Egyptian Journal of
Biological Pest Control

RESEARCH Open Access

Field evaluation of two entomopathogenic fungi; *Beauveria bassiana* and *Metarhizium anisopliae* as a biocontrol agent against the spiny bollworm, *Earias insulana* Boisduval (Lepidoptera: Noctuidae) on cotton plants

Dalia El Sayed Lotfy and Hemat Zakaria Moustafa*

Abstract

Background: The *Earias* spp. are devastating pests which reduce cotton yield up to 40% as seed cotton. Efficacy of *Metarhizium anisopliae* and *Beauveria bassiana* as bio-insecticides were investigated against the spiny bollworm, *Earias insulana* Boisduval (Lepidoptera: Noctuidae), under field conditions in Egypt throughout two successive seasons 2018 and 2019.

Results: Results showed *E. insulana* population reduction during both seasons after bio-insecticidal applications. *M. anisopliae* strains (S1, S2) treatments showed significant reductions in the mean numbers of infested bolls with *E. insulana* after 14 days from the 1st spray. Reduction percentages of *E. insulana* infestation reached to 77.74 and 76.51% respectively, after application of *M. anisopliae* strains (S1, S2) treatments; then, the infestation reduction percentages increased to 88.48 and 85.41% after the 2nd spray by the same fungal strains; then, the infestation reduction percentages increased to 90.16 and 90.84% after 3rd spray of the same fungal strains in season 2018. In 2019 season, the infestation reduction percentages of *E. insulana* was 85.48 and 80.75%, after the 1st spray of *M. anisopliae* strains (S1, S2), respectively, which increased to 92.40 and 89.87%, after 14 days from the 2nd spray by the two fungal strains respectively, and then increased to 94.12 and 93.73%, after 14 days from the 3rd spray of *M. anisopliae* (S1 and S2) respectively. In season 2018, the infestation reduction percentage of *E. insulana* by *B. bassiana* strains (S1, S2) recorded 73.09 and 71.89%, respectively and 81.04 and 82.89% respectively, in season 2019, after 14 days of the 1st spray. While after the 2nd spray of the two tested *B. bassiana* strains, the infestation reduction percentage of *E. insulana* was 85.41 and 85.41% respectively, in season 2018, whereas it was 89.16 and 89.16% respectively, in season 2019. Then after 14 days of the 3rd spray by the same fungus strains, the reduction percentage of *E. insulana* increased to 86.56 and 85.35%, respectively, in season 2018, and 90.83 and 90.83% in season 2019.

Conclusions: Tested strains of *B. bassiana* and *M. anisopliae* fungi proved their potential for decreasing *E. insulana* infestation percentages through 2–3 spray treatments under field conditions.

Keywords: *Earias insulana*, Biopesticides, *Metarhizium anisopliae*, *Beauveria bassiana*, Field application, Cotton

* Correspondence: hemat.zakaria@gmail.com
Plant Protection Research Institute, Agriculture Research Center, Dokki, Giza, Egypt

Background

Cotton (*Gossypium barbadense*) (Malvaceae) is the most important industrial crop worldwide (Johnson *et al.*, 2014). In Egypt, cotton plants like most of the field crops are attacked by many lepidopterous pest species (Gaaboub *et al.,* 2012). Insect pests are the most limiting factors that decrease cotton production and cause severe damages to the crop yield (El-Heneidy *et al*., 2015). The spiny bollworm, *Earias insulana* Boisduval (Lepidoptera: Noctuidae), the mid-late season pest, usually threats cotton plants in Egypt (El Hamaky *et al.,* (1990). The damage of fruits (green bolls) is frequently more destruction than the other parts of the cotton plant. *E. insulana* and the pink bollworm, *Pectinophora gossypiella* (Saund.) (Lepidoptera: Gelechiidae), are the most destructive insect pests of cotton in Egypt (Amin and Gergis, 2006). Biopesticides based on bacteria, viruses, entomopathogenic fungi, and nematodes are often considerable scope as a plant protection agent against several insects. Many works extensively investigated the field bio-efficacy of the entomopathogenic fungi (EPF) such as *Beauveria bassiana* (Balsamo) Vuillemin (Deuteromycotina: Hyphomycetes) (Wraight *et al*., 2010).

The present study aimed to evaluate the potential of EPF, as bio-insecticides against the spiny bollworm, *E. insulana*, under field conditions.

The present study aimed to evaluate the potential of EPF, as bio-insecticides against the spiny bollworm, *E. insulana* under field conditions.

Methods

Fungi culture sources

Two tested fungi, *Beauveria bassiana* and *Metarhizium anisopliae*, were obtained from 2 sources: the first (S1), isolate of *B. bassiana* (Balsamo) AUMC NO (5133) and *M. anisopliae* (Metschnikoff) Sorokin AUMC NO (5130), obtained from the Assiut University, Mycological Center Faculty of Science, Egypt. The second source (S2) was the wettable powder of the commercial bioagents Bioranza® WP 10% (*M. anisopliae* Sorok.). Active ingredients 10%, Inert Ingredient 90%, formulated as a wettable powder with the count of 1×10^8 spore/ml and Biovar® WP 10% (*B. bassiana Balsamo*) active ingredients 10%, Inert Ingredient 90%, formulated as a wettable powder with the count of 1×10^8 spore/ml.

Preparation of specific media

The isolates were cultured on Sabouraud dextrose agar medium g/l containing 40 g glucose, 20 g peptone, 20 g agar, 2 gm yeast extract, and 1000 ml of distilled water in flasks. These flasks were autoclaved at 21°C for 15 min.

Preparation of tested bioagent

The two sources were prepared as solutions as follows:

Source 1 (S1): Fungal cultures were grown on Sabouraud dextrose agar medium (Dextrose 40gm, agar 20gr, peptone 10g)/1000 ml distilled water and incubated at 25±2 °C in darkness for 14 days. Conidial suspensions were prepared by scraping cultures with a sterile objective glass and transferred to 10 ml of sterile water containing 0.05% Tween 80 in a laminar flow chamber. The mixture was stirred for 10 min the hyphal debris was removed by filtering the mixture through fine mesh sieve. The conidial concentration of final suspension was determined by direct count $1{\times}10^8$ using hemocytometer with final concentration of $1{\times}10^8$ spore/ml.

Source 2 (S2): To prepare stock solution ($1{\times}10^8$), spore/ml from the commercial bioagent was weighted, 5gram of powder, and dissolved in 900 ml sterile water.

Field application

Field study was conducted at Talaat-El Agamy experimental farm, henno, Kafr El-Sheikh, Egypt. Studies covered two successive cotton growing seasons 2018 and 2019. Cotton variety Giza 96 was cultivated on April 7, 2018, and on April 13, 2019 seasons. The experimental design was complete randomized block, the whole cultivated area (350 m^2) was divided into equally 5 plots each was divided to 3 replicates, each plot was treated by one of the tested 4 bio-insecticides strain individually with a concentration of $1{\times}10^8$ spore/ml)/l water. The last plot was left untreated as control. Cotton seeds were sown at 20-cm distance between hills. Two sources of the 2 EPF were applied against *E. insulana* on infested cotton green bolls when infestation reached 3% (the economic threshold). Spraying of the tested fungi took place on cotton plants 3 times on July, 8th, 22nd and 5th August, respectively through the 2 seasons. Fungal applications were carried out at 4 pm using a knapsack motor sprayer (20-l capacity). To evaluate the effect of the 4 EPF strains against *E. insulana*, samples of 25 bolls/plot were picked randomly before and 1 week after applications. Sampling continued weekly until harvest. The collected bolls were carefully dissected, and the numbers of larvae, infested bolls, and reduction percentages were recorded. The reduction percentages in the field experiment were calculated.

Statistical analysis

Data of *E. insulana* infestation after 7 and 14 days of treatments were statistically analyzed by one-way analysis of variance (ANOVA) ($P < 0.05$ %) Duncan's multiple range test of means (Duncan, 1955). The reduction percentages of *E. insulana* at different application time interval were calculated according to Henderson and Tilton (1955):

$$= 1\text{-}\frac{(\text{No.in control before treatment} \times \text{No.in treatment after treatment})}{(\text{No.in control after treatment} \times \text{No.in treatment before treatment})} \times 100$$

Results

Effect of the tested entomopathogenic fungi on *E. insulana* under field conditions

In the control plot, larval population increased gradually throughout all the inspection weeks in the 2 studied growing seasons (Tables 1 and 2).

Data in Table 1 showed that applications of *M. anisopliae* (S1, S2) treatments significantly reduced the mean numbers of infested bolls with *E. insulana* after 7 days post the 1st spray with the reduction percentages of 60.69 and 51.74%, respectively, while 77.74 and 76.51% after 14 days from the 1st spray the reduction percentages increased to 88.37 and 61.89%, 7 days from the 2nd spray. The reduction % by *M. anisopliae* (S1, S2) applications attained 88.48 and 85.63%, respectively, after 14 days from the 2nd spray. Afterward, the percentages of infestation reduction reached 92.5 and 90.16% after 7 days from the 3rd spray: 90.13 and 90.84% after 14 days from the 3rd spray in season 2018.

The reduction percentage of *E. insulana* in case of *B. bassiana* (S1, S2) in season 2018 recorded 54.61 and 49.24%, respectively, after 7 days from the 1st spray, and 73.09 and 71.89%, respectively, after 14 days, while it was 85.09 and 83.57%, respectively, after 7 days from the 2nd spray, and 85.41 and 85.41%, respectively, after 14 days from the 2nd spray; then, the reduction percentage increased to 89.00 and 88.12%, respectively, after 7 days from the 3rd spray, and 86.56 and 85.35%, respectively, after 14 days from the 3rd spray.

Results in Table 2 clarified that application of *M. anisopliae* (S1, S2) treatments showed significant reductions in the mean numbers of infested bolls with *E. insulana* than the control after 7 days from the 1st spray in season 2019 and these percentages reached to 79.54 and 72.72%, respectively, and then increased to 85.48 and 80.75%, respectively, after 14 days from the 1st spray, whereas, the reduction percentages were 91.02 and 87.59%, respectively, after 7 days from the 2nd spray, and they were 92.40 and 89.87%, respectively, after 14 days from the 2nd spray of *M. anisopliae* (S1, S2) treatments in season 2019. Afterward, the reduction percentages of *E. insulana* Infestation increased to 95.42 and 95.12%, respectively, after 7 days from *M. anisopliae* (S1, S2) spray, and reached to 94.12 and 93.73%, 14 days from the 3rd spray.

Also, Table 2 shows the reduction percentages of *E. insulana* by the 1st application of *B. bassiana* (S1, S2) (70.61 and 74.69%, respectively), after 7 days and (81.04 and 82.89%, respectively) after 14 days of treatments. While for the 2nd spray of *B. bassiana* (S1, S2) treatments, the reduction percentages of *E. insulana* were 86.48 and 88.42%, respectively, after 7 days whereas they were 89.16 and 89.16%, respectively, after 14 days from treatments. Then, the reduction percentages of *E. insulana* increased to 92.93 and 92.78%, respectively, after 7 days from the 3rd spray of *B. bassiana* (S1, S2) whereas they were 90.83 and 90.83%, respectively, after14 days from the 3rd spray by the same strains, during season 2019.

Data in Table 3 shows that there were significant differences between means of infestations after 7 and 14 days treatments when the p-value is <0.05).

Discussion

Obtained results showed that both *M. anisopliae* and *B. bassiana* strains were elicited effects toward *E. insulana* under field experiment. *M. anisopliae* (S1, S2) showed the best effect toward *E. insulana* infestation reduction percentage after 3 sprays of each treatments, under field conditions followed by *B. bassiana* S1, S2. In this respect, Abd-ElAzeem et al. (2019) investigated the biological activities of spores and metabolites of some fungi against the newly hatched larvae of *E. insulana*. Results showed that the fungi *M. anisopliae* had more effectiveness to the newly hatched larvae. Also, spore suspensions of the all fungal isolates had the highest larval mortality than fungal metabolites. Moustafa et al. (2019) found that toxicity of *M. anisopliae* was high in case of *E. insulana* treatment. Dar et al. (2020) reported that *M. anisopliae* and *B. bassiana* either isolates or (W.P.) with Economy Micron ULVA (15 L./Fed.) were the most effective applications in reduction percentage of boll infestation with *E. insulana* followed by *Bacillus thuringiensis*, whereas, Hegab and Zaki (2012) found that *B. bassiana* gave a low larval mortality against *E. insulana*.

Conclusion

It could be concluded that *B. bassiana* and *M. anisopliae* proved to be efficient EPF against *E. insulana*. These fungi induced significant reductions for the mean numbers of infested bolls with the pest. *B. bassiana* and *M. anisopliae* are commonly used as pathogens against many insect species. Generally, the tested strains can be suggested as promising fungi to be used in biological control program of *E. insulana* in the field application.

Table 1 Reduction percentages of *Earias insulana*-infested cotton bolls through 2018 season

Treatments		Inspection before treatments	Days after treatments											
			1st spray (July 8)				2nd spray (July 22)				3rd spray (August 5)			
			Mean	% Reduction	Mean	% Reduction	Mean	% Reduction	Mean	% Reduction	Mean	% Reduction	Mean	% Reduction
			7 days		14 days		7 days		14 days		7 days		14 days	
Control		9.50^{a}	14.50^{a}	-	20.50^{a}	-	$31.^{a}$	-	35.50^{a}	-	38.00^{a}	-	41.50^{a}	-
Metarhizium anisopliae	S1	10.00^{a}	6.00^{bc}	60.69	4.85^{c}	77.74	3.75^{c}	88.37	4.85^{b}	88.48	3.00^{c}	92.5	4.30^{c}	90.16
	S2	9.50^{a}	7.00^{c}	51.74	4.85^{c}	76.51	3.62^{c}	61.89	5.10^{b}	85.63	3.75^{bc}	90.13	3.80^{c}	90.84
Beauveria bassiana	S1	10.00^{a}	6.93^{bc}	54.61	5.85^{bc}	73.09	4.81^{bc}	85.09	5.45^{b}	85.41	4.40^{bc}	89	$5..87^{b}$	86.56
	S2	10.00^{a}	7.75^{b}	49.24	6.11^{b}	71.89	5.30^{b}	83.57	5.45^{b}	85.41	4.75^{b}	88.12	6.40^{b}	85.35
LSD		0.674	1.266		0.763		0.767		1.630		0.948		0.904	

Means in the same column followed by different letters are significantly different, $P \leq 0.05$

Table 2 Reduction percentages of *Earias insulana* infested cotton bolls through 2019 season

Treatments		Inspection before treatment	Days after treatments											
			1st spray (July 8)				2nd spray (July 22)				3rd spray (August 5)			
			Mean	% Reduction	Mean	% Reduction	Mean	% Reduction	Mean	% Reduction	Mean	% Reduction	Mean	% Reduction
			7 days		14 days		7 days		14 days		7 days		14 days	
Control		5.50^{ab}	17.00^{a}	-	23.00^{a}	-	28.00^{a}	-	32.00^{a}	-	35.00^{a}	-	39.00^{a}	-
Metarhizium anisopliae	S1	6.00^{a}	3.80^{b}	79.54	3.63^{b}	85.48	2.75^{b}	91.02	2.65^{b}	92.40	1.75^{b}	95.42	2.50^{b}	94.12
	S2	4.50^{b}	3.80^{b}	72.72	3.61^{b}	80.75	2.85^{b}	87.59	2.65^{b}	89.87	1.40^{b}	95.12	2.00^{b}	93.73
Beauveria bassiana	S1	5.00^{ab}	4.55^{b}	70.61	3.95^{b}	81.04	3.45^{b}	86.48	3.15^{b}	89.16	2.25^{b}	92.93	3.25^{b}	90.83
	S2	5.50^{ab}	4.31^{b}	74.69	3.92^{b}	82.89	3.25^{b}	88.42	3.15^{b}	89.16	2.30^{b}	92.78	3.25^{b}	90.83
LSD		1.122	0.913		1.833		1.360		0.019		1.290		0.965	

Means in the same column followed by different letters are significantly different, $P \leq 0.05$

Table 3 *T*-test comparison between the mean infestations of *Earias insulana* after using the two fungal after 7 and 14 days at each treatment and control in 2018 and 2019 seasons

Treatment		Days after treatments of season 2018						Days after treatments of season 2019					
		1st spray (July 8)		2nd spray (July 22)		3rd spray (August 5)		1st spray (July 8)		2nd spray (July 22)		3rd spray (August 5)	
		Mean		Mean		Mean		Mean		Mean		Mean	
		7 days	14 days	7 days	14 days	7 days	14 days	7 days	14 days	7 days	14 days	7 days	14 days
Control		*		NS		NS		*		NS		NS	
Metarhizium anisopliae	S1	NS		NS		NS		NS		NS		NS	
	S2	NS		NS		NS		NS		NS		NS	
Beauveria bassiana	S1	NS		NS		NS		NS		NS		NS	
	S2	NS		NS		NS		NS		NS		NS	

*Significant (≤0.05), *NS* not significant

Acknowledgements
Not applicable

Authors' contributions
Equally contribution for DEL and HZM. All authors have read and approved the manuscript.

Funding
Not applicable

Availability of data and materials
All data in the tables are so clear and these numbers are the raw data.

Declarations

Ethics approval and consent to participate
Not applicable

Consent for publication
Not applicable

Competing interests
The authors declare that they have no competing interests.

Received: 1 December 2020 Accepted: 19 April 2021
Published online: 06 May 2021

References

Abd-ElAzeem EM, El-Medany WAZ, Sabry HM (2019) Biological activities of spores and metabolites of some fungal isolates on certain aspects of the spiny bollworms Earias insulana (Boisd.) (Lepidoptera: Noctuidae). Egypt J Biol Pest Control 29(90). https://doi.org/10.1186/s41938-019-0192-y
Amin AA, Gergis MF (2006) Integrated management strategies for control of cotton key pests in middle Egypt. Agron Res 4:121–128
Dar RAA, Lotfy DE, Moustafa HZ (2020) Field application of bio-insecticides on spiny bollworm, *Earias insulana* (Bosid.) On Cotton by using recent low volume ground spraying equipment, Egypt. Acad J Biolog Sci 13(1):47–57
Duncan DB (1955) Multiple range and multiple F test. Biometrics 11(1):1–42. https://doi.org/10.2307/3001478
El Hamaky MA, Moawad GM, Radwan SM, Oman MS (1990) Effect of pheromone mixed with cheated foliar fertilizers of spiny boll worm. Bull Soc Egypt Econ 19(1):113–119
El-Heneidy AH, Khidr AA, Taman AA (2015) Side-effects of insecticides on non-target organisms: 1- In Egyptian Cotton Fields. Egypt J Biol Pest Control 25(3):685–690. https://doi.org/10.1186/s41938-018-0081-9
Gaaboub IA, Halawaa S, Rabiha A (2012) Toxicity and biological effects of some insecticides, IGRs and Jojoba oil on cotton leafworm *Spodoptera littoralis* (Boisd.). J Appl Sci Res 2:131–139
Hegab ME, Zaki AA (2012) Toxicological and biological effects of bacteria, *Bacillus thuringiensis* on *Pectinophora gossypiella* and entomopathogenic fungi, *Beaveria bassiana* on *Earias insulana*. J Plant Prot Path., Mansoura Univ 3(3): 289–297
Henderson CF, Tilton EW (1955) Tests with acaricides against the brown wheat mite. J Econ Entomol 48(2):157–161. https://doi.org/10.1093/jee/48.2.157
Johnson J, MacDonald S, Meyer L, Norrington B, Skelly C (2014) The world and United States Cotton Outlook. Agricultural Outlook Forum 2014:16. https://doi.org/10.22004/ag.econ.168329
Moustafa HZ, Lotfy DE, Hassan KA (2019) Effect of entomopathogenic fungi on *Pectinophora gossypiella* (Lepidoptera: Gelechiidae) and *Earias insulana* (Lepidoptera: Noctuidae) and their predators. Egypt J Plant Prot Res Inst. 2(1): 9–15
Wraight SP, Ramos ME, Avery PB, Jaronski ST, Vandenberg JD (2010) Comparative virulence of *Beauveria bassiana* isolates against lepidopteran pests of vegetable crops. J Invertebr Path 103(3):186–199. https://doi.org/10.1016/j.jip.2010.01.001

Publisher's Note