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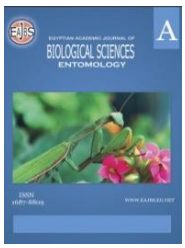

The Role of Entomopathogenic Fungus, *Beauveria bassiana* (Bals.) Vuill. in Reducing Population Densities of Refugee Insect Pests to Dry Okra Pods During Winter

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ABSTRACT

The present work was carried out during two successive seasons, being 2019/20 and 2020/21 in three locations in Gharbia governorate to explain the role of entomopathogenic fungi (EPF) in decreasing insect pest populations inhabiting okra dry pods during the winter period. Three insect pest species were found in the okra dry pods: the cotton seed bug, *Oxycarenus hyalinipennis* (Costa) (Hemiptera: Lygaeidae), the cotton pink bollworm, *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae) and the cotton spiny bollworm, *Earias insulana* (Boisd.) (Noctuidae: Lepidoptera). The EPF, *Beauveria bassiana* (Bals.) Vuill. was isolated from the 3 insect species in the winter months. The seasonal fungal incidence percentages were 3.04 and 10.73% in nymphs and adults of *O. hyalinipennis*, respectively in the first season and were 13.53 and 16.53% in the second one. The seasonal fungal incidence percentages on *P. gossypiella* larvae were 4.64 and 7.96% for the two seasons, respectively. Natural mortality with the fungus ranged from 4.32 to 14.16 and 4.32 to 20.63% in the two seasons, respectively. Whilst the seasonal fungal incidence percentage on *E. insulana* larvae were 6.02 and 5.23 % for the first and second seasons and the fungus was isolated for one time in each season. Results explained and approved the role of EPF as natural mortality factors against insect pest species in their winter microhabitats in Egypt. Accordingly, it encourages biological control advocates to utilize such natural enemies as biological control agents.

INTRODUCTION

Entomopathogenic fungi (EPF) are widespread in nature and play a considerable role as natural mortality factors of many arthropod pests (Shapiro-Ilan, 2003 and Meyer *et.al*., 2005). Many virulent local isolates of fungal pathogens, in particular those belonging to class Sordaiomycetes, were isolated from active and diapaused insect populations (El-Sufty and Boraei, 1987 and El-Sheikh 2003). *Beauveria bassiana* (Bals.) Vuill. is one of the highly virulent EPF to many insect species and some arthropods pests and have been applied for their control (Ramoutar *et. al.*, 2010, El-Sheikh 2012 and Perinotto *et. al.*, 2012). This fungus is a pathogen causing acute diseases leading to pest population suppression and maintaining the fungus in the environment for permanent control.

The cottonseed bug, *Oxycarenus hyalinipennis* (Costa) (Hemiptera: Lygaeidae) is one of the insect pests that infest cotton seeds in Egypt. Kirkpatrick (1923) recorded three

complete generations, and almost certainly a partial and very possibly complete fourth, from July to October, a single breeding season the quiescent period is not connected with either hibernation or aestivation but is merely a period of rest between breeding seasons, which are influenced only by the time of year that the seeds on the different plants are available. However, Schmutterer (1969) and Dimetry (1974) recorded 4-5 annual generations during the period from November to April. It has in the year with an overwintering period during both adult and nymphal stages. Kirkpatrik (1923) stated ,"this bug appears to be immune from either parasites or predatory enemies; he added that weather conditions are probably the only natural means whereby its multiplication is checked. He further indicated that a fungus has been found growing on the dead bugs, the presence of which does increase the rate of mortality, but apparently only by choking up the spiracles. "The current work proves the opposite and proves that continual research always reveals the reality of biological interrelationships".

The pink bollworm, *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae) is one of the most serious insect pests infesting cotton in many cotton-producing areas of the world. It causes serious damage in cotton bolls resulting in a high reduction in cotton quantity and quality (Saadiya, Said *et al*., 2017).

The spiny bollworm (SBW), *Earias insulana* (Boisd.) (Noctuidae: Lepidoptera) is another destructive polyphagous insect. In Egypt, it attacks several summer crops such as cotton and okra (Nada *et al*., 2010). Each SBW larva can damage up to 3 squares and 1 boll in 1-2 plant (Durán *et al.*, 2000).

The present study was carried out to study the incidence and the role of EPF in suppressing the 3 pest species that attack dry okra pods during winter.

MATERIALS AND METHODS

The study was carried out in 3 cities in Gharbia Governorate, Egypt: (Tanta, El-Santa and Quator) during the months from November to April through 2 successive seasons of 2019/20 and 2020/21. In each season and each location, a number of dry okra plants were collected during November and kept in piles under the field's conditions. A sample size of 10 okra pods was randomly selected from the previously preserved plants and at 15 days intervals. The pods were placed individually in paper sacks and completely closed. Plant samples were transferred to the laboratory of the Plant Protection Department, Faculty of Agriculture, Tanta University for further examination.

Population Density of Insect Pests:

Population densities of *O. hyalinipennis*, *P. gossypiella* and *E. insulana*, immature stages and either live or died as well the associated EPF, which inhabited the pods during winter months were estimated by dissecting the pods and kept on filter papers inside Petri dishes for identification and EPF isolation.

Mycosis Test:

Cadavers of larvae, nymphs and adults were externally cleaned by immersing them in 0.5% sodium hypochorite suspension for 10 sec. to remove external contamination, and then cadavers were immersed in distilled water for 30 sec. and transferred to a saturated filter paper inside sterilized dishes. Cadavers were incubated at 25±1ºC and about 100% R.H. daily filter papers were saturated by water and preserved until conidia of fungi formation. All cadavers which were killed by the fungi were counted and the obtained symptoms of the disease were compared as recorded by Cantwell (1974) and El-Sheikh (2012). Insect cadavers showed an external fungal growth was considered killed by this pathogen. The EPF was identified in the Mycology Center, Faculty of Science, Assiut University (AUMC), Egypt.

Statistical Analyses:

Computation was done using computer software Minitab 16.

RESULTS AND DISCUSSION

The population density of *O. hyalinipennis, P. gossypiella* and *E. insulana* in the dried stored okra pods and the incidence of the EPF, *B. bassiana* during the two winter seasons 2019/20 and 2020/21 was presented in tables (1 and 2).

Oxycarenus hyalinipennis

1. Population Density:

In the first season, the population size of nymphs and adults of *O. hyalinipennis* in okra pods were found all over the winter seasons with an average of 880.06±61.2 and 838.9±95.8 insects sampled through the season for nymphs and adults, respectively. There were 3 peaks of nymphs 362.6, 201.0 and 82.0 insects on 27 Nov., 27 Dec. and 26 Jan., respectively. While the adults had 2 peaks, 192.3 and 163.6 insects, recorded on Nov. 27th and Jan. 11th, respectively (Table 1).

In the second season, the population of nymphs and adults of *O. hyalinipennis* in okra pods was noticeable throughout the entire season. The seasonal means were 356.39±26.4 and 601.6±48.4 individuals of nymphs and adults, respectively. There were 3 peaks of nymphs 69.6, 158 and 27 insects on Nov. 6th, Dec. 8th and Feb. 9th, respectively. A similar trend was noticed for adults where 3 peaks were recorded (95.3, 172.6 and 82.6) adults on Nov.6th, Dec. 8th and Jan. 26th, respectively (Table 2).

Bugs become active in periods of warm humid weather and usually leave the winter site, wandering around on the okra plant and soil (Gandhi, 1973). Also, (Ibrahim 1996) reported that generally, the population density of *O. hyalinipennis* in okra pods increased gradually from October to December and then decreased gradually to reach the lowest population during early May.

2. Incidence of the Fungus:

A considerable number of adults and last nymphal instar of *O. hyalinipennis* were found infected and killed by *B. bassiana*. In the first season, the natural mortality by the fungus ranged between 1.4-16.2 and 3.2 - 31.7% in nymphs and adults sampled, respectively. The fungus was observed from Dec. 27th to Feb. 25th for nymphs. While in the adults it was found from 27th of Dec. to 27th of Mar. The highest incidence percentages of the fungus were on 10th and Feb.25th with 16.2 and 31.7% and the seasonal fungal incidence percentages were 3.04±0.23 and 10.73±1.32 % in nymphs and adults sampled, respectively (Table 1).

In the second season, the natural mortality by *B. bassiana* ranged from 9.7 to 39.5 and 2.9 to 43.7% in nymphs and adults sampled, respectively. The fungus was found from Nov 6 th to 26th of Jan. for nymphs and from Nov. 6th to 9th of Feb. in adults. The highest incidence percentage of the fungus was on Nov. 22nd in both stages with 39.5 and 43.7 % and the seasonal fungal incidence percentages were 13.53±1.31 and 16.53±2.67% in nymphs and adults sampled, respectively (Table 2).

The present findings agree with those of (Ibrahim, 1996) who found that the incidence of the fungus on *O. hyalinipennis* in okra pods was first detected in the first half of November in two localities, increased gradually throughout the winter season to reach its highest values in March 22.4 and 32.7% at Kafr El-Sheikh and Desouk, Egypt, respectively. The fungus incidence decreased gradually until disappeared at the beginning of May.

Pectinophora gossypiella

1. Population Density:

In the first season population size of diapaused larvae of *P. gossypiella* in okra pods was found all over the season with a total average of 40.66±2.9 larvae sampled through the season. There were 2 peaks of larvae (6.6 and 7.3) on 27 Nov. and 10 Feb. While in the second season the diapaused larvae of *P. gossypiella* in okra pods were found from the beginning of the season until the 24th of March with an average of 94.63±6.8 larvae sampled through the season. There were four peaks of larvae 11, 13.3, 18.6 and 17.6 larvae on Nov.6th, 22nd of Dec., 26th of Jan and 23th of Feb. (Table 1 and 2).

2. Incidence of the Fungus:

A moderate number of diapaused larvae of *P. gossypiella* in okra pods were found infected and killed by the entomopathogenic fungus, *B. bassiana*. In the first season, the natural mortality by the fungus ranged 4.32-14.16% in sampled larvae. The fungus was found from Jan.11th to Mar.12th. The highest incidence percentage of the fungus was on the 12th of March with 14.16 % while the seasonal fungal incidence percentage was 4.64±0.32 % (Table 1).

In the second season, the natural mortality by the fungus ranged 4.32 - 20.63 % in sampled larvae. The fungus was found from Nov. 22nd to Mar. 10th.The highest incidence percentage of the fungus was on Nov. 22nd with 20.63 % and the seasonal fungal incidence percentage was 7.96±0.7% (Table 2).

In another study, Rashad and Aly (1997) who used laboratory bioassay of the fungus, *B. bassiana*, included eggs and larvae of *P. gossypiella* under different concentrations. They stated that the fungus proved virulent against the two stages of the insect. Treatment of the eggs resulted in a high reduction rate of hatchability. The eggs appeared more susceptible to infection than larvae, and about 90% of the hatched larvae from treated eggs failed to develop into adults and died. LC_{50} for eggs was $1.75x10^3$ spores/ml, and for larvae this was $23x10^3$ spores/ml. These results may suggest a promising potential of the fungus as a biological control agent against *P. gossypiella*.

Earias insulana

1. Population Density:

In the first season, the population size of *E. insulana* larvae in okra pods was found from Dec. 27th to Mar 27th with an average of 4.65±0.26 larvae sampled through the season. There were 2 peaks of larvae 2 and 0.33 larvae on 27 Dec. and 25 Feb. While in the second season population size of *E. insulana* larvae in okra pods was found from 22 Nov. until 9 Feb. with a total average of 5.97±0.41 larvae sampled through the season. There were 2 peaks of larvae 1.66 and 2.33 larvae on Dec. 8th and Jan. 11th (Tables 1 and 2).

2. Incidence of the Fungus:

A few numbers of *E. insulana* larvae in okra pods were found infected and killed by *B. bassiana*. In the first season, the natural mortality by the fungus was only recorded in the larval population on Dec. 27th with 14.16 % and the seasonal fungal incidence of larvae was 6.62±1.17%. In the second season, the natural mortality by the fungus was only recorded in larval population on 11 Jan. with 12.4 % and the seasonal fungal incidence of larvae was 5.23±0.23 % (Tables 1 and 2).

In a similar study, Kashif *et al.,* 2015 assessed a laboratory strain of B. *bassiana* (Bals.) Vuill. and a commercial formulation of *Bacillus thuringiensis* (Berl.) against three field populations of *Earias vittella* F. (Lepidoptera: Noctuidae) in the laboratory. Three dose rates of *B. bassiana* (1.5×10^6, $1.5\times10^7$and 1.5×10^8conidia mL-1) and one of *B. thuringiensis* (0.5 µg g-1) were applied alone and in combination against 2nd and 4th larval instars. The mortality was observed until pupation. The highest larval mortality was observed and recorded in the population in Faisalabad, India with the lowest pupation rate,

adult emergence and egg hatching treated with combined concentrations of *B. bassiana* and *B. thuringiensis*. The lowest mortality was observed in the population from Pakpattan among all the tested populations. Overall results demonstrated that all the treatments gave significant control of *E. vittella* and both microbial agents may become an integral part of the successful IPM program of *E. vittella* in the okra crop.

Table 1: Population density of *O. hyalinipennis*, *P. gossypiella* and *E. insulana* in dried stored okra pods and the incidence of the fungus, *B. bassiana* at Gharbia governorate in 2019/20 season.

Sampling date	Mean number of insects / sample													
	Oxycarenus hyalinipennis								*Pectinophora gossypiella*			*Earias insulana*		
	Nymphs			Adults			Total fungal mortality		Larvae			Larvae		
	Live	Fungal mortality		Live	Fungal mortality				Live	Fungal mortality		Live	Fungal mortality	
		No.	%		No.	%	No.	%		No.	%		No.	%
Nov.27'2019	362.6	0.0	0.0	192.3	0.0	0.0	0.0	0.0	6.6	0.0	0.0	0.0	0.0	0.0
Dec.12'	84.3	0.0	0.0	63.6	0.0	0.0	0.0	0.0	6.3	0.0	0.0	0.0	0.0	0.0
Dec.27'	201.0	3.0	1.47	121.6	4.0	3.2	7	1.66	3.0	0.0	0.0	2.0	0.33	14.16
Jan.11'2020	69.6	5.0	6.7	163.6	21.3	11.5	26.3	10.13	3.6	0.33	8.39	1.0	0.0	0.0
Jan.26'	82.0	6.0	6.8	151.3	13.3	8	19.3	7.64	6.6	0.66	9.1	0.66	0.0	0.0
Feb.10'	51.6	10	16.2	108.3	48.0	30.7	58	26.61	7.3	0.33	4.32	0.0	0.0	0.0
Feb.25'	23.3	3.6	13.3	29.3	13.6	31.7	17.2	24.64	4.6	0.33	6.69	0.33	0.0	0.0
Mar.12'	3.0	0.0	0.0	3.6	0.33	8.4	0.33	4.76	2.0	0.33	14.16	0.33	0.0	0.0
Mar.27'	1.66	0.0	0.0	5.0	0.33	6.2	0.33	4.72	0.33	0.00	0.0	0.33	0.0	0.0
Apr.11'	1.0	0.0	0.0	0.3	0.0	0.0	0.0	0.0	0.33	0.0	0.0	0.0	0.0	0.0
Total No. in season	880.06±6 1.2	27.6± 4.6	3.04± 0.23	838.9± 59.8	100.8± 5.5	10.73± 1.32	128.46 ±4.53	6.95±0. 74	40.66 ±2.9	1.98± 0.06	4.64±0.3 2	4.65± 0.26	0.33±0. 02	6.62± 1.17

Table 2: Population density of *O. hyalinipennis*, *P. gossypiella* and *E. insulana* in dried stored okra pods and the incidence of the fungus, *B. bassiana* at Gharbia governorate in 2020/21season.

Sampling date	Mean number of insects / sample													
	Oxycarenus hyalinipennis								*Pectinophora gossypiella*			*Earias insulana*		
	Nymphs			Adults			Total fungal mortality		Larvae			Larvae		
	Live	Fungal mortality		Live	Fungal mortality				Live	Fungal mortality		Live	Fungal mortality	
		No.	%		No.	%	No.	%		No.	%		No.	%
Nov.6'2020	69.6	9.6	12.1	95.3	13.0	12	19.6	12.25	11.0	0.0	0.0	0.0	0.0	0.0
Nov.22'	29.6	19.3	39.5	33.0	25.6	43.7	44.9	41.76	5.0	1.3	20.63	0.66	0.0	0.0
Dec.8'	158.0	16.3	10.3	172.6	27.0	13.5	43.3	11.58	4.6	0.33	6.69	1.66	0.0	0.0
Dec.22'	52.3	5.6	9.7	71.3	8.0	10	13.6	9.91	13.3	1.0	6.99	0.66	0.0	0.0
Jan.11'2021	11.0	3.0	21.4	48.3	14.0	22.5	17	22.28	7.3	0.33	4.32	2.33	0.33	12.4
Jan.26'	6.6	2.0	23.3	82.6	15.0	15.4	17	16	18.6	1.3	6.53	0.33	0.0	0.0
Feb.9'	27	0.0	0.0	53.3	14.0	20.8	14	14.84	10.3	2.0	16.26	0.33	0.0	0.0
Feb.23'	1.3	0.0	0.0	33.3	1.0	2.9	1	2.8	17.6	1.6	8.33	0.0	0.0	0.0
Mar.10'	0.33	0.0	0.0	7.3	0.0	0.0	0.0	0.0	5.33	0.33	5.83	0.0	0.0	0.0
Mar.24'	0.33	0.0	0.0	2.3	0.0	0.0	0.0	0.0	1.6	0.0	0.0	0.0	0.0	0.0
Apr.7'	0.33	0.0	0.0	2.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Total No. in season	356.39±26 .4	55.8± 3.8	13.53 ±1.31	601.6± 48.4	117.6 ±1.6	16.53± 2.67	170.4 ±9.62	15.06± 1.87	94.63 ±6.8	8.19± 1.67	7.96±0.7	5.97± 0.41	0.33±0. 02	5.23±0. 23

Recently, Hemat, Moustafa *et al*., 2019 applied EPF on insect pests in the greenhouse as agents for biological control and study the toxicity of serial concentrations of fungal spore suspension of both *Metarhizium anisopliae* and *Paceilomyces lilicanus* against the newly hatched larvae of *P. gossypiella* and *E insulana*. Results showed that the toxicity of *P. lilicanus* was higher on *P. gossypiella* treatment; whereas the toxicity of *M. anisopliae* was higher in case of *E. insulana*.

These results indicate that the entomopathogenic fungus is an effective natural pathogen of the cottonseed bug, *O. hyalinipennis*, diapaused larvae of *P. gossypiella* and larvae of *E. insulana* population in Gharbia governorate in okra pods and considered to be one of the promised mortality agents suppress the population size of the insects during quiescent period. The density independence of the fungus incidence indicates that the fungus exists in the habitat of the quiescent insects in okra pods by the continuous presence of its conidia resulting from the mycosed individuals at the beginning of the winter period.

The temperatures and relative humidity prevailing throughout the quiescent period permit successful infections of *B. bassiana* (Müller-Kõgler, 1965; El-Sufty and Boraei, 1987). The infections may be enhanced by the aggregations' behavior of the insects in the

dry okra pods and by high relative humidity characterized in the winter months.

The fungus seems to be widely distributed in the populations of insect pests of Egypt. This study added to previous works which throw light on the importance of entomopathogenic fungus, *B. bassiana* as a natural element against insects in their winter shelters. Accordingly, it encourages to use of this natural element as a biological control agent.

Conclusion

The Entomopathogenic fungus, *B. bassiana* (Bals.) Vuill. was isolated from three insect pest species in winter they were, *O. hyalinipennis, P. gossypiella* and *E. insulana*. The seasonal fungal incidence percentages were 3.04 and 10.73 %. in nymphs and adults of *O. hyalinipennis*, respectively in the first season. While they were 13.53 and 16.53 % in the second one. The seasonal fungal incidence percentages on *P. gossypiella* larvae were 4.64 and 7.96 % for the two seasons. Also, the natural mortality by the fungus ranged from 4.32-14.16 % and 4.32-20.63 % in the two seasons, respectively. Whilst the seasonal fungal incidence percentage on *E. insulana* larvae were 6.02 and 5.23 % for the first and second seasons and the fungus was isolated for one time in each season. Results explained and approved the role of entomopathogenic fungi as natural mortality factors against insect pest species in their winter quiescence in Egypt.

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ARABIC SUMMARY

دور الفطر الممرض للحشرات *Beauveria bassiana* (Bals.) Vuill. في خفض أعداد الآفات الحشرية اللاجئة لقرون الباميا الجافة أثناء فصل الشتاء

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تمت الدراسة الحالية خلال موسمي 2019-2020 و2020-2021 في ثلاث مراكز بمحافظة الغربية لتوضيح دور الفطريات الممرضة للحشرات في التقليل من أحجام عشائر الافات الحشرية المتواجدة في قرون الباميا الجافة اثناء فترة الشتاء. وجدت ثلاث آفات حشرية في قرون الباميا الجافة وهن: بق بذرة القطن، دودة اللوز القرنفلية ودودة اللوز الشوكية. تم عزل الفطر الممرض للحشرات *B. bassiana* من الافات الحشرية الثلاثة خلال أشهر الشتاء. بلغت نسبة التواجد السنوية للفطر 3.04 و10.73 % في حوريات وبالغات بق بذرة القطن على التوالي في الموسم الاول. بينما كانت 13.53 و16.53 % في الموسم الثاني. وكانت نسبة تواجد الفطر السنوية ليرقات دودة اللوز القرنفلية 4.64 و7.96 % للموسمين. وتراوحت نسبة الموت الطبيعية بالفطر بين 4.32 -14.16 و4.32 – 20.63% للموسمين على التوالي. بينما كانت نسبة تواجد الفطر السنوية ليرقات دودة اللوز الشوكية 6.02 و5.23 % للموسم الاول والثاني وتم عزل الفطر لمرة واحدة في كل موسم. تظهر النتائج دور الفطريات الممرضة للحشرات كعوامل موت طبيعية ضد الحشرات في ملاجئها الشتوية في جمهورية مصر العربية. ومما تقدم يقترح تشجيع إستخدام هذه العوامل الطبيعية كعوامل مكافحة حيوية.