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Pathogenicity of the Entomopathogenic Fungi *Beauveria bassiana* and *Metarhizium anisopliae* to the Striped Rice Stem Borer, *Chilo suppressalis*

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Original Research Article

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Abstract: The striped stem borer, *Chilo suppressalis* is one of the most important pests in the rice fields distributed in all rice producing regions of Iran (except for Khuzestan province). Three strains of *Beauveria bassiana* and *Metarhizium anisopliae* were evaluated for potential use as bio-control agents against *C. suppressalis*. Virulence of three isolates were tested based on dose-response mortality bioassays and showed that *B. bassiana* isolate Mcb18 had the lowest LC_{50} values to egg, larva and pupa of 6.64×10^4, 1.78×10^3 and 4.94×10^4 conidia mL^{-1}, and with lower LT_{50} values ranged from 5.22 to 11.90 days, from 5.07 to 11.53 and from 6.09 to 19.08 days for concentrations that resulted in 50% mortality during three weeks for *B. bassiana* Mcb18, for egg, larva and pupa of *C. suppressalis*, respectively. Based on these results *B. bassiana* strain Mcb18 has the highest potential as a bio-control agent for *C. suppressalis*. However, *B. bassiana* Mcb18 isolate showed the ability to grow, sporulate and produce mycosis under laboratory conditions, indicating the potential for causing an epizootic in treated rice fields.
Keywords: Entomopathgen, Fungi, Mortality, Pathogenecity, rice.

INTRODUTION

The rice crop is subjected to sustain a considerable damage by a number of insect pests. Chaudhary *et al.*, [5] pointed out that of about 20 species of stem borers that are rice pests in Asia, *Scirpophaga incertulas* (walker), *Scripophaga innotata* (walker), *C. suppressalis* (walker), *Chilo polychrysus* (Meyrick) and *Sesamia inferens* (walker) are most prominent. Among the stem borers, the striped rice stem borer, *C. suppressalis* is the most destructive insect pests of rice crop [22-38].

In Iran, among the rice insect pests, striped stem borer (SSB), Green semilooper, *Naranga aenescense* (Moore) and armyworm, *Cirphis unipuncta* Haworth, are important constraints, which affect rice productivity [26]. The striped stem borer is the most serious insect pest of rice fields in Iran. Globally, striped rice stem borer alone causes yield losses of one tones and accounts for 12000 tones of all insecticides is used in rice field of Iran [28]. Yield losses are reported to 33% in Iran, Khosrowshahi *et al.*, [18] due to SSB. This insect attacks the crop from the seedling stage to the harvest time and thus causes complete loss of affected tillers [34]. Dead hearts are produced when the insect attacks at vegetative stage, while white heads occur when the stem borer attack at time of heading [23-31]. This borer has been implicated as the major constraint to rice production worldwide. Control of this pest has largely been through the use of insecticides, especially granular insecticides such as, Diazinon 10%, Padan 4% and Regent 0.2%. However, the striped stem borer is difficult to control with insecticides because of a prolonged emergence pattern, multiple generations (2-3), and its cryptic feeding behavior [29]. In addition, chemical pesticides are associated with many ecological and environmental problems in rice agro-ecosystem in North of Iran. The use of chemical insecticides has created many problems such as environmental hazards, resistance in target species and is also a heavy burden for the economy of a country [13]. Chemicals pesticides and their ingredients have so far to be imported and paid for in foreign exchange. For many farmers insecticides are too expensive in Iran. One of the important components in pest management is biological suppression of insect pests employing pathogens like viruses, fungi, bacteria, protozoa and nematodes as bio-control agents. These pathogens when conserved and augmented would keep some of the pests under check within the economic injury levels. Biological control of the stem borer using entomopathogens such as bacteria, *Bacillus thuringiensis* (L.), viruses (NPV) and

Hyphomycetes fungi is promising [33]. Two important parasitic fungi of potential importance in the management of rice stem borers that belong to class Deuteromycetes are *Paecilomyces farinosus* (Holmsk.) and *B. bassiana* (Balsamo) Vuillmin. The former species which occurs in soil, is a cosmopolitan insect pathogen and has been reported on *C. suppressalis* and is easily mass produced, but its efficacy needs evaluation [33]. *B. bassiana* is being used for agricultural pest control worldwide including stem borers, leafhoppers, plant hoppers, skipper and leaf folders of rice [33].In other cases, the pathogens cultured *in vitro* are employed either for short-term control equivalent to chemical control or long-term biological control [16]. The entomopathogenic fungus *B. bassiana* is widely regarded as one of the most promising species known for potential development into a practical insect bio-control agent [17]. The fungus *B. bassiana* has a great potential as a myco insecticide and also more effective than chemical insecticides. The objective of this study was to measure the effect of various strains of the widely used fungi *B. bassiana* and *M. anisopliae* entompathogenic upon *C. suppressalis* under laboratory conditions [20]. Coudron *et al*., [7] suggested that it has a well developed chitinolytic system, which has been considered to be important for causing pathogenicity in Lepidoptera pests [35]. The fungus *B. bassiana* has a great potential as a myco insecticide and also more effective than chemical insecticides. The objective of this study was to measure the effect of various strains of the widely used fungi *B. bassiana* and *M. anisopliae* entompathogenic upon *C. suppressalis* under laboratory conditions.

MATERIALS AND METHODS

The experiments were conducted during May, 2012 to September, 2013 in the biology laboratory in Department of Plant Protection, Rice Research Institute of Iran, Rasht.

Entomopathogenic fungi Culture

The virulence of three isolates of *B. bassiana* and *M. anisopliae* to eggs, larvae and pupae of the striped rice stem borer, *C. suppressalis*, were tested in the first screening. Fungi were grown for 3 weeks at 25±2^{o}C on Sabouraud Dextrose Agar (SDAY) (40g dextrose, 10 g peptone, 15g agar, 10g yeast extract, 0.3g streptomycin) in Difco 1000 ml of distilled water under room controlled conditions (25±1^{o}C, 75±5% RH) and a photoperiod of 14:10 │L:D │h. Conidia were harvested by surface scraping 21 day-old culture plates (Fig. 1).

Fig-1: Culture of *Beauveria bassiana* native isolate Mcb18 grown on Sabouraud Dextrose Agar yeast (SDAY) medium

Subsequently the spore suspension was filtered through several layers of cheesecloth to remove mycelium and then suspended in 10mL sterile distilled water containing 0.02% Tween 80 in Erlenmeyer flasks. Conidial suspensions were vortexed for 5 min to produce a homogeneous suspension. The number of conidia mL^{-1} were determined using a haemocytometer chamber based on the formula, $Y= 5\times10^4$ (Y=spore concentration in conidia mL^{-1}, X=number of spores in 5 squares of haemocytometer, 10^4=correction factor). Serial dilutions were prepared to obtain the desired concentrations. Viability of conidia was determined before each bioassay by spread plating 0.1 ml of conidial suspension titrated at 1×10^5 conidia mL^{-1} on SDA plates in Petri dishes. Sterile microscope cover slips were placed on each plate and plates were incubated at 25±1^{o}C and examined after 16–19 h. Percentage of germination was determined from 100 spore counts at 40× magnification. Each plate was replicated four times. Each treatment had four replications. They were used for insect treatment, and more than 92% of the spore were found to be viable. A spore was considered to be germinated when it had formed a germ tube which was at least half as long as the diameter of the spore [9]. All the fungal isolates used in this study were obtained from the Rice research institute of Iran and different area of Guilan province (Table 1).

Table-1: Fungal isolates used against *C. suppressalis*

Species	Isolate	Origin of Isolate	Locality/Coun.	%Germination
*B. bassiana**	Mcb18*	*Chilo suppressalis*	Rahst- Iran	98
B. bassiana	Msb18	Soil of rice field	Rahst- Iran	92
M. anisopliae	Mcm1	*Chilo suppressalis*	Anzali- Iran	95

*M= Majidi c= Chilo b= Beauveria s=Soil m= Metarhizium

Insects rearing

Rearing of the *C. suppressalis* was continued in the captivity at the entomology laboratory of Rice Research Institute of Iran. The culture started with specimens (adults) collected from light traps around Rasht city, Guilan province in the North of Iran. Newly emerging adults were sexed (August), and then each couple of virgin females and males were kept in 500 ml glass Erlenmeyers each containing leaf of rice for oviposition. The culture was maintained at 25 ± 2°C and 75 ± 5% RH. The laid eggs were collected daily by cutting rice leaf into small pieces, with the aid of sterilized scissors. The eggs were maintained under similar condition in tubes with a height of 10 cm were stored in a refrigerator at 4°C. The immature stages (larvae and pupae) of the pest were collected from the experimental area of Rice Research Institute, Iran, Rasht, Guilan province. Eggs, larvae and pupae of the striped rice stem borer, *C. suppressalis*, were used for the present experiment. Each stage of *C. suppressalis* was dipped in the inoculums for 20-30 seconds and treated insects (eggs, larvae and pupae) were carefully transferred to rice plant and the treated insects were maintained under similar condition in Petri-dishes of 9cm diameter with wet filter paper, until the next stage. The larvae of the striped rice stem borer were placed in ten new pieces of rice plant stems. In experiments, rice variety Hashemi (aromatic rice group) was used. Variety Hashemi is susceptible to striped rice stem borer. The control insects (eggs, larvae and pupae) of *C. suppressalis* were treated with distilled water. There were three replications. The eggs, larvae and pupae were used 150, 30 and 30 numbers respectively in each replication. The numbers of egg hatching were recorded daily up to 10 days after application. Mortality of larvae and pupae were noted daily for two and three weeks respectively after conidial treatment.

Statistical analysis

Four concentrations of the fungus *B. bassiana* and *M. anisopliae* from the each isolate were used; 10^2, 10^4 10^6, 10^8(conidia mL^{-1}) and 0 (Control) for eggs, larvae and pupae of the striped rice stem borer. The LC50 of the three isolates of entomopathogenic fungus *B. bassiana* and *M. anisopliae* was computed from the data obtained on the egg hatching, larvae and pupae percentage of kill for each of the concentrations tested through probit analysis with 95% confidence limit [3]. The data were also analyzed for differences among the fungal doses (conidia mL^{-1}), stages of *C. suppressalis* and their possible interactions using single factorial ANOVA [15]. Fungal infection of larvae was determined by microscopic examination. Observations were recorded daily on treated pupae until the adults emerged or the pupae died. Lethal time and lethal concentration to 50% mortality (LT_{50} and LC_{50}) were estimated with repeated measures logistic regression using generalized estimating equations [37]. Data obtained from the bioassay to determine LC_{50} and LT_{50} were corrected using, Abbott's formula (1] and then analyzed with the Probity analysis [4] which compared the confidence limits at 95%.

RESULTS

In viability tests, germination of conidia ranged from 92% to 98% after 16–19 h is in Table 1. Virulence of three selected fungi based on dose-response mortality bioassays were showed in Table 2. Among the three isolates tested, *B. bassiana* isolate Mcb18 had the lowest LC50 values to eggs, larvae and pupae as 6.64×10^4, 1.78×10^3 and 4.94×10^4 conidia mL^{-1}, respectively. *B. bassiana* Msb18 and *M. anisopliae* isolate Mcm1 had the highest LC_{50} values as 2.12×10^5 and 3.11×10^5 to eggs, 7.11×10^4 and 6.47×10^4 to larvae and 1.20×10^5 and 2.4×10^5 conidia $mL^{-1}$to pupae of SSB, respectively (Table 2).

According to Lagunes [19], the greater the value of the slope, the greater the uniformity of response of the population. Although strain Mcb18 had lower slope value, but it showed very high level of virulence toward SSB as shown by the LC_{50} value. The LT_{50} values for eggs of *C. suppressalis* ranged from 5.22 to 11.90 days, from 5.47 to 12.78 days and from 6.56 to 12.84 days for concentrations that received to 50% mortality during 14 days for *B. bassiana* Mcb18, *B. bassiana* Msb18 and *M. anisopliae* Mcm1, respectively (Fig 2 and Table 3).

Table-2: Lethal concentration values of selected isolates of *B. bassiana* and *M. anisopliae* against *C. suppressalis*

Isolate	Stages	LC50	95% confidence limits	Slope(±SE)	X^2 test
Mcb18	Egg	6.64×10^{4}	32249.4244-138432.0683	0.4789(±0.15)	1.90
Msb18	Egg	2.12×10^{5}	96548.8127-484505.7966	0.4181(±0.18)	2.51
Mcm1	Egg	3.11×10^{5}	138284.8670-730068-2819	0.4013(±0.18)	0.62
Mcb18	Larvae	1.78×10^{3}	9132.3321-35439.2754	0.5539(±4.25)	5.76
Msb18	Larvae	7.11×10^{4}	32136.2235-155027.7530	0.4260(±0.17)	0.66
Mcm1	Larvae	6.47×10^{4}	29194.1223-140419-0919	0.4291(±0.17)	2.07
Mcb18	Pupae	4.94×10^{4}	24833.4265-95768.1180	0.5482(±4.69)	2.91
Msb18	Pupae	1.20×10^{5}	54104.9572-265695.6245	0.4203(±0.17)	1.72
Mcm1	Pupae	2.44×10^{5}	101495.5253-589419.9120	0.3757(±0.19)	5.80

*Three replicates/treatments of 150 eggs, 30 larvae and pupae of insect.

Fig-2: Infection symptoms on an SSB egg induced by the *B. bassiana* isolate Mcb18

Table-3: LT_{50} values *B. bassiana* and *M. anisopliae* strains against egg 0f *C. suppressalis*

Fungal isolate	Concentration (conidia mL^{-1})	No. of egg treated	LT_{50}(days) (95% Fiducial Limits)	Slope(±SE)	X^2 test
B. bassiana Mcb18	10^{2}	150	11.90(9.99-23.25)	5.8(±0.07)	0.55
	10^{4}	150	7.51(7.04-8.490)	8.50(±0.19)	0.95
	10^{6}	150	6.35(5.95-6.74)	7.96 (±0.02)	20.58
	10^{8}	150	5.22 (5.02-5.39)	13.50(±0.01)	2.46
B. bassiana Msb18	10^{2}	150	12.78(10.74-22.97)	6.89(±0.06)	0.31
	10^{4}	150	10.71(9.65-13.658)	6.36(±0.03)	2.61
	10^{6}	150	7.08(6.77-7.92)	10.12(±0.01)	0.00
	10^{8}	150	5.47(5.26-5.65)	7.61(±0.01)	0.02
M. anisopliae Mcm1	10^{2}	150	12.84(9.77-269.86)	5.05(±0.11)	0.48
	10^{4}	150	10.25(9.47-12.08)	8.19(±0.02)	2.55
	10^{6}	150	7.34(7.09-7.64)	10.59(±0.01)	4.25
	10^{8}	150	6.56(6.34-6.75)	12.74(±0.01)	0.45

Slopes of the regression equation ranged from 5.8 to 13.50, from 6.36 to 10.12 and from 5.05 to 12.74 for *B. bassiana* Mcb18, *B. bassiana* Msb18 and *M. anisopliae* Mcm1 concentrations, The LT_{50} values for larvae of *C. suppressalis* ranged from 5.07 to 11.53 days, from 5.57 to 11.23 days and from 5.57 to 11.23 days for concentrations that received to 50% mortality during 14 days for *B. bassiana* Mcb18, *B. bassiana* Msb18 and *M. anisopliae* Mcm1, respectively (Table 4).

Table-4: LT_{50} values for *B. bassiana* and *M. anisopliae* strains against larvae of *C. suppressalis*

Fungal isolate	Concentration (conidia mL^{-1})	No. of larvae treated	LT_{50} (days) (95% Fiducial Limits)	Slope (±SE)	X^2 test
B. bassiana Mcb18	10^2	30	11.53(9.36-38.11)	5.24(±0.08)	0.03
	10^4	30	8.80(7.91-13.45)	4.65(±0.04)	1.27
	10^6	30	5.56(5.37-5.72)	12.86(±0.01)	6.59
	10^8	30	5.07(4.88-5.22)	16.11(±0.08)	0.73
B. bassiana Msb18	10^2	30	11.23(9.28-31.98)	6.07(±0.07)	1.54
	10^4	30	8.60(7.97-10.35)	6.74(±0.02)	0.60
	10^6	30	6.28(6.09-6.50)	11.91(±0.01)	1.06
	10^8	30	5.57(5.40-5.73)	13.07(±0.01)	0.02
M. anisopliae Mcm1	10^2	30	9.29(8.58-13.05)	14.94(±0.03)	0.00
	10^4	30	8.34(7.98-9.01)	12.02(±0.01)	0.55
	10^6	30	7.16(6.59-7.49)	8.43 (±0.01)	4.50
	10^8	30	6.13(5.94-6.29)	13.24(±0.01)	12.07

Slopes of the regression equation ranged from 4.65 to 16.11, from 6.07 to 13.07 and from 8.43 to 14.94 for *B. bassiana* Mcb18, *B. bassiana* Msb18 and *M. anisopliae* Mcm1 concentrations, respectively (Fig. 3 and Table 4).

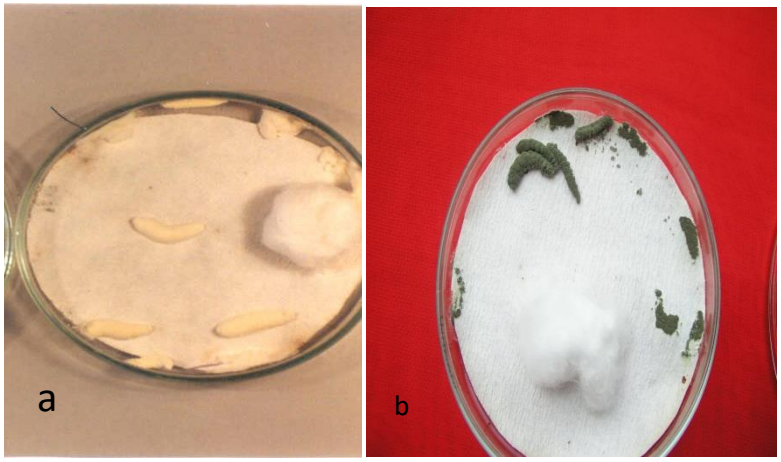

Fig-3: Infection symptoms on SSB larvae induced by the *B. bassiana isolate* Mcb18 (a, left) and *M. anisopliae* isolate Mcm1 (b, right)

The LT_{50} values for pupae of *C. suppressalis* ranged from 6.09 to 19.08 days, from 6.82 to 19.82 days and from 6.47 to 19.38 days for concentrations that received to 50% mortality during 14 days for *B. bassiana* Mcb18, *B. bassiana* Msb18 and *M. anisopliae* Mcm1, respectively (Table 5). Slopes of the regression equation ranged from 2.94 to 12.54, from 8.41 to 21.96 and from 3.33 to 12.19 for *B. bassiana* Mcb18, *B. bassiana* Msb18 and *M. anisopliae* Mcm1 concentrations, respectively (Fig. 4 and Table 5).

Fig-4: Infection symptoms on SSB pupae induced by the *B. bassiana* isolate Mcb18

Table-5: LT_{50} values for *B. bassiana* and *M. anisopliae* strains against pupae of *C. suppressalis*

Fungal isolate	Concentration (conidia mL^{-1})	No. of pupae treated	LT_{50} (days) (95% Fiducial Limits)	Slope (±SE)	X^2 test
B. bassiana Mcb18	10^2	30	19.08(1.57-231.29)	2.94(±0.08)	0.00
	10^4	30	9.03 (8.46-10.83)	5.84(±0.02)	1.96
	10^6	30	6.84(6.63-7.04)	12.54(±0.01)	4.35
	10^8	30	6.09 (5.57-6.61)	4.09(±0.02)	76.74
B. bassiana Msb18	10^2	30	19.82 (8.77 -23.87)	12.47(±0.04)	0.00
	10^4	30	9.28 (8.78-10.41)	8.41(±0.02)	1.22
	10^6	30	7.49 (7.31-7.71)	15.38(±0.01)	0.04
	10^8	30	6.82(6.67-6.95)	21.96(±0.01)	13.13
M. anisopliae Mcm1	10^2	30	19.38(2.15-174.98)	3.33(±0.49)	0.00
	10^4	30	9.07(8.66-9.91)	9.20(±0.01)	7.07
	10^6	30	7.32(7.11-7.57)	12.19 (±0.01)	6.22
	10^8	30	6.47(6.21-6.68)	11.40(±0.01)	1.52

The results of these experiments show that a dose (1×10^8 conidia mL^{-1}) of the Mcb18 isolate of the entomopathogenie fungus *B. bassiana* was the most pathogenic to the immature stages of *C. suppressalis* and dose 1×10^2 conidia mL^{-1} gave the lowest pathogenicity. Such variations have already been reported with different host species including Lepidoptera [2 - 32]. The highest mortality from the three isolates was recorded at the higher spore concentration. Investigations Majidi-Shilsar *et al*. [24) has shown that the least LT_{50} values in various temperatures on germination, mycelium radial growth and virulence of *B. bassiana* to larvae of *C. suppressalis* with average 5.35 day in Mcb18 strain in 25˚C. Majidi-Shilsar *et al*. [27) in the evaluation of fungicidal activity of *B. bassiana* on the overwintering larvae suggested that the larvae of stem borer were infected after the rice harvest in the paddy field. Work Hajek and Leger [14] has shown that the low pathogenesis of these fungi against many insect species was due to the nature of the cuticle, in terms of its density and thickness and degree of sclerotization, among other factors. Some researchers [6] commented that first to third instars of the corn borer were highly susceptible to two white muscardine fungi, indicating that applications of these fungi should be carried out during the early stages of the life history. There is no previous report concerning the effects of *B. bassiana* and *M. anisopliae* on *C. suppressalis*, but Yadava *et al.* [39] mentioned the first record on the susceptibility of stem borers larvae, *S. incertulas*, *C. auricilius* Dudgn and *S. inferens* to *B. brongniartii* Sacc. Ferron [10] suggested that virulence, measured in terms of lethal concentration, will depend on insect species and strain, and mode of application. These enzymes are considered an essential prerequisite for successful fungal infection [12 - 36]. Work Dhuyo and Soomro [8] showed that two isolates (274 and 373) with concentrations ranging from 10^5 to 10^9 conidia mL^{-1} of the entomopathogenic fungus *B. bassiana* were evaluated for pothogenicity against immature stages of yellow rice stem borer, *S. incertulas* under laboratory conditions at Rice Research Institute, Dokri, Sindh Pakistan. Not only the highest rates of mortality of *S. incertulas* were recorded at higher concentrations of conidia mL^{-1}, but also reduced percentage egg hatching of yellow rice stem borer. Log dose (conidia mL^{-1}) of the fungus *B. bassiana* isolate No. 274 and isolate No.373 on egg, larva and pupa of *S. incertulas* was recorded as 5.12, 5.46, 6.10 and 6.23, 8.33, 8.33, respectively. The isolate No. 274 was more pathogenic than the isolate No. 373 to control of *S. incertulas*. In the research N'Doye and M'Baye [30]

was found that *C. suppressalis* mature larvae and pupae reared on a semi-synthetic medium were infected by spraying titrated spore suspensions of *B. bassiana*. Potentiality of imago having survived mature larvae contaminated with a spore suspension titrated at 3×10^7 conidia mL^{-1} as the pupae and imago, were attacked by the muscardine disease. Also, they mentioned that the fertility of the eggs laid by the surviving insects (25% of the total) was very low compared with the control insects. Some pupae, contaminated under the same conditions but with concentrations of inoculate varying between 3.3×10^5 to 3.3×10^6 conidia mL^{-1}, also showed mycosis in the pupal and adult stages (53 to 87% of the insects were contaminated, according to the spore concentration). The fungus *B. bassiana* could be used as bio-control agent in the field [11].

DISCUSSIONS

All the fungal isolates tested were pathogenic to *C. suppressalis*, however, there was variation in the virulence between the fungal isolates. Although strain Mcb18 had lower slope value, but it showed very high level of virulence toward SSB as shown by the LC_{50} value. The difference in virulence observed between the strains tested in these experiments may be due to differences in the production of enzymes, which degrade the cuticle of the potential host, such as chitinase, chyemolestase, chymotrypsin, and esterase. The results revealed that mean mortality and sporulation of larvae by *B. bassiana* and *M. anisopliae* were 75.55, 71.11% and 64.44, 62.22%, respectively [25]. The present study showed that the fungus *B. bassiana* has great potential to control *C. suppressalis*. The isolate Mcb18 was more pathogenic to control striped rice stem borer, *C. suppressalis*, than the isolates *B. bassiana* Msb18 and *M. anisopliae* Mcm1. *B. bassiana* has great potential as bio-control agent against insect pests which has been highly pathogenic to the larvae of lepidopterous pests [21]. The present study showed that the fungus *B. bassiana* has great potential to control *C. suppressalis*. The isolate Mcb18 was more pathogenic to control striped rice stem borer, *C. suppressalis*, than the isolates *B. bassiana* Msb18 and *M. anisopliae* Mcm1.

CONCLUSION

Our study demonstrates that there are differences within strains of the same fungal species and between species in relative pathogencity and time required for mortality in *C. suppressalis*. The present study showed that the fungus *B. bassiana* has great potential to control of *C. suppressalis*. Our current results suggest that *B. bassiana* isolate Mcb18 was more pathogenic than the *B. bassiana* other isolates and *M. anisopliae* of the entomopathogenie fungus. However, *B. bassiana* isolate Mcb18 can be a particularly promising candidate and as a biological control agent for use integrated pest management of SSB in paddy field.

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