球孢白僵菌对小猿叶甲的致病力测定

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摘要：在室内研究了分离自小猿叶甲的一株球孢白僵菌(SCAU-BB01D)对小猿叶甲的致病力。结果表明，该菌株能感染小猿叶甲的成虫和各龄幼虫，但对不同虫期的致病力存在差异。在 10^5～10^8 孢子/ml 的浓度范围内，随着处理浓度的升高，各虫期小猿叶甲的感病死亡率增加，在最高浓度 1×10^8 孢子/ml，处理后成虫第 14 天及 1～3 龄幼虫第 10 天的累计死亡率分别为 84.7%、94.0%、96.0%和 81.0%。用 TDM 模型对成虫和各龄幼虫的致病力数据进行模拟，所建模型均顺利通过 Hosmer-Lemeshow 拟合异质性检验，表明模型拟合良好，并由模型估计出了该菌株对小猿叶甲各虫期的致死剂量与致死时间。在处理后第 10 天，成虫和 1～3 龄幼虫的致死中浓度(LC_{50})分别为 2.68×10^7、1.07×10^6、1.63×10^5 孢子/ml 和 8.31×10^6 孢子/ml，而第 14 天成虫的 LC_{50} 为 2.38×10^6 孢子/ml。随着浓度的增加，各虫期所需的感病死亡时间缩短，在最高浓度 1×10^8 孢子/ml，球孢白僵菌对小猿叶甲成虫及 1～3 龄幼虫的致死中时(LT_{50})分别为 9.28、4.29、4.40d 和 5.06 d。综合分析白僵菌对各虫期的致死剂量及致死时间可以看出，不同虫期的小猿叶甲对球孢白僵菌敏感性不同。结果表明该菌株在小猿叶甲生物防治中具有一定的潜力。

关键词：球孢白僵菌；小猿叶甲；致病力；生物防治

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Pathogenicity of a *Beauveria bassiana* (Balsamo) Vuillemin isolate to daikon leaf beetle, *Phaedon brassicae* Baly (Coleoptera: Chrysomelinae)

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Abstract: The daikon leaf beetle, *Phaedon brassicae*, is an important insect pest of cruciferous vegetables in South China. It is typically controlled by synthetic insecticides. A search was made for alternative control methods, and diseased daikon leaf beetles were collected and screened for potentially useful pathogens. A *Beauveria bassiana* isolate, SCAU-BB01D, originally derived from *P. brassicae* adults was found to infect all stages of the beetle under laboratory conditions. The pathogenicity of the isolate was investigated by immersing *P. brassicae* larvae and adults into serial dilutions of *B. bassiana* (10^5—10^8 conidia/ml). The highest concentration (10^8 conidia/ml) treatment caused an adult mortality of 84.7% on day 14, and larval mortalities of 94.0%, 96.0%, and 81.0% for instars Ⅰ, Ⅱ, Ⅲ on day 10, respectively. A time-dose-mortality model was used to analyze the bioassay data and the model fitted the data well, resulting in parameters for estimating the time and dose effects. The LC_{50} values of instar Ⅰ—Ⅲ and adult were 1.07×10^6, 1.63×10^5, and 8.31×10^6, 2.68×10^7 conidia/ml, respectively, on day 10 after treatment. The LC_{50} value on day 14 was 2.38×10^6 conidia/ml for adults. The lethal time for the isolate on *P. brassicae* varied with the insect stage. At a concentration of 10^8 conidia/ml, the LT_{50} values of the isolate against adult and

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instar Ⅰ, instar Ⅱ, and instar Ⅲ larvae were 9.28, 4.29, 4.40 and 5.06 d, respectively. The results indicated the potential of the isolate for use in microbial control of *P. brassicae*.

Key words: *Beauveria bassiana*; *Phaedon brassicae*; pathogenicity; biological control

The daikon leaf beetle, *Phaedon brassicae* Baly (Coleoptera: Chrysomelinae), is distributed throughout China, Japan, Korea, and Vietnam[1]. It frequently damages cruciferous vegetables including cabbage, Chinese flowering cabbage, leaf mustard, kale and especially watercress and radish[2]. The daikon leaf beetle can complete five generations within a year in the Guangzhou area of South China and causes most severe damage in early summer and autumn. The most damaging stages are adults and third instar larvae feeding on foliage and stems of vegetables[3]. Watercress is a major cruciferous vegetable in South China and is usually grown in the Pearl River Delta area from autumn through spring but not over summer. In recent years due to increasing demand watercress has been widely grown in valley fields near streams or down stream of reservoirs in summer months. The daikon leaf beetle occurred frequently and resulted in severe damage to watercress production[4].

A common technique for control of *P. brassicae* is the application of synthetic chemical pesticides in watercress fields. Repeated application of chemicals can cause high levels of insecticide resistance and excessive residues on the watercress plants. Public concerns for vegetable safety have increased interest in alternative management approaches. *Bacillus thuringiensis* (*Bt*) and entomopathogenic nematodes, which were not derived from daikon leaf beetle, were used for control of *P. brassicae*[5—8], but research results indicated limited efficacy of *Bt* and variable efficacy of entomopathogenic nematodes to the beetles. There is a great need to develop alternative methods to manage the daikon leaf beetle in the region.

Beauveria bassiana (Balsamo) Vuillemin (Ascomycota: Hypocreales) is one of the most widely distributed and extensively studied entomopathogenic fungi. It can infect more than 700 species of insects and mites belonging to 149 families and 15 orders[9] and is the active agent in many products currently in use and under development worldwide[10~12]. Because of its attributes of easy mass culture, desiccation stability, compatibility with some chemicals, broad host range, and safety to vertebrates, it has been used to successfully control many important insect pests such as corn borers, pine caterpillars, Colorado potato beetle, grasshoppers and locusts, *Bemisia*, etc.[11,13,14]. However until now there has been no published report on the use of *B. bassiana* against *P. brassicae*.

In the summer of 2001 a number of *P. brassicae* adults infected with a fungus were discovered in watercress fields in Yanshan County, northern Guangdong. The dead infected *P. brassicae* adults had typical visible external white mycelia. Diseased beetles collected in the field were brought back to the laboratory for isolation and identification of the pathogen, which was found to be *B. bassiana*. Six isolates of A, B, C, D, E, and F were collected and held in the Laboratory of Insect Ecology, Department of Entomology, South China Agricultural University. The isolate D, SCAU-BB01D, was evaluated as the best for further study after preliminary comparison of its biology (spore yield, germination rate and mycelium growth rate) with the other *B. bassiana* isolates as well as its pathogenicity to *P. brassicae* adults[15].

The determination of time-dose-mortality relationships (TDM) is of importance in selecting the pathogen isolates that have the greatest potential for controlling the target host[16]. TDM models consider both time and dose effects and can not only estimate the traditional parameters such as LT_{50} and LC_{50}, but also the time trend for each dose and the dose trend for each time simultaneously. They have been used to evaluate the effectiveness of several pathogens to their target hosts[16~20]. The objectives of this study were to determine the TDM relationships between *B. bassiana* isolate SCAU-BB01D and various stages of *P. brassicae*, and to evaluate its potential for use in control of *P. brassicae*.

1　MATERIAL AND METHODS

1.1　Insects for Bioassay

An experimental population of *P. brassicae* initiated from field-collected adults was maintained at the Laboratory of Insect Ecology, South China Agricultural University at Guangzhou, Guangdong Province. Adult beetles were reared on potted watercress plants inside screened cages (25 cm×25 cm×40 cm) at (25±1)℃ and 12 L: 12 D in the laboratory. After 3 to 5 days of oviposition, *P. brassicae* adults were transferred to new potted watercress plants. The watercress plants with eggs were reared in cages for more than 1 month development, first, second and third instar larvae and adults were selected for use in assays.

1.2 Fungal Isolate and Inocula

The isolate *B. bassiana* SCAU-BB01D was derived from a *P. brassicae* adult cadaver collected in a field of Yangshan County, Guangdong during summer 2001 and was grown on Potato Dextrose Agar (PDA) in Petri dishes (10 cm diameter) in a growth chamber at (25±1)℃ and a 12 L:12 D photoperiod. Conidia were harvested from a 15-day-old culture by flooding the plate with 10 ml of distilled water and then suspended in 0.01% Tween 80. The suspension was vortexed on a magnetized stirrer for about 30 min and then filtered through a piece of double-layer medical gauze to remove the debris. The conidial concentration in the suspension was determined using a hemacytometer under a compound microscope and subsequent dilutions of 1×10^5, 1×10^6, 5×10^6, 1×10^7, 5×10^7 and 1×10^8 conidia/ml were made by adding 0.01% Tween 80. All suspensions were used immediately after preparation.

1.3 Bioassay

Six concentrations of 1×10^5, 1×10^6, 5×10^6, 1×10^7, 5×10^7 and 1×10^8 conidia/ml plus a control of 0.01% aqueous Tween 80 were included in the bioassays against first, second and third instar larvae, and adult beetles. The larvae and adult beetles were collected from the screened cages in the laboratory just before the bioassay. For each treatment 15～20 adult beetles or 20 larvae of each instar were removed from the host plant and placed in a 90mm plastic Petri dish. For inoculation, each batch of insects was immersed in a Petri dish with 10 ml of 1×10^8 conidia/ml suspension for 10s and then moved to a piece of dry filter paper for absorption of extra suspension on the insects. Each treatment consisted of 5 replicates and one control, which was immersed in 0.01% Tween 80 in distilled water. The experiment was conducted with a completely randomized design. After inoculation, treated larvae and beetles were transferred to a plastic Petri dish and fresh greenhouse-grown watercress was provided as food. The Petri dishes were covered with a piece of nylon mesh and then transferred to growth chambers at 25℃, 70% *RH* and 12 L: 12 D.

Treated insects were observed daily for 10 days in the case of larvae and 14 days in the case of adults to record mortality. The watercress plants were replaced daily with fresh ones. All cadavers in all treatments were removed daily and placed in Petri dishes with a damp cloth cover and kept under high humidity to determine the number of sporulating cadavers. Visual observations were also recorded on the time until post-mortem mycelial appearance and sporulation.

1.4 Statistical Analysis

A time-dose-mortality modeling technique[21, 22] was used to fit the data from the bioassay of the selected isolate of *B. bassiana* to four stages of *P. brassicae* (3 larval instars and adult).

The procedures, including modeling, estimation of time and dose effect parameters for both conditional and cumulative models, test for goodness of fit, and estimation of virulence indices (LC_{50} and LT_{50}) using the parameters, were conducted using DPS data processing system software[23].

2 RESULTS

2.1 Bioassay Result

All tested stages of *P. brassicae* were susceptible to *B. bassiana* infection in the laboratory. The mycosis-caused deaths caused by infection of *B. bassiana* occurred first on days 5 to 6 for adults and on days 2 to 3 for larvae and most mortality occurred on days 9 to 11 for adults and on days 4 to 6 for larvae (Figure 1). Both infected adults and larvae displayed typical symptoms of *B. bassiana* infection with mycelial outgrowths from inter-segmental membranes of the adult or integument of the larvae. The cadavers were able to sporulate well under high humidity. The cumulative mortality increased with concentration. The mortalities at the concentration of 10^8 conidia/ml were 84.7% on day 14 for adults and 94.0%, 96.0%, and 81.0% on day 10 for Ⅰ—Ⅲ instar larvae, respectively. No death was observed in the control of the first and second instar larvae. In the adult and third instar larvae control, less than 10% insects were observed dead, but none of them had the typical symptoms of *B. bassiana* infection.

2.2 Modeling and Goodness of Fit

The bioassay data were corrected using the background mortality of the control and then were fitted to the time-dose-mortality model. The parameters of the conditional mortality probability model and those for the cumulative mortality probability model were listed in Table 1. The t test for all parameters estimated were significant ($p<0.001$), indicating the significance of the time-dose-mortality relationship of *B. bassiana* to all tested stages of *P. brassicae*. The Hosmer-Lemeshow

test for heterogeneity of goodness of fit[16,21] was insignificant for all models of four bioassays (adult: $C=10.19$, $p=0.25$, first instar: $C=8.29$, $p=0.41$, second instar: $C=9.75$, $p=0.28$, third instar: $C=6.66$, $p=0.57$; $df=8$, $\chi^2_{0.05}=15.51$), indicating that the data fit the model very well.

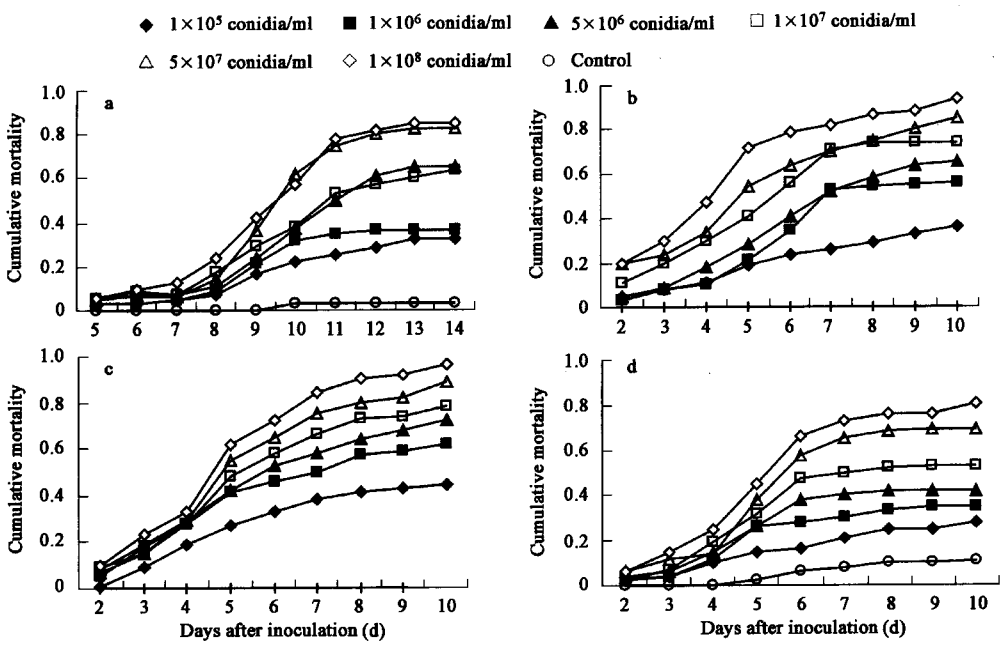

Fig. 1　The cumulative mortality of *P. brassicae* after treatment with a *B. bassiana* isolate. (a) Adult; (b) First instar larvae; (c) Second instar larvae; (d) Third instar larvae.

The slopes for the dose effect (β) of the adult and the three instars were 0.648, 0.657, 0.446 and 0.767, indicating third instar larvae were most sensitive and second instar larvae least sensitive to the increase in concentration. The parameters for the conditional time effects in the assay of adults increased from day 7 to 11(γ_7—γ_{11}) and peaked on day 11, suggesting the true mortality peak of adults occurred at least 10 days after treatment. The parameters for the conditional time effects in the bioassays of first, second and third instars peaked on day 5 or 6. This implies that the true mortality peak of larvae occurred 5—6 days after inoculation, and coincided with the observed mortalities in Fig. 1.

2.3　Lethal Dose and Time

The parameters estimated for the cumulative time-dose-mortality models were used to compute virulence indices using formulae given by Feng[21] and Nowierski *et al.*[16]. The relationship between $\log_{10}$(LC_{50} and LC_{90}) with standard errors and time after inoculation are shown in Fig. 2. As time after treatment increased, the LC_{50} values estimated decreased in all bioassays. On day 10 after inoculation, LC_{50} values for *B. bassiana* against *P. brassicae* were 1.07×10^6 conidia/ml for the first instar, 1.63×10^5 conidia/ml for the second instar, 8.31×10^6 conidia/ml for the third instar, and 2.68×10^7 conidia/ml for the adult, respectively. On day 14 after inoculation, LC_{50} values for *B. bassiana* against *P. brassicae* adults were 2.38×10^6 conidia/ml. The relevant LC_{90} estimates for the three instar larvae and the adults were 7.11×10^7, 8.4×10^7, 3.06×10^8, and 1.9×10^9 conidia/ml, respectively.

The values of LT_{50}(d) for *B. bassiana* against different stages of *P. brassicae* under different concentrations are listed in Table 2. The lethal time of *B. bassiana* to *P. brassicae* varied with the insect stage tested and shortened with increased concentration. At the low concentration of 1×10^5, LT_{50} values of *B. bassiana* for all tested stages of *P. brassicae* were unable to be estimated because the true mortalities were below 50%.

3　DISCUSSION

The *B. bassiana* SCAU-BB01D derived from the *P. brassicae* cadaver can infect both larvae and adults of *P. brassicae*, but the infectivity varied with the tested insect stages. At the highest concentration of 1×10^8 conidia/ml, LT_{50} for adults was 9.28 d, much longer than those for larvae (4—5 d). Both the dose and the time effects of *B. bassiana* on different stages of *P.*

brassicae indicate that the first two instars were more susceptible to the infection of *B. bassiana* than third instar that the adult stage was apparently less susceptible. The differences in susceptibility may be due to variation in morphological characteristics. It is well known that older larvae, with thicker cuticles and a well-developed immune system, may have a stronger defensive reaction to fungal penetration of the integument thus decreasing the susceptibility to pathogens[24—26].

Table 1 Parameters estimated by fitting the time-dose-mortality model to assay data of the *B. bassiana* SCAU-BB01D against different stages of *P. brassicae*

Conditional mortality model				Cumulative mortality model				Conditional mortality model				Cumulative mortality model			
Para-metera	Value	S. E.	t^b	Para-metera	Value	Var (τ_i)	Cov (β,τ)	Para-metera	Value	S. E.	t^b	Para-metera	Value	Var (τ_i)	Cov (β,τ)
Adult								First instar larvae							
β	0.648	0.195	3.32	β	0.648	0.005	0.005	β	0.657	0.327	2.01	β	0.657	0.014	0.014
γ_5	−7.776	0.537	14.48	τ_5	−7.776	0.442	−0.038	γ_2	−6.761	0.898	7.53	τ_2	−6.761	0.889	−0.108
γ_6	−8.451	0.537	15.73	τ_6	−7.364	0.420	−0.038	γ_3	−7.379	0.890	8.20	τ_3	−6.330	0.873	−0.108
γ_7	−9.275	0.538	17.25	τ_7	−7.226	0.435	−0.038	γ_4	−6.736	0.900	7.50	τ_4	−5.819	0.846	−0.108
γ_8	−7.178	0.536	13.38	τ_8	−6.508	0.336	−0.038	γ_5	−6.002	0.893	6.72	τ_5	−5.213	0.819	−0.107
γ_9	−6.306	0.534	11.80	τ_9	−5.709	0.298	−0.038	γ_6	−6.261	0.900	6.99	τ_6	−4.913	0.814	−0.107
γ_{10}	−6.077	0.535	11.39	τ_{10}	−5.183	0.289	−0.038	γ_7	−6.372	0.896	7.11	τ_7	−4.704	0.812	−0.107
γ_{11}	−5.799	0.532	10.90	τ_{11}	−4.751	0.284	−0.038	γ_8	−6.596	0.897	7.35	τ_8	−4.563	0.812	−0.107
γ_{12}	−6.548	0.535	12.23	τ_{12}	−4.60	0.284	−0.038	γ_9	−7.149	0.899	7.95	τ_9	−4.491	0.812	−0.107
γ_{13}	−7.011	0.536	13.08	τ_{13}	−4.512	0.284	−0.038	γ_{10}	−6.285	0.916	6.86	τ_{10}	−4.337	0.811	−0.107
γ_{14}	−9.067	1.470	6.169	τ_{14}	−4.501	0.285	−0.038								
Second instar larvae								Third instar larvae							
β	0.446	0.052	8.58	β	0.446	0.001	0.001	β	0.767	0.256	3.00	β	0.767	0.009	0.009
γ_2	−5.763	0.403	14.28	τ_2	−5.763	0.088	−0.010	γ_2	−8.780	0.706	12.43	τ_2	−8.780	0.664	−0.065
γ_3	−5.275	0.387	13.62	τ_3	−4.796	0.078	−0.010	γ_3	−8.513	0.706	12.06	τ_3	−7.945	0.562	−0.065
γ_4	−5.016	0.383	13.10	τ_4	−4.207	0.075	−0.010	γ_4	−8.142	0.706	11.54	τ_4	−7.345	0.527	−0.065
γ_5	−4.284	0.379	11.30	τ_5	−3.552	0.074	−0.010	γ_5	−7.116	0.703	10.13	τ_5	−6.531	0.502	−0.065
γ_6	−4.740	0.386	12.28	τ_6	−3.286	0.073	−0.010	γ_6	−6.900	0.701	9.84	τ_6	−6.006	0.494	−0.065
γ_7	−4.687	0.390	12.03	τ_7	−3.065	0.073	−0.010	γ_7	−7.695	0.705	10.92	τ_7	−5.836	0.494	−0.065
γ_8	−4.727	0.391	12.09	τ_8	−2.892	0.072	−0.010	γ_8	−8.307	0.705	11.77	τ_8	−5.755	0.495	−0.065
γ_9	−5.544	0.441	12.47	τ_9	−2.823	0.072	−0.010	γ_9	−10.03	0.707	14.19	τ_9	−5.741	0.495	−0.065
γ_{10}	−4.831	0.409	11.81	τ_{10}	−2.697	0.071	−0.010	γ_{10}	−8.379	0.759	11.04	τ_{10}	−5.672	0.495	−0.065

a The subscripts represent the number of days or the ith day after treatment; *b* The t statistics were highly significant for all parameters estimated ($p<0.001$)

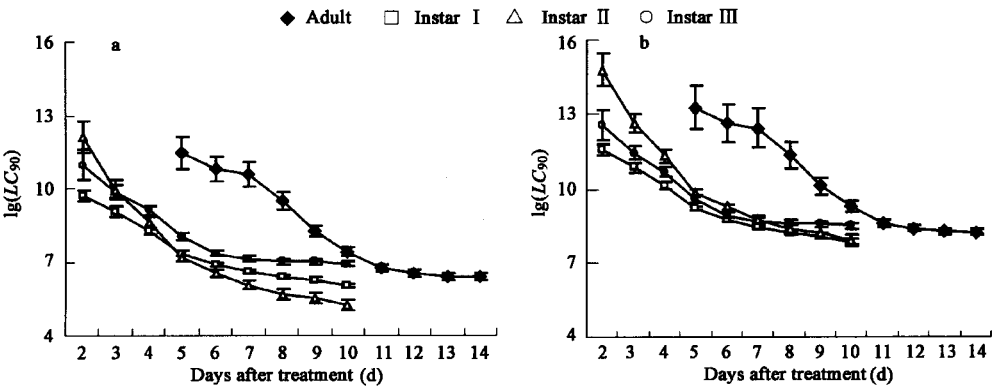

Fig. 2 Logarithms of the time-dependent LC_{50}(a) and LC_{90}(b) values (no. conidia/ml) for *B. bassiana* SCAU-BB01D against different stages of *P. brassicae*.

Usually, adults of Coleoptera are less susceptible to fungal infection than larvae due to their special elytra and sclerotized body structure. For example, Chikwenhere and Vestergaard[27] reported that LC_{50} values of *B. bassiana* to larvae and adults of the Chevroned water hyacinth weevil, *Neochetina bruchi*, were 7.6×10^5 and 8.9×10^6 conidia/ml, respectively, on day 12 post-treatment. This implies larvae were about 10 times more susceptible to *B. bassiana* than adults and thus was well in accord with our results. A higher susceptibility of the larval stage was also observed in the brinjal spotted beetle, Henosepilachna *viginlioctopunctata*[28], and the Colorado potato beetle, *Leptinotarsa decemlineata*[29, 30].

Table 2　The values of LT_{50}(d) for *B. bassiana* SCAU-BB01D against different stages of *P. brassicae*

Stages	LT_{50}(d) at the concentration(conidia/ml)					
	1×10^5	1×10^6	5×10^6	1×10^7	5×10^7	1×10^8
Adult	—	—	11.26	10.63	9.65	9.28
instar Ⅰ	—	—	6.64	5.78	4.61	4.29
instar Ⅱ		7.14	5.74	5.24	4.60	4.40
instar Ⅲ	—	—	—	9.12	5.48	5.06

In watercress fields, the adults and the third instar larvae of *P. brassicae* were primarily damaging stages, accounting for more than 90% of the total food consumption of all stages[2]. To avoid damage to cruciferous crops, it is necessary to control *P. brassicae* populations before the third instar. This study indicated that the first two instars of *P. brassicae* larvae were most susceptible to *B. bassiana*. More than 90% of larvae died from infection at a concentration of 1×10^8 conidia/ml. Taking the virulence and culture aspects[15] into consideration, *B. bassiana* SCAU-BB01D has a high potential for use in microbial control of *P. brassicae* in the future.

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