INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
19–0164/202x/00–0–000–000
DOI: 10.17957/IJAB/15.1492
http://www.fspublishers.org

Full Length Article

Efficacy of a New Strain of *Beauveria bassiana* against the Melon Fruit Fly, *Zeugodacus cucurbitae* (Diptera: Tephritidae)

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Received 25 January 2019; Accepted 28 April 2020; Published _____

Abstract

The melon fruit fly, *Zeugodacus cucurbitae* Coquillett (Diptera: Tephritidae) is an important pest in tropical and subtropical regions of the world. The biocontrol effect of *Beauveria bassiana* (Bals.) Vuill. (Hypocreales: Cordyciptaceae) BC-B1 strain on *Z. cucurbitae* was evaluated. One suspenso-emulsion (SE) formulation of *B. bassiana* was prepared and was applied against *Z. cucurbitae* under laboratory conditions. The results showed that the LC_{50} of BC-B1 on the adults and larvae of *Z. cucurbitae* were 0.65×10^5 and 1.08×10^5 conidia/mL, respectively. *B. bassiana* conidia were not affected by abamectin and imidacloprid at field dose, which is strong in compatibility. In comparison to using single biopesticide (*B. bassiana*), using the mixed formulation could improve efficacy. There were high synergistic interactions of *B. Bassiana*-abamectin and *B. bassiana*–imidacloprid to larvae of *Z. cucurbitae*. The larval mortalities reached 88.75%. The soil inoculation of *B. bassiana* is proved to be a great help to increase mortality of larvae. These results suggested that *B. bassiana* BC-B1 strain could be useful for control of *Z. cucurbitae*. © 2020 Friends Science Publishers

Keywords: *Zeugodacus cucurbitae*; *Beauveria bassiana*; Entomopathogen; **V**irulence; Synergistic interactions

Introduction

The melon fruit fly, *Zeugodacus cucurbitae* Coquillett (Diptera: Tephritidae) is a fruit fly of the family Tephritidae. It is a serious pest of vegetables and fruits in the tropical area. It can infest over 125 host plants (Vargas *et al.* 2009). It has been causing severe damage to cucurbit vegetables such as the bitter gourd (*Momordica charantia*) in China. This fly could cause severe damage to the fruits when females lay their eggs inside fruits and the eggs hatch into larvae feeding on the fruit flesh. Furthermore, the fruits attacked by larvae in early stages fail to ripen and drop or rot on the plant (Dhillon *et al.* 2005; Sapkota *et al.* 2010). The melon fruit fly control mainly depended on the chemical pesticide. Currently, the large-scale use of insecticides to control melon fly is the main control method in China, which is less effective due to the larvae burrow into and feed in fruits.

Crop protection based on biological control of pests with microbial pathogens has been recognized as a valuable tool in pest management (Rao *et al.* 2006; Anand *et al.* 2009). The entomopathogenic fungus has an important position in biological control of pests since they are effective and present a low risk for non-target organisms (Hussein *et al.* 2012). Nowadays, formulations of entomopathogenic fungi have become an effective bioinsecticides for pest control in agriculture and forestry. Many entomopathogenic fungi occur frequently in the soil (Bidochka *et al.* 2002; Goble *et al.* 2010). One of the most famous of fungus is *Beauveria bassiana* (Balsamo) Vuillemin that grows naturally in soils throughout the world (Hussein *et al.* 2010). There were many reports that the *B. bassiana* were widely used due to its high specificity and low environmental impact (Glare *et al.* 2002; Zimmermann 2007). Several *Beauveria* based products are available around the world for some pest species, such as scarabs, aphids, corn borer and pine caterpillar *etc*. (Sivasundaram *et al.* 2007; Kim *et al.* 2013).

The mature larvae of *Z. cucurbitae* leave the host plant to pupate in the soil in fields. Pupation occurs in the soil at 0.5 to 15 cm below the soil surface. So, the optimum period of controlling *Z. cucurbitae* is at the time of larval descent to the soil. Entomogenous pathogens could become a useful alternative to chemical insecticides for fruit fly control. It could be used as a main factor in the IPM strategy to enhance control efficacy of the fly. However, there have been few published research articles on the use of entomopathogenic fungi to control the melon fruit fly (Sookar *et al.* 2008; Amala *et al.* 2013). A strain of *B. bassiana* against the melon fly (*Z. cucurbitae*) was selected from soil in Hainan, China. The objective of the study was to evaluate the effect of *B. bassiana* and its application on *Z.*

cucurbitae under laboratory conditions: (1) to test virulence *of B. bassiana* conidia against *Z. cucurbitae*, (2) to test conidial viability of *B. bassiana* in the presence of insecticides, and (3) to evaluate the effects of mixtures of *B. bassiana* and insecticides on *Z. cucurbitae* larvae.

Materials and Methods

The melon flies *Zeugodacus cucurbitae*

The culture of *Z. cucurbitae* was reared following the method (Yang *et al.* 2015). The adults and larvae were reared on the fresh pumpkin in rearing containers. Sandy soil was provided as pupation site. Pupae were collected from the sand, and the emerging adults were placed in another rearing container. Temperature and relative humidity (RH) of the rearing room were maintained at 30 ± 2°C and 70–80%, respectively.

The entomopathogenic fungus BC-B1 strain morphological and molecular identification

The BC-B1 strain was isolated from the soil of Haikou Suburb in Hainan Province, China. It was inoculated on sabouraud's dextrose agar medium (SDAY) at 28°C for 7–15 d. After cultivated in the above media for 15 d, the conidia were harvested from the surface of the colony. The morphological characteristics of the colonies and microscopic characteristics were observed. The strain was identified by using ITS (internal transcribed spacer) sequence method. The genomic DNA was extracted using a universal genomic DNA extraction kit (TaKaRa Biotechnology Dalian Co., Ltd., China) according to the manufacturer's instructions. The ITS of nuclear rDNA was amplified with the primer pair (ITS1: 5′-TCCGTAGGTGAACCTGCGG-3′, ITS4: 5′-TCCTCCGCTTATTGATATGC-3′). The PCR products were sequenced by Sangon Biotech (Shanghai, China). The sequences of the ITS was submitted to NCBI Genbank database and analyzed using the NCBI database and BLAST program.

Virulence assay of BC-B1 strain on fly

The number of conidia in the suspension were counted using haemocytometer and adjusted to required concentrations with 0.1% Tween-80 solution. To evaluate the virulence level of *B. bassiana* BC-B1 strain to adults and larvae of *Z. cucurbitae*, tests were performed with different conidia concentrations, *viz*., 1.0×10^4, 1.0×10^5, 1.0×10^6, 1.0×10^7 and 1.0×10^8 conidia/mL. The same batch of adults was placed in the rearing box. The flies directly were sprayed with 8 mL of conidia suspension, and the treated adults were transferred to other rearing box with fresh pumpkin. The mature larvae (3rd instar) were collected and applied using larval immersion method. The larvae were then transferred to the rearing box which contained fresh pumpkin and moist sandy soil. Those flies sprayed with sterilized water used as controls. Each treatment contained 30 adults (one day old) or 30 mature larvae (3rd instar). Each bioassay was repeated thrice. The melon flies were reared normally at room temperature (30 ± 2°C). The data for mortality was recorded daily for 8 days, and calculated the median lethal concentrations (LC_{50}). Dead flies were picked out and put on sterile wet filter paper in Petri dishes at 28°C, so as to observe the presence of external growth of fungi. Disease symptoms of flies were observed. The experiment was repeated three times.

Compatibility between insecticides and *Beauveria bassiana* conidia

The commercial insecticides were employed in the bioassays, as follows: acetamiprid (5% EC), imidacloprid (5% EC), abamectin (1.8% EC), trichlorphon (30% EC), beta-cypermethrin (4.5% EC), bifenthrin (10% EC). Six insecticides were supplied by Hainan Zhengye Zhongnong High-tech Co., Ltd., China.

The SDAY medium was autoclaved and cooled to 45–50°C, and thoroughly mixed with acetamiprid or other insecticides with recommended field dose (FD). Then the medium was poured into each Petri dish. A drop (10 μL) of conidial suspension (1×10^8 conidia/mL) of *B. bassiana* was placed on medium. All dishes were incubated at 28°C for 5 days. The colony diameter of *B. bassiana* was measured, the rates of growth inhibiting were assessed on the basis of growth in control dishes (SDAY medium alone). Three dishes were made for each of the treatments. The experiment was repeated three times.

Synergism between *Beauveria bassiana* and insecticide

The suspoemulsion (SE, 10%) formulation of *B. bassiana* was prepared in our laboratory. It was composed of spore powder of *B. bassiana*, paraffin oil, octylphenol polyoxyethylene (10) ether (OP-10) and sodium carboxymethyl cellulose (Sinopharm Chemical Reagent Co., Ltd, China), ascorbic acid (Guangzhou Chemical Reagent Factor, China), and the oils mixture were directly emulsified into the suspension using a magnetic stirrer. The following six treatments were assayed against adults and larvae of *Z. cucurbitae* in the laboratory: (1) 500 times diluent of *B. bassiana* 10% SC with dosage equivalent to 1×10^7 conidia/mL; (2) imidacloprid at 2 mg/L; (3) 500 times diluent of *B. bassiana* and 2.0 mg/L imidacloprid; (4) abamectin at 0.72 mg/L; and (5) 500 times diluent of *B. bassiana* and 0.72 mg/L abamectin.

The soil inoculation of *B. bassiana* was applied following the method (Erler and Ates 2015). The mature larvae (3rd instar) were collected. The sand soil sample was sieved through 0.5 × 0.5 cm sieve. Foam boxes (60 ×60× 30 cm), each box including 7 kg of sterilized sand soil were

used as test medium. Thirty mature larvae were introduced into each box, and then sprayed with 1000 mL of testing liquid in the sand soil. All of the treatments were applied by soil drench for sufficient wetting of the top 10–12 cm soil surface layer. Each treatment included three replicates. The experiment was repeated two times. The box including the sand soil treated with tap water served as control. The laboratory temperature and relative humidity (RH) were kept at 30 ± 2°C and 75–80%, respectively. The counts were made on the 14th d after application, and the boxes were poured onto a plastic sheet, then the larvae were collected from the soil. The larvae were considered dead which the color of insect tuned black with conspicuously shrunk and had white flocculent-like mycelia. Dead larvae were checked for mycosis and uneclosioned pupae were recorded.

Data analysis

The mean value was calculated with the use of cumulative mortality data. Data analysis was processed by with ANOVA of the program S.P.S.S. 24.0. The *t*-test was applied to analyze the differences of treatments at $P < 0.05$. The median lethal concentrations (LC_{50}) were calculated by probit analysis.

Results

Morphological and molecular identification of strain BC-B1

Strain BC-B1 formed white, snow-form and hollow colonies on SDAY culture medium. Based on microscopic observation, it produced hyaline, smooth, globose to ovoid conidia in shape and 1.8–2.2 μm in size. It is a typical characteristic of *Beauveria bassiana*. The ITS gene fragments of strain were amplified successfully. The sequence was submitted to NCBI Genbank database, which analysis revealed that the nucleic acid sequence homology between tested BC-B1 strain and *B. bassiana* was 100%. And the accession number at GenBank is KM006491. Strain BC-B1 was identified as *Beauveria bassiana* with morphological and molecular biology methods.

Symptoms of *Z. cucurbitae* infected by *B. bassiana*

The adult flies began to die about 2–3 days later by *B. bassiana* infection. The mycelia and conidiation structures gradually appeared on the adults at 4–7 days after inoculation. The white flocculent-like mycelia were observed on the surface of on the dead adults at 4 days after inoculation. Mycelium colonized the whole body and a small amount of white powder-like conidia emerged from dead adults at 5–6 days after inoculation. A great amount of conidia grows out in the stiff bodies of adults for approximately 7 days (Fig. 1A), which is a typical symptom of white muscardine. The white powdery conidia also

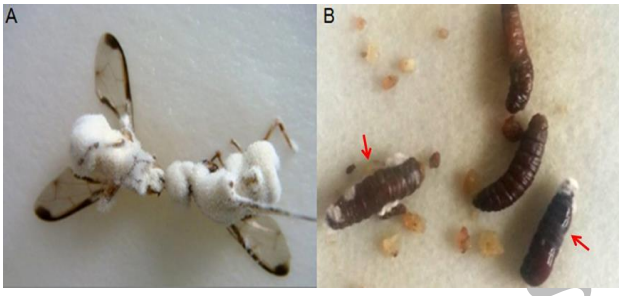

Fig. 1: The typical symptom of *Zeugodacus cucurbitae* infected with BC-B1 strain. **A**: Dead adults. **B**: Dead larvae and pupae

occurred on the dead larvae and pupal surface, and the color of insect became black with ankylosis (Fig. 1B). There was no evidence of mycosis in any control cadavers.

Virulence of *B. bassiana*

Flies infected with conidia of BC-B1 strain, the linear regression equations were obtained using mortality probability and concentration logarithm values. The LC_{50} values of the adults and larvae were 0.65×10^5 and 1.08×10^5 conidia/mL at 8 days after inoculation, respectively (Table 1). This result indicated that BC-B1 strain was highly effective against *Z. cucurbitae*.

Compatibility between *B. bassiana* and insecticides

In order to improve insecticidal activity of *B. bassiana* to larvae, experiments were conducted by using combinations of *B. bassiana* and chemical insecticides. It is necessary to examine their compatibilities of the conidia of *B. bassiana* and chemical insecticides. The results of the *in vitro* development of *B. bassiana* with the additions of 6 insecticides are illustrated in Table 2. The mycelial growth was not affected by abamectin ($P < 0.05$). There were few affected of the *B. bassiana* cultures when they were grown with imidacloprid and beta-cypermethrin, the average inhibiting rate were less than 4.0%. On the contrary, acetamiprid, trichlorphon and bifenthrin significantly inhibited colony growth at FD ($P < 0.05$), these insecticides showed a strong inhibition of *B. bassiana* at 5 days after treatment. The results showed that the viability of conidia wasn't affected by abamectin and there were few affected by imidacloprid and beta-cypermethrin. According to the test results, *B. bassiana* can be used with some insecticides (such as abamectin, imidacloprid and beta-cypermethrin), which is strong in compatibility.

Synergistic interactions between *Beauveria bassiana* and insecticide

The dead larvae had fungal outgrowths on the surface in sandy soil with *B. bassiana* after 8–10 days' inoculation (Fig. 2A–B). Some larvae were still able to pupate

Table 1: The Virulence of *Beauveria bassiana* BC-B1 against *Zeugodacus cucurbitae* treated with multiple-concentration (1.0×10^4-1.0×10^8 conidia/mL) after 8 days of exposure

Bactrocera cucurbitae	*Regression equation y=ax+b	Correlation coefficient (r)	LC_{50} (95% CL) ($\times10^5$ conidia/mL)	χ^2	*df*	*Sig.*
Adults	y = 0.6622x + 1.1366	0.99	0.65 (0.17~1.63)	1.43	3	0.70
Larvae	y = 0.4761x + 1.8196	0.95	1.08 (0.07~5.03)	0.71	3	0.87

* Regression equation: y is dead probability and x is concentration logarithmic value

Table 2: Compatibility of *Beauveria bassiana* with 6 commercial insecticides *in vitro*

Treatments	Dose (ml/lit)	*Average inhibiting rate (%)
		BC-B1
Abamectin 1.8% EC	3.6	0a
Imidacloprid 5% EC	20.0	1.54 ± 0.35a
Beta-cypermethrin 4. 5% EC	22.5	2.86 ± 0.23ab
Acetamiprid 5% EC	10.0	7.69 ± 1.01b
Bifenthrin 10% EC	20.0	20.00 ± 2.52c
Trichlorphon 30% EC	375.0	25.67 ± 1.04c

*Each value represent mean of three replicates and ± SE. Different letters within columns are significantly different ($P < 0.05$)

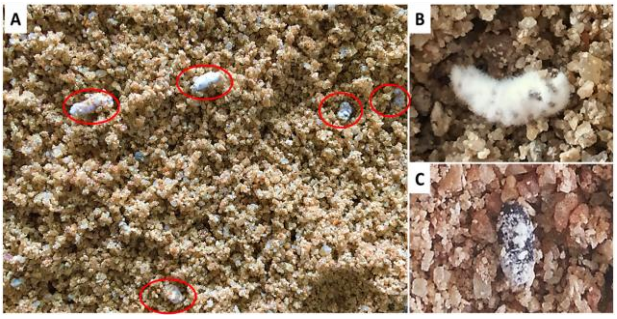

Fig. 2: Effect of *Beauveria bassiana* and abamectin against the larvae of *Zeugodacus cucurbitae* in soil. **A**: The dead larvae and pupae. **B**: Moldy larvae; **C**: Uneclosioned pupae

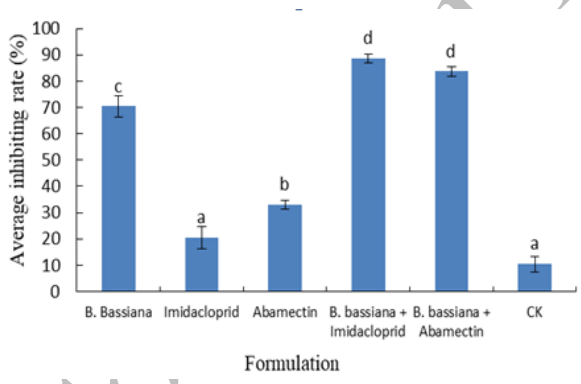

Fig. 3: Efficiency of *Beauveria bassiana* with two insecticides against *Zeugodacus cucurbitae*. Error bars show standard error of the mean. Different letters indicate that mortality is significantly different ($P < 0.05$)

normally, but the pupae were unable to emerge as adult flies and died (Fig. 2C). The larval mortalities using box experiment were from 10.42% to 88.75% when treated with control and 5 formulations (Fig. 3). The mortalities were higher when treated with a combination of *B. bassiana* and imidacloprid (or *B. bassiana* and abamectin) in comparison to treatments of *B. bassiana* respectively ($P < 0.05$). The application of insecticide with *B. bassiana* conidia revealed a clear synergy so as to enhance the mortality of ready-to-pupate larvae (mature larvae).

Discussion

It is reported that *Beauveria* spp. is the most important entomopathogenic fungi against Dipteran insects (Watson *et al.* 1995). Our results are in agreement with those of Ibrahim *et al.* (2014) who reported that *B. bassiana* are suitable candidates for the control of two adult flies of family Tephritidae, such as *Ceratitis capitata* and *Bactrocera zonata*. Moreover, Castillo *et al.* (2000) reported 100% mortality in adult flies of *C. capitata* by using several strains of *B. bassiana*. It is noteworthy to mention that humidity is the most important factor affecting efficacy of entomogenous fungus in the field, with water being essential for conidial germination, infection and subsequent sporulation on insect cadavers (Devi *et al.* 2005; Mishra *et al.* 2015). Conidia are the most efficient propagules during infection. In the laboratory experiments, inoculations were carried out by spraying a spore suspension on the melon fruit fly, this incubation conditions were optimized to allow fungal infection. So, the mortality of flies under laboratory conditions may be higher than that of field conditions. The soil is an important reservoir for entomopathogenic fungi in the fields, which increases the persistence of conidia and their ability to thrive (Jackson *et al.* 2000; Ekesi *et al.* 2003; Jaronski 2010). Our results showed that the mortality of larvae was only 55.33% when sprayed directly with 1×10^7 conidia/mL concentration of *B. bassiana*. In the same treatment concentration, the mortality of larvae was 70.41% after soil application of *B. bassiana*. At the same time, the soil inoculation of *B. bassiana* is proved to be a great help to increase mortality of larvae. Moreover, test using foam boxes with moist sand soil was much closer to the nature's condition than direct inoculation experiments.

In order to improve the biocontrol agents efficiently, the laboratory studies were developed to evaluate the synergism of abamectin and imidacloprid with *B. bassiana* against *Z. cucurbitae*. The results showed that there were high synergistic interactions of *B. bassiana*-abamectin and *B. bassiana*–imidacloprid to larvae of *Z. cucurbitae*. The mortality of larvae was only 20.42% when treated with 2 mg/L imidacloprid (the normal concentration was diluted by 10-fold). However, adding low dose of abamectin to *B. bassiana* could enhance efficiency of *B. bassiana*. In comparison to using single *B. bassiana*, using the mixture of *B. bassiana* and abamectin could improve efficacy, and the mortality of larvae has improved from 70.41% to 88.75%. The observed synergetic effect would allow the use of lower concentrations of chemical pesticides contributing to a decline in the likelihood of resistance. And the use of lower concentrations in turn lessens risks to natural enemies by chemical pesticides.

Conclusion

This research showed that the new isolated *B. bassiana* BC-B1 had high toxicity to *Z. cucurbitae*. Based on the analysis of data on virulence, compatibility and synergistic interactions between *B. bassiana* and synthetic insecticides, the *B. bassian*a would be a potential biocontrol agent of *Z. cucurbitae*. These results suggested that BC-B1 strain could be useful for the development of environmentally benign IGR insecticide to control *Z. cucurbitae*. Our study will provide an important basis to develop *B. bassiana* BC-B1 as a bioinsecticide against *Z. cucurbitae*.

Acknowledgements

The corresponding author acknowledges the supported by Special Fund for Agro-scientific Research in the Public Interest, Ministry of Agriculture, China: Invasive Insects Comprehensive Prevention and Control Technology Research and Demonstration to Promote (2014).

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