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Effect of the fungus, *Beauveria bassiana* (Balsamo) Vuillemin, on the beet armyworm, *Spodoptera exigua* (Hübner) larvae (Lepidoptera: Noctuidae), under laboratory and open field conditions

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Abstract

A local isolate of the fungus *Beauveria bassiana* was tested against the beet armyworm *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae) larvae reared on artificial diet. Seven successive increased conidiospore concentrations (2×10^1, 2×10^2, 2×10^3, 2×10^4, 2×10^5, 2×10^6, and 2×10^7 spores/ml) were tested against larvae of L_3 and L_4. Larval mortality increased by increasing the conidiospore concentrations. The larvae of L_3 were more susceptible to the treatment with *B. bassiana* conidiospores than larvae of L_4. The infected larvae survived the tested low concentrations more than 5 days and died later in the fifth larval instar. The LC_{50} for L_3 and L_4 was 18,463 (slope 0.414) and 35,990 (slope 0.387) spores/ml, while the LC_{90} was 37,806 (slope 0.345) and 74,391 (slope 0.387) for the two larval instars, respectively. Applying *B. bassiana* (6×10^7 conidiospore/ml) for controlling the beet worm, *S. exigua*, in sugar beet fields in Fayoum Governorate, Egypt, resulted to a suppression in its larval populations through 5 applications by 54.5–70% in season 2016/2017 and 66.6–80% in season 2017/2018.

Keywords: *Beauveria bassiana*, *Spodoptera exigua*, Pathogenicity, Field application

Background

Larvae of the beet armyworm, *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae), is considered one of the most damaging pests to many field crops and vegetables as a leaf feeder causing economic losses (Taylor and Riley 2008). Among its plant hosts in Egypt are lettuce, cabbage, spinach, and sugar beet (El-Husseini et al. 2008). Traditionally, chemical insecticides are used to control the pest. Modern pest control strategies are concerned with minimizing the use of chemical insecticides with their known hazards to the environment, beneficial fauna, and human health. Thus, the use of biological control became an important alternative, especially those bioagents that can be applied with the same machineries of insecticide application (spraying and dusting), i.e., entomopathogenic viruses, bacteria, and fungi.

The fungus *Beauveria bassiana* (Balsamo) Vuillemin is known as a highly virulent entomopathogenic fungus (EPF) having a very wide range of insect hosts mostly agricultural pests (Tanada and Kaya 1993). It is a soil-borne fungus (Klingen et al. 2002) that is distributed worldwide. Laboratory and field experimental results showed that this fungus is one of the most promising microbial control agents combating different insect pests (Lacey and Goettel 1995). Keller (2000) reported scarabs, weevils, locusts, and immature stages like caterpillars, larvae, pupae, and adults among hosts of *B. bassiana*. In most cases, field application of *B. bassiana* had no harm on the non-target organisms (Goettel and Hajek 2001). After the death of the infected host insects, *B. bassiana* produces and releases all its infective stages (conidiospores) in a single spell (Bartlett and Jaronski 1988 and Wraight et al. 2001). The successful invasion and

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propagation of *B. bassiana* inside its host insect depends on its capability to penetrate the body wall (cuticle) and reach the hemocoel, followed by suppressing the host's immune response (Götz 1991). The use of EPF within the strategies of integrated pest managements seems promising and potential. At the same time, it could minimize the undesired side effects of chemical insecticides on the environment and on other biocontrol agents as well as other beneficial microbial agents. Liu and Li (2004) mentioned that there are about 66 products representing at least 38 taxonomically diverse species or varieties of EPFs that have been developed or are being developed for insect pest's control.

In the present study, the virulence of a local *B. bassiana* isolated from the mole cricket *Gryllotalpa gryllotalpa* (Linn.) was tested in different conidiospores concentrations under the laboratory against third and fourth larval instars of the sugar beet armyworm, *S. exigua*, reared on artificial diet. Also, the fungus isolate was propagated and tested for controlling this pest on sugar beet in open field in seasons 2016/2017 and 2017/2018 at Fayoum Governorate, Egypt.

Material and methods

Rearing of *Spodoptera exigua*

Larvae of *S. exigua* were reared in the laboratory on the semi-artificial diet developed by Shorey and Hale (1965) and modified by Mabrouk et al. (2001) at the Centre of Biological Control, Faculty of Agriculture, Cairo University, Giza, Egypt. The initial culture started with the larvae collected from sugar beet field in Sennores, Fayoum Governorate. To avoid growth of bacteria and saprophytic fungi on the diet, 0.05 g of commercial streptomycin and 0.2 ml of formaldehyde were mixed in the already prepared diet (ca 2 kg). The diet was poured into trays (20 × 30 cm) in a layer of 2 cm in thickness. A plastic grid of 80 (8 × 10) square cubic cells (2 × 2 × 3 cm) was pressed in the diet plate. One larva was confined in each cell on the diet, and the structure was covered with a flat plastic plate perforated with four fine openings over each cell for aeration and fitted in place by two broad rubber bands. The diet amount in each cell was enough for the larva to complete the development and pupate inside the cell. Pupae were collected from the rearing units, rinsed with water shower on a sieve, and placed each 20 ones in a 2-l jar furnished with filter paper until adult moth emergence. Jars were provided with cotton wool wetted by bee honey solution in water (1:50 w/w) placed in a bottom or cover of a small Petri dish (3 cm in diameter) as the nutrient for the moths. The glass jars were provided by broad stripes of filter paper as an oviposition site. Egg masses were collected daily by cutting from the stripes and kept in Petri dishes on moistened filter paper. Just prior to hatching (egg color turned dark), they were placed on diet in the trays without the grid. Thus, the newly hatched larvae were first reared gregarious on the diet until reaching third instar larvae, then transferred individually on diet in the grid cells of rearing trays. The rearing and infectivity tests versus L_3 and L_4 were carried out under laboratory conditions of 25° C and 50–60% relative humidity.

Production of *Beauveria bassiana* conidiospores

A local strain of the fungus *B. bassiana*, originally isolated from a naturally infected mole cricket, *Gryllotalpa gryllotalpa* L. by El-Husseini et al. (2008), was propagated on Czapek's Dox agar medium composed of sucrose 20 g, K_2HPO_4 1 g, KNO_3 2 g, yeast extract 2 g, KCL 0.5 g, $MgSO_47H_2O$ 0.5 g, $FeSO_47H_2O$ 0.003 g, and agar-agar 20 g/L completed to 1 l of distilled water and adjusted at pH 5.5–6.5. The medium was autoclaved at 120 °C for 20 min. The sterilized medium was poured into sterilized Petri dishes (12 cm in diameter). When cooled, the Petri dishes were inoculated by the spore suspension of *B. bassiana* and incubated for 15 days at 25 °C. The conidiospores were harvested from the Petri dishes by scraping with a spatula and suspended in distilled sterilized water with 0.02% Tween 80 as a wetting agent in the primary stock suspension following Rombach et al. (1986). The spore count was determined by using a Neubauer Hemocytometer, and the stock was kept in the refrigerator till needed.

Laboratory experiments

Bioassay of *B. bassiana* versus *S. exigua* L_3 and L_4

Seven concentrations of 2×10^1, 2×10^2, 2×10^3, 2×10^4, 2×10^5, 2×10^6, and 2×10^7 conidiospore/ml were prepared in distilled water from the stock spore suspension. For standardization of the tested insect material for the bioassay test, the second and third larval instars of *S. exigua*, which reached the molting stage, characterized by staying motionless on the diet or on the filter paper cover of the rearing plates, with the head erected forward showing the coronal sutures clearly with the servicum translucent swollen behind, were picked up gently and harmlessly by very soft forceps and placed onto moistened filter paper in large Petri dishes (15 cm in diameter) and left to the next day where they molted to the third and fourth larval instars (L_3 and L_4). For each tested concentration, four replicates each of 25 larvae of the tested instar were treated by direct spray, using a fine perfume atomizer. Thereafter, the treated larvae were placed in the cells of the grid pressed in the diet trays and left to feed on under a perforated cover plate similarly to those in the rearing technique. For the control, each tested instar was sprayed by distilled water containing 0.02% Tween 80 and kept on diet as those of the treatments. Mortality was recorded daily for 10 days post-treatment. The dead larvae were kept on moistened

Table 1 Mortality (%) of *Spodoptera exigua* (L_3 and L_4) treated with different concentrations of *Beauveria bassiana* conidiospores

Concentration/ml	Larval instars	Mortality (%) in days post treatment									
		1	2	3	4	5	6	7	8	9	10
2×10^1	L_3	0	0	0	0	11	11	15	24	30	40
	L_4	0	0	0	0	5	6	12	18	25	33
2×10^2	L_3	0	0	0	5	15	30	30	50	70	90
	L_4	0	0	0	5	12	21	26	41	58	78
2×10^3	L_3	0	0	0	10	17	32	43	62	98	100
	L_4	0	0	0	7	13	23	30	51	80	91
2×10^4	L_3	0	0	0	16	41	62	80	91	100	
	L_4	0	0	0	10	30	50	70	80	90	98
2×10^5	L_3	0	0	0	22	46	68	98	100		
	L_4	0	0	0	15	42	61	80	96	100	
2×10^6	L_3	0	0	0	30	70	90	100			
	L_4	0	0	0	25	65	86	94	100		
2×10^7	L_3	0	0	0	42	80	100				
	L_4	0	0	0	30	75	98	100			
Control	L_3	0	0	0	0	0	0	0	0	0	0
	L_4	0	0	0	0	0	0	0	0	0	0

filter paper in Petri dishes and followed daily for developing mycosis proving the death by *B. bassiana*.

Field tests

In seasons 2016/2017 and 2017/2018, an area of one feddan (4200 m^2) located in Fayoum Governorate, Egypt, and cultivated with sugar beet, *Beta vulgaris* L. (*Chenopodiaceae*), was selected for the field experiment. Half of the field was sprayed with 6×10^7 conidiospores/ml of *B. bassiana* + 0.02% Tween 80 as detergent, using a 600-l spraying motor. Meanwhile, the other half feddan was sprayed only by water containing 0.02% Tween 80. Inspection of the beet plants for the number of *S. exigua* larvae was carried out on randomly sampled 50 plants directly in the field. The first treatment of both areas started at the early season on 8 November 2017. During the period from January 2018 to the late of March 2018, the number of larvae was very low during this 2-week inspection times in winter. Thus, no control measure was needed. The second application took place on 26 March 2018 by the occurrence of more numbers of the larvae and followed with three sprayings at 7-day intervals. The numbers of larvae were counted in both the treatment and the control fields, and the reduction rates due to the treatment were calculated only for the first inspection after application of the fungi following El-Husseini et al. (2008).

Data analysis

LC_{50} and LC_{90} were calculated, using the software "Ldp Line" software (Bakr 2005). Data were processed by analysis of variance (one-way classification ANOVA), followed by a least significant difference, L.S.D., at 5% (Costat Statistical Software 1990).

Results and discussion

Bioassay under laboratory conditions

Treating *S. exigua* L_3 and L_4 with the lowest conidiospores concentration (2×10^1 conidiospores/ml) of *B. bassiana* showed higher mortality (11%) among larvae of L_3 than those of L_4 (5%), beginning from the fifth day post-treatment (Table 1). Mortality rates of L_3 and L_4 larvae started on the fourth day post-treatment. The 100% mortality in the treated larvae of L_3 was recorded for the concentrations 2×10^3, 2×10^4, 2×10^5, 2×10^6, and 2×10^7 conidiospores/ml at the tenth, ninth, eighth, seventh, and sixth day post-treatment. Meanwhile, the 100% mortality for L_4 was recorded at the concentrations 2×10^5, 2×10^6, and 2×10^7 at the ninth, eighth, and seventh day post-treatment. Mortality rates differed significantly in relation to the tested concentration. The present results showed that larval mortality increased by increasing the conidiospores concentrations and that the larvae of L_3 were more susceptible to the treatment with *B. bassiana* than larvae of L_4. The infected larvae survived more than 5 days and died later in the fifth larval

Fig. 1 Cadavers of *S. exigua* larvae covered with the white mycelium and white spores (white muscardine) proving death with *B. bassiana* in the bioassay test

Table 2 Number of *Spodoptera exigua* larvae (/50 plants) post treatments with *Beauveria bassiana* in sugar beet field in season 2016/2017

Dates of inspections	Numbers of larvae/50 plants		Reduction** (%)	Dates of inspections	Numbers of larvae		Reduction** (%)
	Treat.	Cont.			Treat.	Cont.	
18 November 2016*	9	9		26 March 2017*	5	8	
3 December 2016	5	11	54.5	4 April 2017*	3	9	66.6
18 January 2017	2	5		11 April 2017*	3	10	70
27 January 2017	1	4		18 April 2017*	3	8	62.5
20 February 2017	2	3		25 April 2017	1	2	50
3 March 2017	4	6		2 May 2017	0	0	

*Applications with *Beauveria bassiana* (6×10^7 spores/ml)
**Reduction (%) by the only count after application

instar (L_5) showing the typical symptoms for death by *B. bassiana* known as "white muscardine" (Fig. 1). The calculated LC_{50} for L_3 and L_4 was 18,463 (slope 0.414) and 35,990 (slope 0.387) spores/ml, while the LC_{90} was 37, 806 (slope 0.345) and 74,391 (slope 0.387) for the two larval instars, respectively. These results are in line with those of El-Husseini et al. (2008) and El-Banna et al. (2014).

Experimental field

Season 2016/2017

Data showed that the first application with *B. bassiana* in season 2016/2017 decreased the number of *S. exigua* by 54.5% after 14 days from the application where the plant leaves were still small in size. No applications were carried out during the period from 3 December 2016 to 26 March 2017 due to very low numbers of *S. exigua* larvae (Table 2). During the late winter and early summer (late of March), four successive applications, with 1-week intervals, were practiced (Table 2). The second *B. bassiana* application (26 March 2017) reduced the larvae of *S. exigua* by 66.6%, 1 week after application. Meanwhile, the third application (2 April 2017) caused an insignificant increase in the reduction of larvae (70%). The fourth application (14 April 2007) decreased numbers of the larvae by (62.5%). The fifth application seemed to be not necessary as the larval population was low (1–2 larvae/50 plants), even though it decreased the larval population statistically by 50%.

Season 2017/2018

In season 2017/2018, the numbers of the *S. exigua* larvae was relatively less than that of season 2016/2017. Although the larval population was low at the beginning of the season, the damage was noticeable on leaves of the young plants. Thus, a control action was necessary at the early season (during November), which traditionally occurs by applying chemical insecticides. The first application with *B. bassiana* at the beginning of the season (8 November 2017) induced a suppression of 66.6% in the larval population after 1 week (Table 3). As the population of *S. exigua* remained stable during January and February till late March due to winter cold weather, no control action was done till late of March (26 March 2018), where four successive applications with *B. bassiana* were carried out in weekly intervals (Table 3). The reduction in larval population recorded 75, 50, 80, and 75% for the second, third, fourth, and fifth applications, respectively (Table 3).

The present results for controlling the beet worm, *S. exigua*, with *B. bassiana* in sugar beet are in line with those of Sabbour and Abdel-Rahman (2007) and El-Husseini et al. (2008).

Table 3 Number of *Spodoptera exigua* larvae (/50 leaves) post treatments with *Beauveria bassiana* in sugar beet field in season 2017/2018

Dates of inspections	Numbers of larvae		Reduction** (%)	Dates of inspections	Numbers of larvae		Reduction** (%)
	Treat.	Cont.			Treat.	Cont.	
8 November 2017*	4	4		26 March 2018*	3	4	
3 December 2017	2	6	66.6	4 April 2018*	1	4	75
18 January 2018	0	3		11 April 2018*	2	4	50
27 January 2018	1	3		18 April 2018*	1	5	80
20 February 2018	3	4		25 April 2018	1	4	75
3 March 2018	3	4		2 May 2018	1	2	

*Applications with *Beauveria bassiana* (6×10^7 spores/ml)
**Reduction (%) by the only count after application

Conclusion

The present results showed the capability of the entomopathogenic fungus *B. bassiana* to induce acceptable mortality rates in treated larvae of the sugar beet worm, *S. exigua*, in the laboratory tests. The application of the fungal conidia by spraying on sugar beet plants in the field caused a reduction in the pest larval population between 66.6–80%. It is common in Egypt that the farmers control this pest in sugar beet by applying different insecticides. It is suggested that *B. bassiana* could replace the use of the insecticides and suppress the pest population without pausing hazards to the environment which is associated with the use of chemical pesticides.

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Ethics approval and consent to participate
I agree to all concerned regulations.

Consent for publication
I agree to publish this scientific paper in the EJBPC.

Competing interests
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References
Bakr EM (2005) A new software for measuring leaf area, and area damaged by *Tetranychus urticae* Koch. J Appl Entomol 129(3):173–175
Bartlett MC, Jaronski ST (1988) Mass protection 191 of entomogenous fungi for biological control of insects. In: Burge MN (ed) Fungi in Biological Control System. Manchester University Press, Manchester, pp 61–85
Costat Statistical Software (1990) In: Howard PH (ed) In Hand Book of Environmental Degradation Rates. CRS Press, Mar 28, 1991, p 776
El-Banna A, Abd El-Wahab S, El-Akad S, Amin S (2014) A laboratory study on the efficacy of *Metarhizium Anisopliae* (Metsch.) Sorokin and *Beauveria Bassiana* (Bals.) Vuill against *Spodoptera exigua* (Hb.) (Lepidoptera: Noctuidae). Int J Environ Scis 9(1):41–45
El-Husseini MM, Agamy EA, Mesbah AH, El-Fandary O, Abdallah MF (2008) Using *Beauveria bassiana* (Bals.) Vuillemin in spraying and dusting applications for biological control of sugar beet insect pests in Egypt. Egypt J Biol Pest Control 18(2):369–375
Goettel MS, Hajek AE (2001) Evaluation of nontarget effects of pathogens used for management of arthropods. In: Wajnberg E, Scott JK, Quimby PC (eds) Evaluating Indirect Ecological Effects of Biological Control. CAB International, New York, pp 81–94
Götz P (1991) Invertebrate immune response to fungal cell wall components. In: Latge LP, Boucias D (eds) Fungal Cell and Immune Response, vol 53. Springer, Berlin, pp 317–330
Keller S (2000) Use of *Beauveria brongniartii* in Switzerland and its acceptance by farmers. IOBC WPRS Bull. 23:67–71
Klingen I, Eilenberg J, Meadow R (2002) Effects of farming system, field margins and bait insect on the occurrence of insect pathogenic fungi in soils. Agric Ecosyst Environ 91:191–198
Lacey LA, Goettel MS (1995) Current developments in microbial control of insect pests and prospects for the early 21st century. Entomophaga 40:3–27
Liu XZ, Li SD (2004) Fungi secondary metabolites in biological control of crop pests. In: An ZQ (ed) Handbook of Industrial Mycology. Marcel Dekker, New York, pp 723–744
Mabrouk A, Bekheit HKM, El Husseini MM (2001) Economic diet for rearing *Spodoptera littoralis* (Boisd.), used for production of the nuclear polyhedrosis virus. Egypt J Biol Pest Control 11(2):127–134
Rombach MC, Aguda RM, Shepard BM, Roberts DW (1986) Infection of rice brown plant hopper, *Nilaparvata lugens* (Homoptera: Delphacidae), by field application of entomopatho genic Hyphomycetes (Deuteromycotina). Environ Entomol 15:1070–1073
Sabbour MM, Abdel-Rahman A (2007) Evaluation of some terpenes and entomopathogenic fungi on three sugar beet insect pests. Egypt J Biol Pest Control 17(2):125–130
Shorey HH, Hale RL (1965) Mass rearing of the larvae of nine noctuid species on a simple artificial medium. J Econ Entomol. 58:522–524
Tanada Y, Kaya HK (1993) Insect pathology. Academic Press, London, pp 319–385
Taylor JE, Riley DG (2008) Artificial infestations of beet armyworm, *Spodoptera exigua* (Lepidoptera: Noctidae) used to estimate an economic injury level in tomato. Crop Protection 27:268–274
Wraight SP, Jackson MA, de Kock SL (2001) Production, stabilization and formulation of fungal biocontrol agents. In: Butt TM, Jackson C, Magan N (eds) Fungi as Biocontrol Agents: Progress, Problems and Potential. CAB International, Wallingford, pp 253–287

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