachlon did. For example, in 2004, the average disease index in dry land control plots at Xishui county was 46.92 %, the highest YI by *C. minitans* was 27.3 %, but it was 66.8 % by dimethachlon (Table 1 and Table 2); in 2005, the average disease index in rice field control plots at Xishui county was 44.68 %, the highest YI by *C. minitans* was 25.63 %, but it was 52.52 % by dimethachlon (Table 3 and Table 4). A similar result was observed in the field experiments at Tianmen county and Wuxue county in 2006. One exception was in the field experiments at Tianmen county in 2005, the YIs of both *C. minitans* treatments and dimethachlon treatments were not significantly different, but the YIs of dimethachlon treatments were relatively lower than expected.

Table 3 The severities of rapeseed stem white rot caused by S. sclerotiorum in C. minitans and dimethachlon treated plots in 2005

Treatment	Xishui County				Wuxue County				Tianmen County			
	Dry land (%)*	Disease index reduced (%)	Rice field (%)	Disease index reduced (%)	Dry land (%)	Disease index reduced (%)	Rice field (%)	Disease index reduced (%)	Dry land (%)	Disease index reduced (%)	Rice field (%)	Disease index reduced (%)
Control	31.34^{A}		44.68^{A}		13.07^{A}		26.37^{A}		45.60^{A}		41.83^{A}	
CM 10% bloom	30.28^{A}	0.02	39.04^{AB}	12.62	10.87^{B}	16.83	24.37^{B}	7.59	41.60^{A}	-	37.07^{AB}	-
CM 50% bloom	22.60^{B}	27.89	33.52^{AB}	24.98	10.83^{B}	17.14	18.58^{D}	29.54	43.40^{A}	-	37.60^{B}	10.11
CM10% bloom and 50% bloom	18.22^{BC}	41.86	27.68^{BC}	38.05	8.10^{C}	38.02	17.18^{E}	34.85	40.87^{A}	-	36.07^{B}	13.77
Dimethachlon 10% bloom	14.83^{CD}	52.68	19.16^{C}	57.12	10.99^{B}	15.91	21.84^{C}	17.18	35.93^{A}	-	30.83^{B}	26.30
Dimethachlon50 % bloom	10.41^{D}	66.78	10.61^{D}	76.25	6.90^{D}	47.2	5.98^{F}	77.32	40.00^{A}	-	31.17^{B}	25.48
Dimethachlon 10% bloom and 50% bloom	5.63^{E}	88.42	4.94^{D}	88.94	5.47^{E}	58.15	5.57^{F}	78.88	35.70^{A}	-	30.43^{B}	27.25
L.S.D	4.664		6.824		0.559		0.646		5.907		5.213	
P=0.05												

Application at 5 % bloom and 10 % bloom might get high efficiency; however, the time asked for delivering *C. minitans* is possibly a self-limited factor for high efficiency. Rapeseed petals are very important for *S. sclerotiorum* infection, initially ascospores germinate on senescent petals and develop to infectious hyphae, and then the infectious hyphae fall on leaves, branches, pods and stems to cause secondary infection; furthermore, the release of ascospores would last a quite long time to be effective inocula. Conidia of *C. minitans* could not germinate on plants well without the stimulation of *S. sclerotiorum*, it also could not inhibit the infection of pathogen with large amount of inocula (such as mycelial agar and infectous petals) (Jiang et al, 2000). Application *C. minitans* at 5 % bloom or 10 % bloom give little chance for *C. minitans* to stay on later opening petals, which take up 90-95 % of total petals, and subsequently fails to inhibit secondary infection initiated by infected petals which open lately. This may be the reason to explain why *C. minitans* could not reduce the disease severity and enhance seed production with high efficiency as dimethachlon do.

Table 4 The seed yield of rapeseed in *C. minitans* and dimethachlon treated plots in 2005

Treatment	Xishui County				Wuxue County				Tianmen County			
	Dry land (kg)	Yield improved (%)	Rice field (kg)	Yield improved (%)	Dry land (kg)	Yield improved (%)	Rice field (kg)	Yield improved (%)	Dry land (kg)	Yield improved (%)	Rice field (kg)	Yield improved (%)
Control	2.58^{E}		2.38^{E}		1.88^{D}		6.05^{E}		3.10^{B}		3.23^{B}	
CM 10% bloom	2.62^{DE}	1.55	2.55^{E}	7.14	2.05^{BC}	9.04	6.77^{C}	11.90	3.4^{AB}	9.68	3.60^{AB}	11.46
CM 50% bloom	2.97^{CD}	15.12	2.77^{DE}	16.39	1.95^{CD}	3.72	6.33^{D}	4.63	3.27^{AB}	5.48	3.57^{AB}	10.53
CM10% bloom and 50% bloom	3.23^{BC}	25.19	2.99^{CD}	25.63	2.10^{B}	11.70	7.00^{AB}	15.70	3.53^{A}	13.87	3.73^{AB}	15.48
Dimethachlon 10% bloom	3.31^{BC}	28.29	3.22^{BC}	35.29	2.02^{BC}	7.45	6.40^{D}	5.79	3.53^{A}	13.87	3.50^{AB}	8.36
Dimethachlon50 % bloom	3.61^{AB}	39.92	3.40^{AB}	42.86	2.22^{A}	18.09	7.13^{A}	17.85	3.37^{AB}	8.71	3.60^{AB}	11.46
Dimethachlon 10% bloom and 50% bloom	3.83^{A}	48.44	3.63^{A}	52.52	2.27^{A}	20.74	6.90^{BC}	14.05	3.57^{A}	15.16	3.77^{A}	16.72
L.S.D	0.388		0.391		0.115		0.190		0.354		0.510	
P=0.05												

Table 5 The severities of rapeseed stem white rot caused by *S. sclerotiorum* in C. minitans and dimethachlon treated plots in 2006

Treatment	Xishui County				Wuxue County				Tianmen County			
	Dry land (%)	Disease index reduced (%)	Rice field (%)	Disease index reduced (%)	Dry land (%)	Disease index reduced (%)	Rice field (%)	Disease index reduced (%)	Dry land (%)	Disease index reduced (%)	Rice field (%)	Disease index reduced (%)
Control	22.05 ^{A}		29.82 ^{A}		30.60 ^{A}		40.63^{A}		54.03 ^{A}		32.53^{A}	
CM 10% bloom	14.12^{BC}	35.96	24.06 ^{AB}	19.32	26.27 ^{B}	14.15	29.47^{C}	27.48	49.07 ^{AB}	9.18	21.73 ^{B}	33.20
CM 50% bloom	15.30 ^{B}	30.61	29.43 ^{AB}	1.31	29.16 ^{B}	4.70	34.95^{B}	13.98	48.23 ^{AB}	10.73	23.13^{AB}	28.90
CM10% bloom and 50% bloom	12.37 ^{C}	43.90	20.34 ^{B}	31.79	21.42 ^{C}	30.00	33.47^{BC}	17.62	44.47 ^{BC}	17.69	9.17 ^{C}	71.81
Dimethachlon 10% bloom	13.45^{BC}	39.00	22.95 ^{AB}	23.04-	26.65 ^{B}	12.90	33.71^{BC}	17.03	43.13 ^{BC}	20.17	29.10^{AB}	10.54
Dimethachlon50 % bloom	1.25 (6.56)^{D}	94.33	4.27 ^{C}	85.68	4.90 ^{D}	56.30	14.10^{D}	65.30	37.03 ^{C}	31.46	11.17^{C}	65.66
Dimethachlon 10% bloom and 50% bloom	0.88 (5.22)^{D}	96.00	1.63 (7.79)^{C}	94.53	3.47 (10.68)^{D}	88.66	11.17^{D}	72.51	25.43 (30.26)^{D}	52.93	9.50^{C}	70.80
L.S.D	1.954		6.164		2.529		3.074		5.018		6.985	
P=0.05												

Table 6 The seed yield of rapeseed in *C. minitans* and dimethachlon treated plots in 2006

Treatment	Xishui County				Wuxue County				Tianmen County			
	Dry land (kg)	Yield improved (%)	Rice field (kg)	Yield improved (%)	Dry land (kg)	Yield improved (%)	Rice field (kg)	Yield improved (%)	Dry land (kg)	Yield improved (%)	Rice field (kg)	Yield improved (%)
Control	3.47^{A}		3.26^{C}		5.43^{E}		5.17^{E}		2.40^{D}		3.00^{C}	
CM 10% bloom	3.51^{A}	-	3.34^{BC}	2.45	5.57^{DE}	25.78	5.57^{D}	7.74	2.77^{BCD}	15.42	3.37^{B}	12.33
CM 50% bloom	3.48^{A}	-	3.28^{C}	0.61	5.77^{CD}	6.26	5.77^{CD}	11.61	2.63^{CD}	9.58	3.33^{B}	11.00
CM10% bloom and 50% bloom	3.63^{A}	-	3.44^{ABC}	5.52	5.70^{DE}	4.97	5.87^{BCD}	13.54	2.93^{BC}	22.08	3.73^{A}	24.33
Dimethachlon 10% bloom	3.50^{A}	-	3.35^{BC}	2.76	6.00^{BC}	20.35	5.97^{BC}	15.47	2.77^{BCD}	15.42	3.40^{B}	13.33
Dimethachlon50 % bloom	3.55^{A}	-	3.68^{AB}	12.88	6.13^{B}	12.89	6.17^{AB}	19.34	3.17^{AB}	32.08	3.23^{BC}	7.67
Dimethachlon 10% bloom and 50% bloom	3.99^{A}	-	3.82^{A}	17.18	6.83^{A}	25.78	6.40^{A}	23.79	3.37^{A}	40.42	3.80^{A}	26.67
L.S.D	0.741		0.323		0.278		0.289		0.374		0.276	
P=0.05												

Conclusions

C. minitans could protect rapeseed against *S. sclerotiorum* with acceptable efficiency, the seed yield of most treated plots might be improved with a range of 6-27.3%; the ideal time for delivery is at 5 % bloom to 10 % bloom stage of rapeseed, and applying twice (one at 5 % bloom, another one at 10 % bloom) could obtain highest effect. Furthermore, rapeseed seed yield as criteria is a reasonable and objective for evaluation of *C. minitans* biocontrol efficiency.

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