Screening of high-virulent entomopathogenic fungal strains to infect *Xylotrechus rusticus* larvae

Yan-chen Wang¹ · De-fu Chi¹

Received: 19 November 2018 / Accepted: 31 January 2019 / Published online: 13 February 2019
© King Abdulaziz City for Science and Technology 2019

Abstract

The gray tiger longicorn beetle, *Xylotrechus rusticus* Linnaeus (Coleoptera: Cerambycidae) is a stem-boring pest that can inhibit not only the transportation of nutrients in the trunk but also the tree growth, increasing the risk of tree breakage and causing economic losses. It is distributed in China, Iran, Turkey, Russia, Korea, Japan, and Southern Europe. This study aimed to investigate selected *Beauveria* strains that could be used as entomopathogenic fungi for the biological control of this pest. The high-virulence strains were screened among the selected strains by cumulative mortality, correct mortality, and lethal time to 50% mortality (LT50). These screened high-virulence strains were Bb01, CFCC83486, and CFCC81428. Bb01 exhibited 96.96% cumulative mortality, with an estimated LT50 of 3.28 days. CFCC83486 and CFCC81428 caused 89.29% and 75.74% cumulative mortality, with an estimated LT50 of 3.45 and 4.28 days, respectively. Pathogenicity at different concentrations and lethal concentration of 50% (LC50) of these high-virulence strains were investigated. The pathogenicity was found to be positively correlated with suspension concentration, and LC50 was negatively correlated with infection time. These suspensions of high-virulence strains at different concentrations were also investigated in the forest by brushing the suspensions on the poplar tree trunk infested with *X. rusticus* L. larvae. The most effective strain was found to be Bb01, whose cumulative mortality reached 76.33% at 1.32×10^8 conidia mL⁻¹, followed by the strain CFCC83486, whose cumulative mortality reached 65.17% at 1.32×10^8 conidia mL⁻¹. This study provides an important basis for using *B. bassiana* in the biological control of *X. rusticus* L.

Keywords *Beauveria bassiana* · *Beauveria brongniartii* · Pathogenicity · Fungal · Gray tiger longicorn beetle · Biocontrol

Introduction

The gray tiger longicorn beetle, *Xylotrechus rusticus* Linnaeus (Coleoptera: Cerambycidae), is one of the most destructive pests. It is a stem-boring pest feeding mainly on poplar trees. It is found in northeast Asia where poplar trees are present, including China (Heilongjiang, Jilin, Liaoning, Inner Mongolia, and Jiangsu provinces), Iran, Turkey, Russia, Korea, Japan, and Southern Europe. *X. rusticus* L. inhibits the transportation of nutrients and growth by boring the trunk of poplar trees, increasing the risk of tree breakage. This pest was listed as a forestry quarantine pest by the

State Forestry Administration of China after 2004 (Wang et al. 2003; Hu et al. 2004; Wang and Liu 2005; Li et al. 2014). Poplar species have strong economic value; the bark and leaves are valuable ingredients in traditional Chinese medicine and the trunk in paper, wood composite board, chopsticks, and so on. *X. rusticus* L. causes serious environmental and economic losses (Zhao 2011).

At present, *X. rusticus* L. can be managed using biological control, which includes inundative releases of the natural enemies [*Dastarcus helophoroides* (Fairmaire) and *Scleroderma guani* Xiao et Wu] (Ding et al. 2016). Chemical methods include spraying or brushing insecticides (omethoate, chlorbenzuron, and sumithion) on the poplar trunk to kill larvae (Wang et al. 2002; Wen and Wang 2010). Long-term use of chemical pesticides increases pest resistance and resurgence to these chemical pesticides, and also causes severe environmental and ecological damage. Therefore, discovering a strategy that can minimize negative

✉ De-fu Chi
chidefu@126.com

Yan-chen Wang
tony196015249@hotmail.com

¹ School of Forestry, Northeast Forestry University, No. 26, Hexing Road, Harbin 150040, China

environmental effects and effectively control *X. rusticus* L. is becoming extremely urgent.

Entomopathogenic fungi have a great potential to be an environment friendly and sustainable alternative to control pests by parasitizing insects. These entomopathogenic fungi include *Beauveria bassiana* (Balsamo) Vuillemin (Hypocreales: Cordycipitaceae), *B. brongniartii* (Saccardo) Petch (Hypocreales: Cordycipitaceae), *Metarhizium brunneum* Petch (Hypocreales: Clavicipitaceae), *M. flavoviride* var. *minus*, *Isaria farinosa* (Holmskjöld) Fries (Hypocreales: Clavicipitaceae), and *I. poprawskii* sp. nov. (Hypocreales: Cordycipitaceae) (Cabanillas and Jones 2009; Popa et al. 2012; Cabanillas et al. 2013; Woruba et al. 2014; Brabbs et al. 2015).

The process of *B. bassiana* infection can be described in five steps as follows: first, *B. bassiana* attaches to the host body by the conidia. Second, an infection structure is formed. Third, *B. bassiana* penetrates into the host body cavity and invades hemolymph. Fourth, mycelium grows within the host body and produces toxins against the immune system of the host, killing the host. Fifth, the conidia are formed on the surface of the host body after a period of parasitic growth, and a new infection cycle is formed (Latge et al. 1987; Clarkson and Charnley 1996; Ding et al. 2015).

The pest control efficiency of *B. bassiana* and *B. brongniartii* has been reported in many studies. *B. bassiana* (Hyphomycetes) has been studied to control turpentine beetle, *Dendroctonus valens* LeConte. It killed all the infected insects at a concentration of 1×10^8 conidia mL^{-1} , and the median lethal time [lethal time to 50% mortality (LT50)] was 4.06 days (Zhang et al. 2011). Sabbahi et al. (2008) found that *B. bassiana* (Bals.) Vuill can be used to control a tarnished plant bug, *Lygus lineolaris* L. Dubois et al. (2004) reported that fiber bands impregnated with *B. brongniartii* can be used to control Asian long-horned beetle, *Anoplophora glabripennis*. Being inspired by these studies, the researchers aimed to explore an ecological and environment-friendly strategy to control *X. rusticus* L. using *B. bassiana* and *B. brongniartii* in the present study.

This study included five strains of *B. bassiana* and one strain of *B. brongniartii* to screen high-virulent entomopathogenic fungal strains infecting larvae of *X. rusticus* L. The most effective entomopathogenic fungal strains (determined

by the cumulative mortality rate, correct mortality rate, and LT50 at the same concentration of each strain) were formulated into various concentrations to evaluate their lethal concentration of 50% (LC50) and their effect (cumulative mortality rate and correct mortality rate) in the laboratory and forest. In this way, the most lethal and the most optimal concentration was determined to serve as a potential biological and environment-friendly strategy to control *X. rusticus* L.

Materials and methods

Preparation of insects

Xylotrechus rusticus L. larvae were collected from the poplar tree infested with *X. rusticus* L. larva, from Bin county, Harbin, China, and transferred to the Key Laboratory of Forest Pest Biology (KLFPB), Northeast Forestry University (Harbin, Heilongjiang Province, China). *X. rusticus* L. larvae were incubated at $25 \pm 0.5^\circ\text{C}$, $75\% \pm 10\%$ relative humidity (RH), darkness, and fed with artificial diet for continuous rearing of *X. rusticus* L. (Ding et al. 2015). The dead or sick larvae were removed. Healthy fifth instar larvae of uniform size were used for the experiments.

Preparation of *Beauveria* strains

The *B. bassiana* strains CFCC83486, CFCC87298, CFCC83116, and CFCC81428 and *B. brongniartii* strain CFCC83487 were obtained from BeNa Culture Collection, Beijing. *B. bassiana* strain Bb01 was obtained from KLFPB (Table 1). All the strains were cultured using an improved Martin culture medium (200 g L^{-1} potato extraction, 20 g L^{-1} glucose, 3 g L^{-1} KH_2PO_4 , 1.5 g L^{-1} $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 8 mg L^{-1} vitamin B1, and 20 g L^{-1} agar, pH 6).

Pathogenicity and LT50 of the six *Beauveria* strains to *X. rusticus* L. larvae

Conidia from the six strains were isolated and scraped into 0.1% Tween-80 sterile solutions to make bacteria suspensions at 1.32×10^8 conidia mL^{-1} . The *X. rusticus* L. larvae

Table 1 The information of fungal strain used in the experiments

Strain no.	Strain	Host	Geographic origin
Bb01	<i>Beauveria bassiana</i>	<i>Xylotrechus rusticus</i> L	Heilongjiang, China
CFCC83486	<i>Beauveria bassiana</i>	Cerambycidae pupa	Liaoning, China
CFCC83487	<i>Beauveria brongniartii</i>	<i>Holotrichia diomphalia</i>	Heilongjiang, China
CFCC87298	<i>Beauveria bassiana</i>	Trunk borer pupa	Jilin, China
CFCC83116	<i>Beauveria bassiana</i>	<i>Anoplophora glabripennis</i>	Inner Mongolia, China
CFCC81428	<i>Beauveria bassiana</i>	Cerambycidae larvae	Anhui, China

were immersed in 1.32×10^8 conidia mL^{-1} suspension for 30 s and then incubated at 26°C , $75\% \pm 10\%$ RH, and darkness for 10 days. The control group was immersed in 0.1% Tween-80 sterile solution for 30 s and incubated at the same condition mentioned earlier. Thirty larvae were tested in each group, and the procedure was repeated 3 times. The post-infection larval mortality was observed on the second, fourth, sixth, eighth, and tenth days. The mortality rates were recorded, and the calculated LT50 was taken as a basis to select the highest virulence for further study. The mortality rates were adjusted as follows. Corrected mortality (%) = $(C_1 - C)/(100 - C) \times 100\%$, where C_1 is the cumulative mortality rate of the treated larvae and C is the cumulative mortality rate of the control (Ding et al. 2015).

Pathogenicity at different concentrations and LC50 of the high-virulence strains to *X. rusticus* L. larvae

The suspensions of high-virulence strains were diluted to 1.32×10^8 , 1.32×10^7 , 1.32×10^6 , 1.32×10^5 , and 1.32×10^4 conidia mL^{-1} using a hemacytometer (XB. K. 25, Qiujiang, Shanghai, China) and 0.1% Tween-80 sterile solution. The 0.1% Tween-80 sterile solution served as the control. The larvae were treated using the same methods described in the “Pathogenicity and LT50 of the six *Beauveria* strains to *X. rusticus* L. larvae” section. The post-infection larval mortality was observed on the second, fourth, sixth, eighth, and tenth days. Ten larvae were tested in each group, and the procedure was repeated three times.

Forest trial of the pathogenicity of high-virulence strains at different concentrations

Forest trial was conducted from June 15 to June 30, 2016, in Lindian county, Daqing, China, where trees infested with *X. rusticus* L. were presented in the forest (124.86°E , 47.17°N). The annual average temperature was 4°C , annual precipitation was 550–600 mm, and altitude was 142.7–172.4 m.

The suspensions of high-virulence strains were diluted into 1.32×10^8 , 1.32×10^7 , 1.32×10^6 , 1.32×10^5 , and 1.32×10^4 conidia mL^{-1} by the same method described in the “Pathogenicity and LT50 of the six *Beauveria* strains to *X. rusticus* L. larvae” section. The Tween-80 sterile solution served as the control. The diluted suspensions were brushed on the poplar tree trunk infested with *X. rusticus* L. larvae (2 m high from the ground) and covered with perforated plastic film. Five larvae-infested poplar trees were tested in each group, and the procedure was repeated three times. Different treated groups were separated by more than 4 m from untreated tree belts. The average height of the tree was 40 m with an average 35 cm diameter at breast height. The post-infection larval mortality was observed by dissecting these treated trees, accounting the living and dead larvae on

the 15th day after treatment. The average climate temperature was 28°C during the forest trial.

Statistical analysis

Cumulative mortality data was conducted using the one-way analysis of variance followed by Duncan's multiple range test. LT50 and LC50 were calculated by SPSS v.19.0 (IBM-SPSS, NY, USA) (Cao and Chi 2017). The cumulative mortality rate was calculated by Microsoft Office 2017 Excel (Microsoft, WA, USA).

Results

Pathogenicity of the six *Beauveria* strains to *X. rusticus* L. larvae

The dynamic changes in the cumulative mortality of *X. rusticus* L. larvae treated by six fungal strains are illustrated in Fig. 1. All the six strains exhibited pathogenicity after the larvae were treated with 1.32×10^8 conidia mL^{-1} suspension. The strain Bb01 appeared to have the highest cumulative mortality among other strains ($P < 0.01$). On the tenth day, the cumulative mortality reached up to $96.96\% \pm 1.19\%$. The cumulative mortality of CFCC83486 increased faster from the second day to the eighth day ($P < 0.05$, $P < 0.01$). On the tenth day, the cumulative mortality reached $89.29\% \pm 0.17\%$. The cumulative mortality of CFCC81428 increased fast from the second day to the sixth day and then increased slowly from the sixth day to the tenth day ($P < 0.05$, $P < 0.01$), respectively. On the tenth day, the cumulative mortality reached up to $75.74\% \pm 0.13\%$. The cumulative mortality of the other three strains (CFCC83116, CFCC87298, and CFCC83487) increased slowly and was lower than 70% on the tenth day. Therefore, the most effective strains were Bb01, CFCC83486, and CFCC81428.

LT50 of the six *Beauveria* strains to *X. rusticus* L. larvae

LT50 of different fungal strains to *X. rusticus* L. is presented in Table 2. Only the LT50 of strains Bb01, CFCC83486, and CFCC81428 was less than 5 days. The LT50 of strains Bb01, CFCC83486, and CFCC81428 was 3.28, 3.45, and 4.28 days, respectively, indicating that these three strains had faster pathogenic speed compared with other strains. The corrected mortality of strains Bb01, CFCC83486, and CFCC81428 was 96.64%, 88.15%, and 73.14%, respectively. The corrected mortality of strains CFCC83116, CFCC87298, and CFCC83487 was lower than 60%.

Fig. 1 Dynamic changes of cumulative mortality of larvae *Xylotrechus rusticus* L. treated by different fungal strains. Capital letters indicate a very significant difference ($P < 0.01$), lowercase letters indicate a significant difference ($P < 0.05$)

Table 2 LT50 of different fungal strains to *Xylotrechus rusticus* L. larvae

Strains	Cumulative mortality rate (%)	Corrected mortality (%)	Regression equation	LT50/days	Correlation coefficient
Bb01	96.96 ± 1.19aA	96.64	$y = -1.198 + 1.008x$	3.28	0.995
CFCC83486	89.29 ± 0.17bB	88.15	$y = -1.199 + 0.114x$	3.45	0.974
CFCC81428	75.74 ± 0.13cC	73.14	$y = -1.206 + 0.827x$	4.28	0.974
CFCC83116	65.73 ± 0.53dD	62.03	$y = -1.199 + 0.743x$	5.01	0.925
CFCC87298	54.57 ± 0.22eE	49.71	$y = -1.481 + 0.702x$	8.25	0.966
CFCC83487	45.64 ± 0.59fF	39.83	$y = -1.397 + 0.578x$	11.23	0.971
CK	9.66 ± 0.11gG				

Capital letters indicate a very significant difference ($P < 0.01$), lowercase letters indicate a significant difference ($P < 0.05$). The data format (mean ± SE)

Strains Bb01, CFCC83486, and CFCC81428 were selected for further experiments as high-virulence strains based on the cumulative mortality and LT50.

LC50 and pathogenicity of the high-virulence strains at different concentrations to *X. rusticus* L. larvae

Cumulative mortality rate of strain Bb01 at different concentrations and its LC50

The dynamic changes of cumulative mortality of *X. rusticus* L. larvae treated with different concentrations of strain Bb01 are illustrated in Fig. 2. The cumulative mortality rate exhibited a significant difference or the most significant difference at all the concentrations ($P < 0.05$, $P < 0.01$) except between 1.32×10^7 and 1.32×10^6 conidia mL^{-1} on the second day, and between 1.32×10^8 and 1.32×10^7 conidia mL^{-1} on the tenth day.

The LC50 of strain Bb01 on the second, fourth, sixth, eighth, and tenth days was 2.32×10^{10} , 6.07×10^8 , 3.05×10^7 , 2.63×10^6 , and 1.91×10^5 conidia mL^{-1} , respectively (Table 3).

Cumulative mortality rate of strain CFCC83486 at different concentrations and its LC50

The dynamic changes of cumulative mortality of *X. rusticus* L. larvae treated with different concentrations of strain CFCC83486 are illustrated in Fig. 3. The cumulative mortality rate exhibited a significant difference or the most significant difference at all the concentrations ($P < 0.05$, $P < 0.01$) except between 1.32×10^8 and 1.32×10^7 conidia mL^{-1} on the tenth day.

The LC50 of strain CFCC83486 on the second, fourth, sixth, eighth, and tenth days was 1.26×10^{12} , 6.97×10^{10} , 4.4×10^4 , 1.15×10^4 , and 1.99×10^3 conidia mL^{-1} , respectively (Table 4).

Cumulative mortality rate of strain CFCC81428 at different concentrations and its LC50

The dynamic changes of cumulative mortality of *X. rusticus* L. larvae treated with different concentrations of strain CFCC81428 are depicted in Fig. 4. The

Fig. 2 Dynamic changes of cumulative mortality of *Xylotrechus rusticus* L. larvae treated by different concentration of strains Bb01. Capital letters indicate a very significant difference ($P < 0.01$), lowercase letters indicate a significant difference ($P < 0.05$)

Table 3 LC50 of Bb01 strain to *Xylotrechus rusticus* L. larvae at different times after treatment

Time/d	Regression equation	LC50 (conidia mL ⁻¹)	Correlation coefficient
10	$y = -0.069 + 0.244x$	1.91	0.571
8	$y = -0.38 + 0.268x$	2.63×10	0.556
6	$y = -0.234 + 0.158x$	3.05×10	0.743
4	$y = -1.184 + 0.135x$	6.07×10^8	0.789
2	$y = -1.268 + 0.122x$	2.32×10^{10}	0.646

cumulative mortality rates exhibited a significant difference or the most significant difference at all the concentrations ($P < 0.05$, $P < 0.01$) except between 1.32×10^7 and 1.32×10^6 conidia mL⁻¹ on the fourth day, and between 1.32×10^8 and 1.32×10^7 conidia mL⁻¹ on the tenth day.

The LC50 of strain CFCC81428 on the second, fourth, sixth, eighth, and tenth days was 4.11×10^{12} , 5.36×10^7 ,

1.83×10^5 , 3.13×10^4 , and 1.5×10^3 conidia mL⁻¹, respectively (Table 5).

The aforementioned results demonstrated that pathogenicity was positively correlated with suspension concentration and LC50 was negatively correlated with infection time, and dose effect increased.

Pathogenicity of the high-virulence strain at different concentrations in the forest trial

All cumulative mortality rates of different concentrations of Bb01 were significantly higher than other strains and the control ($P < 0.01$). The correct mortality rate of the strain CFCC83486 was 63.17%, 51.08%, 31.89%, 30.23%, and 17.39% at 1.32×10^8 , 1.32×10^7 , 1.32×10^6 , 1.32×10^5 , and 1.32×10^4 conidia mL⁻¹, respectively, which were significantly higher than those of strain CFCC81428 and the control ($P < 0.01$) but lower than those of strain Bb01 (Table 6). It means that the strain Bb01 was more effective than the strains CFCC83486 and CFCC81428. The pathogenicity

Fig. 3 Dynamic changes of cumulative mortality of *Xylotrechus rusticus* L. larvae treated by different concentration of strains CFCC83486. Capital letters indicate a very significant difference ($P < 0.01$), lowercase letters indicate a significant difference ($P < 0.05$)

Table 4 LC50 of CFCC83486 strain to *Xylotrechus rusticus* L. larvae at different times after treatment

Time/days	Regression equation	LC50 (conidia mL ⁻¹)	Correlation coefficient
10	$y = -0.906 + 0.275x$	1.99×10^3	0.602
8	$y = -1.112 + 0.274x$	1.15×10^4	0.786
6	$y = -1.07 + 0.23x$	4.4×10^4	0.763
4	$y = -1.129 + 0.104x$	6.97×10^{10}	0.779
2	$y = -1.45 + 0.12x$	1.26×10^{12}	0.716

of each strain was positively correlated with its conidia concentration.

Discussion

The *Beauveria* strains could infect Coleoptera, Diptera, Lepidoptera, Homoptera, Hymenoptera, Orthoptera species, and so on (Su et al. 2009). For instance, *B. bassiana* was effective for control *Monochamus alternatus* Hope; the percentage of the pest-infested trees in the treated plot declined to 31.1% (Hu et al. 2006). Another research reported that *B. bassiana* could also control cowpea weevil, *Callosobruchus maculatus* (Fabricius), in the farm scale (Cherry et al. 2007). In the study by Sabbahi et al. (2008), *B. bassiana* at the rate of 1×10^{13} conidia ha⁻¹ triggered a significant reduction in the number of tarnished plant bug, *Lygus lineolaris* L., in strawberry fruit plots. Verde et al. (2015) found that a strain of *B. bassiana* isolated from infected insects collected on dead palms triggered a significant pathogenicity on *Rhynchophorus ferrugineus* (Olivier). Therefore, *Beauveria* strains could be a biological and environment-friendly strategy to control pests.

In the present study, the pathogenicity of five strains of *B. bassiana* and one strain of *B. brongniartii* was evaluated. These six fungal strains varied in their cumulative mortality rate, corrected mortality rate, and LT50 at a concentration of 1.32×10^8 conidia mL⁻¹. The strain Bb01, which was isolated from infected *X. rusticus* L., triggered the highest cumulative mortality rate ($96.96\% \pm 1.19\%$), corrected mortality rate (96.64%), and the shortest LT50 (3.28 days) compared with the other five strains. Li et al. (2007) also found that the *B. bassiana* strain of Bi05, which was isolated from infected mulberry longicorn, *Apriona germari* Hope, caused the highest cumulative mortality rate and the shortest LT50 to *A. germari* compared with other strains. The reason might be attributed to the fact that both Bb01 and Bi05 were isolated from their original host pest. These two strains had the stronger ability to infect their original host compared with other strains isolated from other host insects. The *B. bassiana* strains of CFCC83486 and CFCC81428 also have an ideal pathological effect. The reason might be that these two strains were isolated from Cerambycidae, which *X. rusticus* L. belonged to. The *B. brongniartii* strain of CFCC83487 triggered the lowest cumulative mortality

Table 5 LC50 of CFCC81428 strain to *Xylotrechus rusticus* L. larvae at different times after treatment

Time/days	Regression equation	LC50 (conidia mL ⁻¹)	Correlation coefficient
10	$y = -0.448 + 0.141x$	1.5×10^3	0.728
8	$y = -0.841 + 0.187x$	3.13×10^4	0.754
6	$y = -1.031 + 0.196x$	1.83×10^5	0.654
4	$y = -1.405 + 0.182x$	5.36×10^7	0.526
2	$y = -1.763 + 0.14x$	4.11×10^{12}	0.710

Fig. 4 Dynamic changes of cumulative mortality of *Xylotrechus rusticus* L. larvae treated by different concentration of strains CFCC81428. Capital letters indicate a very significant difference ($P < 0.01$), lowercase letters indicate a significant difference ($P < 0.05$)

Table 6 The results of infecting experiments in the forest trial with three fungal strains

Concentration (conidia mL ⁻¹)	Strain			
	Bb01	CFCC83486	CFCC81428	Control
1.32 × 10 ⁸				
Cumulative mortality (%)	76.33 ± 0.97aA	65.17 ± 1.6bB	57.89 ± 1.98cC	5.43 ± 0.73dD
Corrected mortality (%)	74.97	63.17	55.47	
1.32 × 10 ⁷				
Cumulative mortality (%)	64.75 ± 0.81aA	53.74 ± 1.32bB	46.81 ± 0.47cC	5.43 ± 0.73dD
Corrected mortality (%)	62.73	51.08	43.76	
1.32 × 10 ⁶				
Cumulative mortality (%)	43.82 ± 0.74aA	35.59 ± 0.34bB	29.49 ± 0.87cC	5.43 ± 0.73dD
Corrected mortality (%)	40.49	31.89	25.47	
1.32 × 10 ⁵				
Cumulative mortality (%)	34.12 ± 0.79aA	34.02 ± 0.84aA	23.61 ± 1.49bB	5.43 ± 0.73cC
Corrected mortality (%)	30.34	30.23	19.22	
1.32 × 10 ⁴				
Cumulative mortality (%)	23.05 ± 1.28aA	21.88 ± 1.59aA	14.55 ± 0.62bB	5.43 ± 0.73cC
Corrected mortality (%)	18.63	17.39	9.64	

Capital letters indicate a very significant difference ($P < 0.01$), lowercase letters indicate a significant difference ($P < 0.05$). The data format (mean ± SE)

rate (45.64% ± 0.59%), corrected mortality rate (39.83%), and the longest LT50 (11.23 days) compared with other *B. bassiana* strains. Gu et al. (2009) found that the pathogenicity of *B. bassiana* was more significant than that of *B. brongniartii*, which was consistent with the results of the present study. The reason might be that *B. brongniartii* could only germinate and expand when it infests its specific host, called host specificity. The reason of host specificity is biological long-term co-evolution, and mutual adaptation limited the choices of parasites for the host (Guillebeau 1994).

Three high-virulent *B. bassiana* strains (Bb01, CFCC83486, and CFCC81428) were selected based on the cumulative mortality rate, corrected mortality rate, and LT50 at concentrations of 1.32 × 10⁸ conidia mL⁻¹. These three fungi varied in their cumulative mortality rate at different concentrations. The most significant pathogenicity appeared at the concentration of 1.32 × 10⁸ conidia mL⁻¹ for each strain. For each strain, the cumulative mortality rate increased as the infection time extended. Cao and Chi (2017) found the same pattern in the pathogenicity of *B. bassiana* to *Cryptorhynchus lapathi* L. These patterns also confirmed the pathogenicity effect of *B. bassiana* to *Xylosandrus crassiusculus* (Motschulsky) and *X. germanus* (Blandford) (Castrillo et al. 2016). The reason might be that these pests belong to Coleoptera or the host specificity of *B. bassiana*. However, Bb01, CFCC83486, and CFCC81428 exhibited no significant increase in the cumulative mortality rate from the eighth day to the tenth day at 1.32 × 10⁸ and 1.32 × 10⁷ conidia mL⁻¹. The reason might be that the survived *X. rusticus* L. had strong resistibility to Bb01

and CFCC81428. However, further studies are required to find out whether other factors are also responsible for this pattern.

In the forest trial, the whole cumulative mortality rate and the corrected mortality rate were lower than those that came from the laboratory, consistent with the results from the forest trial of *B. bassiana* pathogenicity to *C. lapathi* (Cao and Chi 2017). The reason might be that temperature affected the pathogenicity of the *B. bassiana*. In the forest stands, the temperature was fluctuated between day and night time, which was different with the incubation temperature of treating larvae under laboratory condition (26 °C). He et al. (2005) found that temperature could affect the pathogenicity of a strain of beetle-derived *B. bassiana* to daikon leaf beetle and *Phaedon brassicae* Baly. Another reason might be that the larvae were immersed in the diluted suspension for 30 s, which means every larva was exposed to a large amount of conidia in the laboratory. However, in the forest trial, the diluted suspensions were brushed on the trunk, making it almost impossible to ensure that every larva was exposed to as many conidia as in the laboratory condition. The sunlight that contains the ultraviolet ray in the forest could also affect the growth and production spores of *B. bassiana* even though the perforated plastic film was covered on the trunk immediately after brushing the suspension. Qian et al. (2015) found that the growth and production of *B. bassiana* spores decreased with the increase in exposure time to ultraviolet ray.

The present study demonstrated that the mortality of *X. rusticus* L. treated by *B. bassiana* increased with the extension of infection time at the same concentration. The

Bb01 strain, which was isolated from *X. rusticus* L. larvae, exhibited the strongest pathogenicity followed by strains CFCC83486 and CFCC81428. However, the *B. brongniartii* strain, CFCC83487, exhibited the weakest pathogenicity to *X. rusticus* L. larvae. The concentration of the suspension of the high-virulent strains Bb01, CFCC83486, and CFCC81428 exhibited a positive effect with the cumulative mortality rate. In the forest trial, Bb01 strain exhibited the strongest pathogenicity among the other high-virulent strains but lower than the bioassay results obtained in the laboratory. The method to increase pathogenicity, as well as the efficiency and stability of high-virulent *B. bassiana* in the forest, requires further investigation.

Conclusions

This study found that high-virulence strains were Bb01, CFCC83486, and CFCC81428 among the selected strains. The Bb01 strain, which was isolated from *X. rusticus* L. larvae, exhibited the strongest pathogenicity followed by strains CFCC83486 and CFCC81428. The selected strains was also tested in the forest. Bb01 strain exhibited the strongest pathogenicity among the other high-virulent strains in the forest trial. This study provides an important basis for using *B. bassiana* in the biological control of *X. rusticus* L.

Acknowledgements This article was supported by the National Natural Science Foundation of China (NSFC) [Grant number: 31370649].

Compliance with ethical standards

Conflict of interest No potential conflict of interest was reported by the authors.

References

- Brabbs T, Collins D, Hérard F, Maspero M, Eyre D (2015) Prospects for the use of biological control agents against *Anoplophora* in Europe. *Pest Manag Sci* 71(1):7–14
- Cabanillas HE, Jones WA (2009) Pathogenicity of *Isaria*, sp. (Hypocreales: Clavicipitaceae) against the sweet potato whitefly B biotype, *Bemisia tabaci*, (Hemiptera: Aleyrodidae). *Crop Protection* 28(4):333–337
- Cabanillas HE, León JHD, Humber RA, Murray KD, Jones WA (2013) *Isaria poprawskii*, sp. nov. (Hypocreales: Cordycipitaceae), a new entomopathogenic fungus from Texas affecting sweet potato whitefly. *Mycoscience* 54(2):158–169
- Cao QJ, Chi DF (2017) Screening of high virulent entomopathogenic fungal strains to infect larvae of *Cryptorhynchus lapathi* (Coleoptera: Curculionidae). *Sci Silvae Sin* 53(1):119–127
- Castrillo LA, Griggs MH, Vandenberg JD (2016) Competition between biological control fungi and fungal symbionts of ambrosia beetles *Xylosandrus crassiusculus* and *X. germanus* (Coleoptera: Curculionidae): mycelial interactions and impact on beetle brood production. *Biol Control* 103:138–146
- Cherry AJ, Abalo K, Hell K, Korie S (2007) Farm-scale trials to compare the entomopathogenic fungus *Beauveria bassiana* with pirimiphos methyl + deltamethrin and essential oil of lemon grass for protection of stored cowpea against *Callosobruchus maculatus* (Coleoptera: Bruchidae). *Ann Appl Biol* 151(1):1–10
- Clarkson JM, Charnley AK (1996) New insights into the mechanisms of fungal pathogenesis in insects. *Trends Microbiol* 4(5):197–203
- Ding JN, Zhang HH, Chi DF (2015) Effects of a Pathogenic *Beauveria bassiana* (Hypocreales: Cordycipitaceae) Strain on Detoxifying and Protective Enzyme Activities in *X. rusticus* (Coleoptera: Cerambycidae) larvae. *Florida Entomol* 98(4):1148–1156
- Ding JN, Yu J, Chi DF (2016) Study on the biocontrol of *Xylotrechus rusticus* L. by parasitic enemies. *J Nanjing For Univ (Nat Sci Edn)* 40(4):107–112
- Dubois T, Li Z, Hu JF, Hajek AE (2004) Efficacy of fiber bands impregnated with *Beauveria brongniartii* cultures against the Asian longhorned beetle, *Anoplophora glabripennis* (Coleoptera: Cerambycidae). *Biol Control* 31(3):320–328
- Gu LQ, Li CX, Zhang SH (2009) Indoor toxicity test of *Beauveria bassiana* and *Beauveria brongniartii* to *Spodoptera exigua* Hiibner. *Jiangsu Agric Sci* 41(3):118–120
- Guillebeau LP (1994) Risk-benefit analysis of pesticides: the US Environmental Protection Agency perspective. *Am Entomol* 40(3):173–179
- He YR, Lu LH, Kuang ZB, Feng X, Chen HY, Wu YJ (2005) Effect of temperature and humidity on the virulence of beetle-derived *Beauveria bassiana* (Balsamo) Vuillemin (Deuteromycetes: Moniliales) against the daikon leaf beetle, *Phaedon brassicae* Baly (Coleoptera: Chrysomelidae). *Acta Entomol Sin* 48(5):679–686
- Hu CX, Huang YH, Li CJ, Sun JH (2004) Larval spatial distribution pattern of *Xylotrechus rusticus*. *Entomol Knowl* 41(3):241–244
- Hu JF, Miao K, Dong ZH, Han B, Fan MZ, Li ZZ (2006) Field trial of application of *Beauveria bassiana* against *Monochamus alternatus*. *J Anhui Agric Univ* 33(3):332–336
- Latge JP, Sampedro L, Brey P, Diaquin M (1987) Aggressiveness of *Conidiobolus obscurus* against the pea aphid: influence of cuticular extracts on ballistospore germination of aggressive and non-aggressive strains. *Microbiology* 133(7):1987–1997
- Li HP, Huang DZ, Wang ZG, Yan HX, Zheng JW (2007) Screening test of highly virulent strains of entomopathogenic fungi *Beauveria bassiana* against *Apriona germari*. *Larvae Sci Silvae Sin* 43(11):66–71
- Li JW, Shi J, Xue Y, Mao HB, Luo YQ (2014) Major physiological adjustments in freezing-tolerant grey tiger longicorn beetle (*X. rusticus*) during overwintering period. *J For Res* 25(3):653–659
- Popa V, Déziel E, Lavallée R, Baucé E, Guertin C (2012) The complex symbiotic relationships of bark beetles with microorganisms: a potential practical approach for biological control in forestry. *Pest Manag Sci* 68(7):963–975
- Qian JJ, Chi DF, Ding JN (2015) Biological characteristics of *Beauveria bassiana* Bb01 strain. *J Anhui Agric Sci* 43(27):374–379
- Sabbahi R, Merzouki A, Guertin C (2008) Efficacy of *beauveria bassiana* (Bals.) Vuill. against the tarnished plant bug, *Lygus lineolaris* L. in strawberries. *J Appl Entomol* 132(2):124–134
- Su P, Liao XL, Zhang Y, Xue ZJ (2009) Advance in controlling *Holotrichia diomphalia* by *Beauveria bassiana* in peanut field. *Guangxi Agric Sci* 40(4):373–377
- Verde GL, Torta L, Mondello V, Caldarella CG, Burrano S, Caleca V (2015) Pathogenicity bioassays of isolates of *Beauveria bassiana* on *Rhynchophorus ferrugineus*. *Pest Manag Sci* 71(2):323–328
- Wang ZM, Liu GR (2005) Distribution damage and assessment of forestry quarantine pests in Jilin province. *For Pest Dis* 24(5):19–21
- Wang YL, Yang XS, Tan LJ, Wang LJ (2002) Chemical control methods of *Xylotrechus rusticus* L. *For Sci Technol* 27(3):26

- Wang GC, Zhang WD, Song TT, Chen RY (2003) Huangpu port intercepted *Cyrtoclytus capra* (Germar) and *Xylotrechus rusticus* L. Plant Quar 17(2):90
- Wen P, Wang H (2010) Harm and control technology of *Xylotrechus rusticus* L.. Min Ying Ke Ji 1:92
- Woruba DN, Priest MJ, Dewhurst CF, Gitau CW, Fletcher MJ, Nicol HI, Gurr GM (2014) Entomopathogenic fungi of the oil palm pest, *Zophiuma butawengi* (Fulgoromorpha: Lophopidae), and potential for use as biological control agents. Aust Entomol 53(3):268–274
- Zhang LW, Liu YJ, Yao J, Wang B, Huang B, Li ZZ, Fan MZ, Sun JH (2011) Evaluation of *Beauveria bassiana* (Hyphomycetes) isolates as potential agents for control of *Dendroctonus valens*. Insect Sci 18:209–216
- Zhao GH (2011) A study on the real value of poplar. Pract For Technol 8:20–22