



AENSI Journals

Australian Journal of Basic and Applied Sciences

ISSN:1991-8178

Journal home page: www.ajbasweb.com



Virulence Bio-Assay Efficiency of *Beauveria bassiana* and *Metarhizium anisopliae* for the Biological Control of *Spodoptera exigua* Hübner (Lepidoptera: Noctuidae) Eggs and the 1st Instar Larvae.

Wafa A. Al-Kherb

Department of Biology; College of Arts and Sciences in Buraydah, Qassim University, Saudi Arabia

ARTICLE INFO

Article history:

Received 20 January 2014

Received in revised form 10

March 2014

Accepted 16 March 2014

Available online 5 April 2014

Keywords:

Biological control- Beet armyworm-
Spodoptera exigua- Eggs- 1st instar
 larvae – Bioassay- Biocontrol-
 Entomopathogenic fungi - *Beauveria*
bassiana - *Metarhizium anisopliae*

ABSTRACT

(Background) The beet armyworm, *Spodoptera exigua* Hübner (Lepidoptera: Noctuidae), is a serious and persistent agricultural pest worldwide. *S. exigua* is a highly harmful, polyphagous insect that can attack that attacks over 90 plant species in at least 18 families, many of which are crop plants include vegetable, field and flower crops, such as cabbage, pepper, tomato, lettuce, celery, strawberry, eggplant, sugar beet, alfalfa, cotton. **(Objective)** So, this study is aims to determine the efficiency of two entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae* against eggs and 1st instar larvae of *S. exigua*. As well as, discusses the strategies for manipulation of these entomopathogenic fungi as potential microbial tool to reduce the insecticide resistance in sustainable IPM systems. **(Results)** The current research was carried out to study the capabilities of two entomopathogenic fungi, *B. bassiana* and *M. anisopliae*, in controlling eggs and the 1st instar larvae of the beet armyworm, *Spodoptera exigua* Hub. The potential for using the *B. bassiana* and *M. anisopliae* to control *S. exigua* eggs and 1st instar larvae has been established in the laboratory. Eggs and 1st instar larvae of *S. exigua* were treated with different concentrations of conidia of two of entomopathogenic fungi belonging to two species, *B. bassiana* and *M. anisopliae* under laboratory conditions. Three weeks after treatment, spore concentration 10×10^5 spores/ml resulted in the highest mortality (50 and 37.6%) with eggs and (100 and 88%) with 1st instar larvae when treated by *B. bassiana* and *M. anisopliae*, respectively. The lowest mortality (33.6 and 25.6% of eggs) and (73.6 and 57.2% of 1st instar larvae) occurred when *S. exigua* eggs were treated with *B. bassiana* and *M. anisopliae*, respectively. Mortality values resulted from the experiments indicated that *B. bassiana* showed superiority over *M. anisopliae* regarding its effect on controlling eggs and the 1st instar larvae of the beet armyworm. In addition, 4×10^5 spores/ml showed the least virulent effect among the two fungi used in the bioassay. **(Conclusion)** Our findings suggest that the moderate (8×10^5) and the higher (10×10^5) spore concentration/ml of *B. bassiana* and *M. anisopliae* are potent entomopathogens and could be developed into bio-control agents against the beet armyworm, *S. exigua* in IPM programs. Laboratory studies, however, frequently overestimate the level of control achieved by the candidate biological control agents in the glasshouses.

© 2014 AENSI Publisher All rights reserved.

To Cite This Article: Al-Kherb, Wafa A., Virulence Bio-assay efficiency of *Beauveria bassiana* and *Metarhizium anisopliae* for the biological control of *Spodoptera exigua* Hübner (Lepidoptera: Noctuidae) eggs and the 1st instar larvae. *Aust. J. Basic & Appl. Sci.*, 8(3): 313-323, 2014

INTRODUCTION

The beet armyworm, *Spodoptera exigua* Hübner (Lepidoptera: Noctuidae), is a serious and persistent agricultural pest worldwide (Zhou *et al.*, 2011; Saaed *et al.*, 2010). *S. exigua* is a polyphagous insect pest with a worldwide spread, and is considered a serious pest of vegetables, field, and flower crops (Suenaga and Tanaka, 1997; Grennborg *et al.*, 2001; Saaed *et al.*, 2010). This insect is a migrant insect (Mitchell 1979) which takes on long distance migration in Asia, Europe, and North America (Feng *et al.* 2003). The worsening pest problem of the pest on various crops in many regions of the world is at least partially attributed to its strong migratory capacity (Showler, 2001; Han *et al.* 2005).

The beet armyworm is a cosmopolitan species that attacks over 90 plant species in at least 18 families, many of which are crop plants (Pearson, 1982; Grennborg *et al.*, 2001; Showler, 2001; Saaed *et al.*, 2010). This insect pest has no diapauses and can overwinter in the pupal stage such as in California (Zheng *et al.*, 2013), and in the larval stage in Japan (Suenaga and Tanaka, 2000). Some considered outbreak populations are still vague due to the lack of understanding of ecological and biological information about the pest (Feng *et al.* 1985) while

Corresponding Author: Wafa A. Al-Kherb, Qassim University Department of Biology; College of Arts and Sciences in Buraydah, Saudi Arabia.
 Ph: (00966-504280102) E-mail address: w-a-k29@hotmail.com

others insisted that they originated from the local overwintering population (Zheng *et al.*, 2013). Chemical insecticides have been complicated, by its propensity, to develop insecticide resistance with *S. exigua* (Cobb and Bass 1975, Brewer *et al.* 1990; Lui and Su, 2011; Zhou *et al.*, 2011; Tong *et al.*, 2013). Mostly, an outbreak of *S. exigua* and other insect pests are often associated with multiple early-season treatments by broad-spectrum insecticides, especially organophosphates.

Recently, Scientists and agriculturist move to adopt more environmentally benign practices. There is a resurgence of interest to search for biologically compatible forms of pest control options to abate resistance to insecticides in insect pests of crops. In all pest control programs where insecticides are to be used, insecticides management must be paramount importance to: (i) preserve ecological balance, (ii) minimize adverse effects on non-target organisms, and (iii) ensure human safety and health (Deedat, 1994). In this context, the use of microbial agents would be an ideal option for reducing insecticide induced resistance in insects. The need to find environmentally safe insecticides as well as bio-pesticides to combat *S. exigua* has spurred increased interest in alternative insecticides such as utilization of entomopathogenic fungi and bacteria which are currently evaluated for insecticidal efficacy (Pendland *et al.*, 1995). Currently, *Beauveria bassiana* (Balsamo-Crivelli) and *Metarhizium anisopliae* (Metschnikoff) Sorokin are two of the most notable entomopathogenic fungi used widely against insect pests and being the most common species developed as bio-pesticides (Butt *et al.* 2001; Zimmerman 2007a, b). *Beauveria bassiana* (Balsamo) Vuillemin and *M. anisopliae* (Metschnikoff) (Deuteromycotina, Hyphomycetes) are the most common fungal parasite of Arthropods. This ubiquitous entomopathogenic fungus attacks various host-arthropods by causing acute mycoses. These fungi can spread fast among hosts horizontally via aerially produced conidia and infects its host by penetration of the cuticle by its germ hyphae. The fungi grow within the internal fluids of insect integument, sponging degraded proteins and fat bodies, and produces toxins which kill the host. After a host death, the mycelium grows throughout the cadaver and protrudes outside completing the life cycle by rich conidial sporulation (Hajek and St Leger, 1994). Many strains of *B. bassiana* has been isolated and tested against different pests in various cropping systems (Feng *et al.*, 1985 Leland *et al.*, 2005). Recently, selected strains of *B. bassiana* and *M. anisopliae* have been successfully licensed for commercial use against whiteflies, aphids, thrips, and numerous other insect pests [Shah and Pell, 2003]. These fungi, which are regularly observed in natural populations of arthropods in particular with insects [Abdel Rahman *et al.*, 2010], can be effective against larvae when applied to plant foliage (Abdel Rahman *et al.*, 2010; Zaki, and Abdel-Raheem, 2010).

Because of the problems associated with the use of chemical pesticides, safer and more effective methods of controlling insect pests are essential for continued agricultural production. While biological control agents are a reasonable alternative to chemical pesticides, none have been identified as being commercially feasible for controlling lepidopterous pests by direct activity against the egg stage. *B. bassiana* and *M. anisopliae*, for example, are biological control agents designed specifically as an insecticide for control of certain caterpillars (Lepidoptera) (Zaki, and Abdel-Raheem, 2010). The active phase of fungi are spores attacking insect cuticle and/or as a stomach poison and must be eaten by the larvae to be effective. Larvae of lepidopterous insects must, therefore, be actively feeding on treated exposed plant parts (Freed *et al.*, 2012). Recently the developments of bio-insecticides have been directed towards specific insects. No bio-insecticide has, however, been heretofore identified as having ovicidal activity against *S. exigua* eggs (Vega *et al.* 2008). So, the objectives of this study were to determine virulence effects of two entomopathogenic fungi namely; *B. bassiana* and *M. anisopliae* against eggs and 1st instar larvae of *S. exigua*. Additionally, the current study discusses strategies for manipulation of certain important fungal entomopathogens as potential microbial tool to reduce the insecticide resistance management program in sustainable pest management systems.

MATERIALS AND METHODS

A) Rearing technique of *Spodoptera exigua*:

Culture of the beet armyworm, *S. exigua* Hübner was initiated from freshly field collected larvae at Qassim region. Larvae were reared under laboratory condition (27±2 oC, and 60±5 % RH) until emergence adult stage, and then adults allowed mating and getting new egg masses. Egg-masses of *S. exigua* were placed in a cylindrical glass jars (1 lb.) with small pieces of lettuce leaf (*Lactuca sativa* L.) and covered with muslin cloth held with a rubber band. Once eggs were hatched, the newly hatched larvae were transferred into larger rearing jars (4lb.) using a fine hair camel brush. In order to absorb any surplus moisture, a filter paper was provided at the bottom of the jar. Larvae were reared on a fresh lettuce leaf which was provided daily until pupation. As getting close to the end of the 6th instar larvae, moist sawdust was placed at the base of the rearing jars to provide a pupation sites. The formed pupae were eventually collected and placed in clean jars until adult emergence. The newly emerged moths were sexed and kept in pairs in clean jars (1 lb.). Each rearing jar was provided with 10% honey solution soaked in cotton fiber, which was tied with wire for moth feeding. Honey solution was renewed daily to avoid any fermentation and growth of microorganisms. Fresh green leaves of lettuce were introduced daily into clean jars (4 lb.) as oviposition sites of mated adults. The newly laid egg-

masses were collected daily and transferred into the rearing jars (5 egg-masses/jars). All rearing jars were placed under laboratory conditions of 26 ± 2 °C and R.H. 60 ± 5 %.

B) Pathogen (fungi) source:

Entomopathogenic fungi, *Beauveria bassiana* and *M. anisopliae* were obtained from Plant Production and Protection Dept., Fac. Of Agric. and Medicine Veterinary, Qassim University, KSA. These fungi were naturally isolated from soils and decayed insects.

The spores of *B. bassiana* and *M. anisopliae* were routinely grown on potato dextrose agar (PDA) media and incubated at 25 ± 2 °C till the fungi growth developed dense sporulations within 14 to 15 days of incubation. The spores of each fungus were scraped with a spatula and kept in sterile water containing 0.05% of Tween 80. The desired spore concentration was prepared to use a haemocytometer. Hundred microlitre of each spore concentration (4×10^5 , 6×10^5 , 8×10^5 and 10×10^5) was applied topically on the eggs and 1st instar larvae of *S. exigua* using a micro pipette.

C) Treatments:

1. Spray treatment:

The bioassay method was conducted according to Goettel and Johnson (1992). Eggs and 1st instar larvae of *S. exigua* were sprayed using a method of spray application which involved a direct application of conidia (water-based suspension). Each egg group was received 1 ml of fungus suspension with fractional doses (4×10^5 , 6×10^5 , 8×10^5 and 10×10^5 spores/ml/ replicate) respectively. The control was treated with 1 ml of distilled water. Sets of 50 neonate larvae (newly hatched larvae\12 h old) were exposed to four fungal concentrations (4×10^5 , 6×10^5 , 8×10^5 and 10×10^5 spores/ml/ replicate) of each fungus separated as well as to a water-treated control, and five replicates were performed for each concentration and control.

2. Mortality evaluation:

Number of dead eggs and larvae were observed and recorded daily, and the observations were continuously carried out to distinguish between dead eggs and larvae as a result of the fungal infection, as well as, the egg hatchability which escaped from the fungal infection. Replicates and treatments were scored for mortality 3 days after exposure, and the surviving larvae were also monitoring and left for 21 days or until pupation (one larva/ Petri-dish).

Any morphological abnormalities in inoculated eggs and larvae were detected. Microscopic observations and the growth of bio-pesticides on eggs and larvae were confirmed. Dead eggs were removed and surface sterilized according to the method described by Lacey *et al.*, (2007). Dead eggs and larvae were soaked in 75% ethanol solution for few seconds and rinsed in a plenty of sterile distilled water. Abnormal eggs and larvae were then placed into 0.5% sodium hypochlorite for two minutes, followed by two rinses with sterile distilled water then left to dry for 48h. Abnormal eggs and larvae were incubated in a Petri-dish with moist cotton under sterile conditions inside the clean desiccators at room temperature to examine whether they died because of fungus infection or not.

D) Data analysis:

All experiments were repeated 3-4 times. Each treatment included five replicates. The data were recorded, tabulated and subjected to statistical analysis. Treatment means, standard deviations (SDs), and significant differences were analyzed using Costat software (1993) test program. The significance of the main effects was determined by ONE-way analysis of variance (ANOVA). The Significant differences between treatments were determined using Tukey's multiple range tests ($P \leq 0.01$, 0.05). Percent mortality was calculated according to Abbott (1925):

$$\text{Abbott's corrected mortality} = \frac{\% \text{mortality in treatment} - \% \text{mortality in control}}{100 - \% \text{mortality in control}} \times 100$$

Results:

1- *Spodoptera exigua* eggs:

a. Efficacy of *Beuveria bassiana* against *Spodoptera exigua* eggs:

The chronic pathogenicities (mortality within 21 days) associated with four spore concentrations of *B. bassiana* applied against *S. exigua* eggs were assessed *in vivo*. The laboratory studies revealed that the four spore concentrations of the fungus *B. bassiana* were pathogenic to *S. exigua* eggs (Table 1). Although, there were no initial killings (mortality % = 0) among the four spore concentrations, however, their activities showed a moderate effect against *S. exigua* eggs (Table 1, Fig. 1).

Twenty one days of treatment, the use of a low fungal concentration (4×10^5 spores/ml), resulted in lower mortality rates compared to other concentrations after (16.8 ± 1.14 eggs of 50 eggs; which formed 33.6% of the total) (F value = 123.8 with 6/28 df; and $P \geq 0.0001$). With 6×10^5 spores/ml, the accumulative mortality reached

19.6±0.25 eggs of 50 one used, which formed 39.2% (F value = 520.93 with 6/28 df; and $P \geq 0.0001$). Additionally, use of 8×10^5 spores/ml caused accumulative mortality equal 22.4±0.25 eggs of the total (50 eggs), which formed 44.8% (F value = 514.30 with 6/28 df; and $P \geq 0.0001$). On the other hand, the higher fungal concentration (10×10^5 spores/ml) induced 50% mortality within *S. exigua* eggs (25.0±0.63 eggs, N=50; F value = 363.68 with 6/28 df; and $P \geq 0.0001$) (Table1).

Also, it could be concluded from these data that, as in the case of *S. exigua* eggs, the mortality rates were extremely low in the beginning of the treatment and then increased gradually, causing the highest mortality rates among the treated eggs, after 15-21 days of treatment (Fig. 1).

This study revealed that the pathogenic efficiency of *B. bassiana* varies significantly in terms of the initial kill, days after treatment, residual activity, total activity and spore concentrations.

Table 1: Mortality of *S. exigua* eggs after treatment by four spore concentrations of *Beauveria bassiana* under lab conditions in vitro.

spore Concentrations/ml	N	Initial Kill (0 Time)	Mortality average ±SE/replicate (Days after treatment)						Residual Activity % (RA)	Total Activity % (TA)	Statistical analysis	
			3	5	7	10	15	21			F Value	P > F
4 x 10 ⁵	50	0 f	0.8±0.37 Ef	1.8±0.37 e	5.6±0.51 d	10.8±0.66 c	14.6±0.68 b	16.8±1.14 a	16.68	8.35	123.8 with 6/28 DF	<0.0001
6 x 10 ⁵	50	0 f	1.4±0.40 E	2.2±0.37 e	6.6±0.25 d	11.4±0.25 c	16.8±0.58 b	19.6±0.25 a	19.33	9.67	520.93 with 6/28 DF	<0.0001
8 x 10 ⁵	50	0 g	2.2±0.58 f	3.6±0.51 e	8.8±0.2 d	13.8±0.37 c	17.8±0.37 b	22.4±0.25 a	22.87	11.43	514.30 with 6/28 DF	<0.0001
10 x 10 ⁵	50	0 g	3.4±0.68 f	5.2±0.68 e	11.4±0.51 d	17.4±0.51 c	21.6±0.25 b	25.0±0.63 a	28.00	14.00	363.68 with 6/28 DF	<0.0001

*Averages followed by same letter with in a row are not significantly different from each other at 5% (Duncan)

**Averages followed by same letter with in a column are not significantly different from each other at 5% (Duncan)

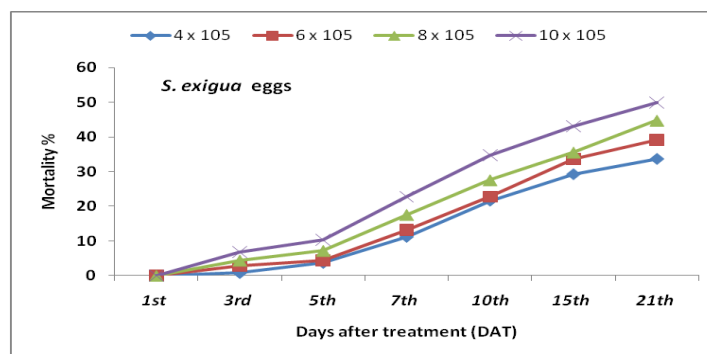


Fig. 1: Daily mortality percentage of *S. exigua* eggs followed treatment by four spore concentrations of *Beauveria bassiana* under lab conditions in vitro.

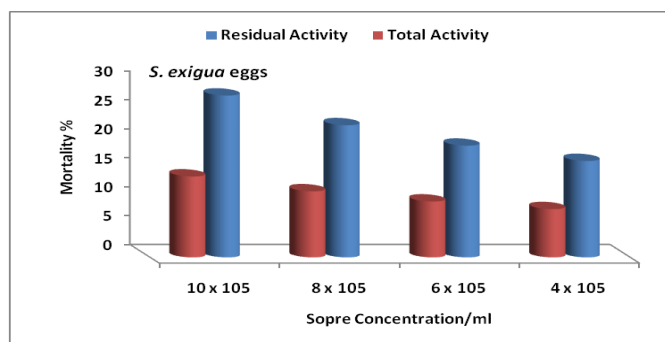


Fig. 2: Pathogenicity of *Beauveria bassiana* against *S. exigua* eggs followed treatment by four spore concentrations of under lab conditions in vitro.

b. Efficacy of *Metarhizium anisopliae* against *Spodoptera exigua* eggs:

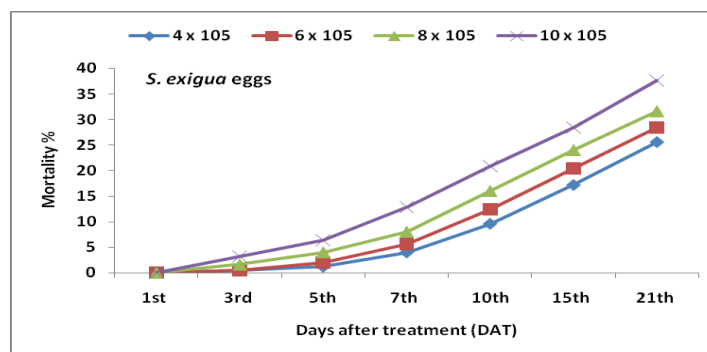
In vivo, mortality rates within 21 days associated with four spore concentrations of *M. anisopliae* applied against *S. exigua* eggs were evaluated. The application of *M. anisopliae* against *S. exigua* eggs on all four spore concentrations used within the laboratory resulted on 25.6 to 36.4% mortality of eggs compared to the control, which achieved significant mortalities of *S. exigua*. Data showed that *M. anisopliae* were pathogenic to *S. exigua* eggs (Table 2) with the four spore concentrations applied. In spite of, the initial killings of the fungus were zero (mortality % = 0) among the four spore concentrations, their activities ranged from lower mortality % to a moderate effect against *S. exigua* eggs (Table 2, Fig. 3).

Table 2: Mortality of *S. exigua* eggs after treatment by four spore concentrations of *Metarhizium anisopliae* under lab conditions.

spore Concentrations/ml	N	Initial Kill (0 Time)	Mortality average \pm SE/replicate (Days after treatment)						Residual Activity % (RA)	Total Activity % (TA)	Statistical analysis	
			3	5	7	10	15	21			F Value	P > F
4 x 10 ⁵	50	0 e	0.2 \pm 0.02 e	0.6 \pm 0.25 e	2.0 \pm 0.32 d	4.8 \pm 0.38 c	8.6 \pm 0.51 b	12.8 \pm 0.80 a	9.7	4.83	136.52 with 6/28 DF	<0.0001
6 x 10 ⁵	50	0 f	0.2 \pm 0.02 f	1.0 \pm 0.00 e	2.8 \pm 0.20 d	6.2 \pm 0.38 c	10.2 \pm 0.20 b	14.2 \pm 0.38 a	11.43	5.77	530.77 with 6/28 DF	<0.0001
8 x 10 ⁵	50	0 f	0.8 \pm 0.20 f	2.0 \pm 0.45 e	4.0 \pm 0.45 d	8.0 \pm 0.32 c	12.0 \pm 0.32 b	15.8 \pm 0.37 a	14.2	7.1	327.61 with 6/28 DF	<0.0001
10 x 10 ⁵	50	0 g	1.6 \pm 0.25 f	3.2 \pm 0.37 e	6.4 \pm 0.25 d	10.4 \pm 0.25 c	14.2 \pm 0.37 b	18.8 \pm 0.37 a	18.2	9.1	566.07 with 6/28 DF	<0.0001

*Averages followed by same letter with in a row are not significantly different from each other at 5% (Duncan)

**Averages followed by same letter with in a column are not significantly different from each other at 5% (Duncan)

**Fig. 3:** Daily mortality percentage of *S. exigua* eggs followed treatment by four spore concentrations of *Metarhizium anisopliae* under lab conditions *in vitro*.

Low fungal concentration (4x10⁵ spores/ml) caused lower mortality rates compared to other concentrations after 21 days of treatment (12.8 \pm 0.80 eggs of 50 eggs; which formed 25.6% of the total) (F = 136.52 with 6/28 df; and P \geq 0.0001). As increasing the fungal concentrations to 6x10⁵ spores/ml, the accumulative mortality was increased to 14.2 \pm 0.38 eggs of 50 eggs, which represents 28.4% (F value = 530.77 with 6/28 df; and P \geq 0.0001). Use of 8x10⁵ spores/ml induced slight increases in accumulative mortality which reached 15.8 \pm 0.32 eggs of 50 eggs, accounting for 31.6% (F value = 327.61 with 6/28 df; and P \geq 0.0001). The higher fungal concentration (10x10⁵ spores/ml) induced 37.6% mortality (18.8 \pm 0.37 eggs, N=50; F value = 566.07 with 6/28 df; and P \geq 0.0001) (Table 2).

In general, it can be concluded that *M. anisopliae* induced a lowest mortality among *S. exigua* eggs in the beginning of the study, and then increased gradually with the prolonging the exposure period, causing varied mortality rates, after 15-21 days of treatment (Fig. 3). Moreover, our data brought out that the four spore concentrations used against *S. exigua* eggs varies significantly in terms of the initial kill, days after treatment, residual activity, total activity and the fungal concentration used (table 3).

Table 3: Mortality of *S. exigua* 1st instar larvae after treatment by four spore concentrations of *Beauveria bassiana* under lab conditions, *in vitro*.

spore Concentrations/ml	N	Initial Kill (0 Time)	Mortality average \pm SE/replicate (Days after treatment)						Residual Activity % (RA)	Total Activity % (TA)	Statistical analysis	
			3	5	7	10	15	21			F Value	P > F
4 x 10 ⁵	50	3.6 \pm 0.45	7.4 \pm 1.72 e	11.8 \pm 2.62 e	20.2 \pm 1.53 d	23.6 \pm 1.72 c	31.2 \pm 1.02 b	36.8 \pm 0.80 a	43.67	25.43	60.28 with 6/28 DF	<0.0001
6 x 10 ⁵	50	4.8 \pm 0.67	11.8 \pm 0.92 ef	18.4 \pm 1.44 e	24.0 \pm 0.71 d	30.8 \pm 1.02 c	34.6 \pm 0.51 b	40.2 \pm 0.66 a	53.33	31.27	202.438 with 6/28 DF	<0.0001
8 x 10 ⁵	50	9.0 \pm 0.45	16.4 \pm 0.75 f	25.2 \pm 0.47 e	33.2 \pm 0.86 d	41.2 \pm 1.02 c	45.4 \pm 1.23 b	49.0 \pm 0.55 a	70.17	44.09	305.072 with 6/28 DF	<0.0001
10 x 10 ⁵	50	12.0 \pm 0.65	23.6 \pm 0.93 h	32.2 \pm 0.66 e	39.0 \pm 0.71 d	47.8 \pm 0.86 c	50.0 \pm 0.0 b	50.0 \pm 0.0 a	80.8	52.4	527.242 with 6/28 DF	<0.0001

*Averages followed by same letter with in a row are not significantly different from each other at 5% (Duncan)

**Averages followed by same letter with in a column are not significantly different from each other at 5% (Duncan)

2- *Spodoptera exigua* 1st instar larvae:

a. Efficacy of *Beauveria bassiana* against the 1st instar larvae of *S. exigua*:

In vivo, the mortality among the 1st instar larvae that treated by *B. bassiana* (four spore concentrations) were assessed. Data showed that the efficiency of *B. bassiana* was more virulence to *S. exigua* 1st instar larvae (Table 3) comparing with eggs. One day after treatment (initial killings), the fungus showed an effect on 1st instar larvae (mortality % ranged from 7.2 to 24.0) among various spore concentrations. Meanwhile, the fungal activities among the four concentrations showed highly significance effects against *S. exigua* 1st instar larvae (Table 3, Fig. 5).

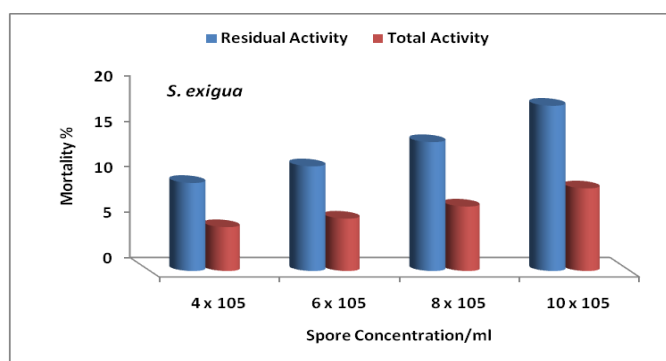


Fig. 4: Pathogenicity of *Metarhizium anisopliae* against *S. exigua* eggs followed treatment by four spore concentrations of under lab conditions *in vitro*.

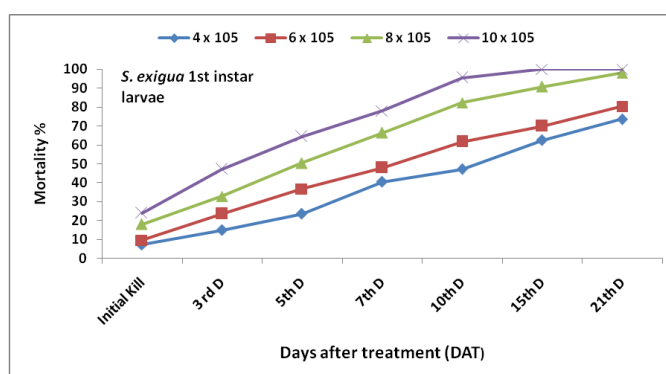


Fig. 5: Daily mortality percentage of *S. exigua* 1st instar larvae after treatment by four spore concentrations of *Beauveria bassiana* under lab conditions *in vitro*.

Twenty one days after application, the dose of 4×10^5 spores/ml caused lower mortality rates in comparison of other concentrations (36.8 ± 0.80 eggs of 50 eggs; which formed 73.4% of the total) (F-value is 60.283 with 6/28 df; and $P \geq 0.0001$). With 6×10^5 spores/ml, the accumulative mortality reached 40.2 ± 0.66 eggs of 50 eggs, and this represents 80.4% of the total (F-value is 202.44 with 6/28 df; and $P \geq 0.0001$). In the same context, use of 8×10^5 spores/ml caused accumulative mortality equal 45.4 ± 0.55 eggs of 50 eggs, which represents 90.8% of the total (F value = 305.07 with 6/28 df; and $P \geq 0.0001$). On the other hand, application of the higher fungal concentration (10×10^5 spores/ml) induced 100% mortality within 15 days of treatment (N=50; F-value is 527.24 with 6/28 df; and $P \geq 0.0001$) (Table3).

It could be concluded that the 1st instar larvae of *S. exigua* were more susceptible to *B. bassania* than eggs and the mortality rates were varied based on the fungal concentrations which was low in the beginning and then increased gradually causing the highest mortality rates among the treated larvae, after 15-21 days of treatment (Fig. 5).

As shown in the table (3) the mortalities among the 1st instar larvae, by the four spore concentrations of *B. bassania*, were varied significantly in terms of the initial kill, days after treatment, residual activity and total activity.

b. Efficacy of *M. anisopliae* against *S. exigua* 1st instar larvae:

M. anisopliae caused various mortality rates among the 1st instar larvae of *S. exigua*. All spore concentrations used of *M. anisopliae* were pathogenic to the 1st instar larvae (Table 4). The initial kill varied based on the fungal concentrations which ranged from 4 to 15.6% and the fungal activity showed an ability of the fungus to cause various mortality percentages among 1st instar larvae of *S. exigua* (Table 4, Fig. 7).

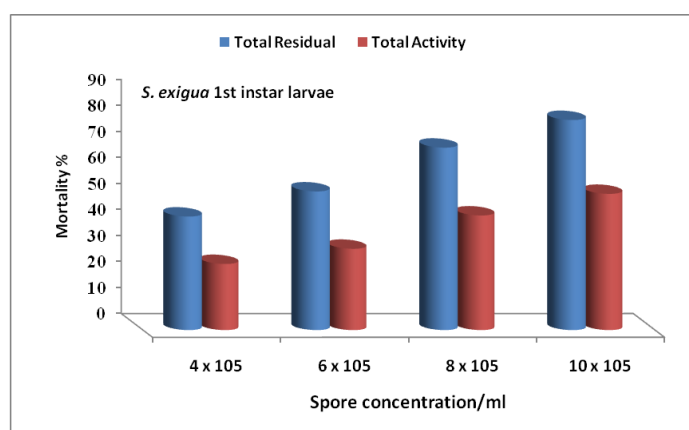
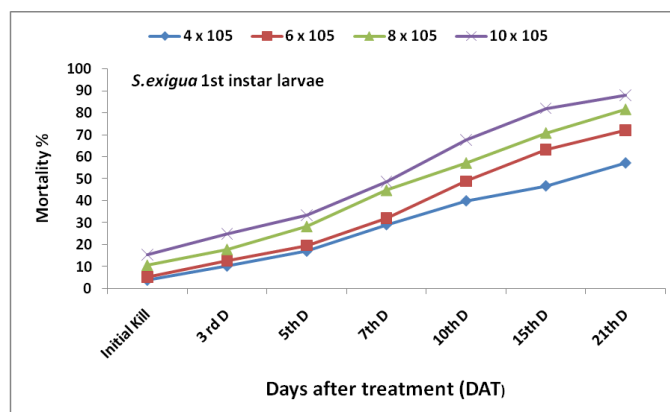
The fungal concentrations caused 57.2% (28.6 ± 0.51 of 50 eggs), 72% (36.0 ± 0.71 of 50 eggs), 81.6% (40.8 ± 0.66 of 50 eggs) and 88% (44.0 ± 0.51 of 50 eggs) mortalities among the 1st instar larvae of *S. exigua* for 4×10^5 , 6×10^5 , 8×10^5 and 10×10^5 spores/ml, respectively, after twenty days of treatment. F-values are 345.12, 332.83, 177.24 and 233.23 with 6/28 df and $P > F = < 0.0001$ for 4×10^5 , 6×10^5 , 8×10^5 and 10×10^5 spores/ml, respectively (Table 4).

Table 4: Mortality of *S. exigua* 1st instar after treatment by *Metarhizium anisopliae* under lab conditions.

spore Concentra tions/ ml	N	Initial Kill (0 Time)	Mortality average \pm SE/replicate (Days after treatment)						Residual Activity % (RA)	Total Activity % (TA)	Statistical analysis	
			3	5	7	10	15	21			F Value	P > F
4 x 10 ⁵	50	2.0 \pm 0.45	5.2 \pm 0.49 e	8.6 \pm 0.40 E	14.6 \pm 0.68 d	20.0 \pm 0.55 c	23.4 \pm 0.60 b	28.6 \pm 0.51 a	33.5	18.75	345.12 with 6/28 DF	<0.0001
6 x 10 ⁵	50	2.6 \pm 0.25	6.4 \pm 0.40 ef	9.8 \pm 0.37	16.0 \pm 0.55 d	24.4 \pm 1.20 c	31.6 \pm 0.93 b	36.0 \pm 0.71 a	41.33	23.27	332.83 with 6/28 DF	<0.0001
8 x 10 ⁵	50	5.4 \pm 0.81	9.0 \pm 0.55 f	14.2 \pm 0.49 E	22.4 \pm 1.69 d	28.6 \pm 1.44 c	35.4 \pm 0.75 b	40.8 \pm 0.66 a	50.17	50.17	177.240 with 6/28 DF	<0.0001
10 x 10 ⁵	50	7.8 \pm 0.58	12.6 \pm 0.6 8 h	16.8 \pm 0.58 e	24.2 \pm 1.03 d	33.8 \pm 0.80 c	41.0 \pm 1.65 b	44.0 \pm 0.71 a	57.5	57.50	233.231 with 6/28 DF	<0.0001

*Averages followed by same letter with in a row are not significantly different from each other at 5% (Duncan)

**Averages followed by same letter with in a column are not significantly different from each other at 5% (Duncan)

**Fig. 6:** Pathogenicity of *Beauveria bassiana* against of *S. exigua* 1st instar larvae after treatment by four spore concentrations of under lab conditions *in vitro*.**Fig. 7:** Daily mortality percentage of *S. exigua* 1st instar larvae after treatment by four spore concentrations of *Metarhizium anisopliae* under lab conditions *in vitro*.

Moreover, data showed that *M. anisopliae* has an ability to induce more virulence to *S. exigua* larvae than eggs, and the mortality rates were varied based on the fungal concentrations which was low in the beginning and then increased gradually causing the highest mortality rates among the treated larvae, after 15-21 days of treatment (Fig. 7).

As shown in the table (4) the mortalities among the 1st instar larvae, by the four spore concentrations of *M. anisopliae*, were varied significantly in terms of the initial kill, days after treatment, residual activity and total activity.

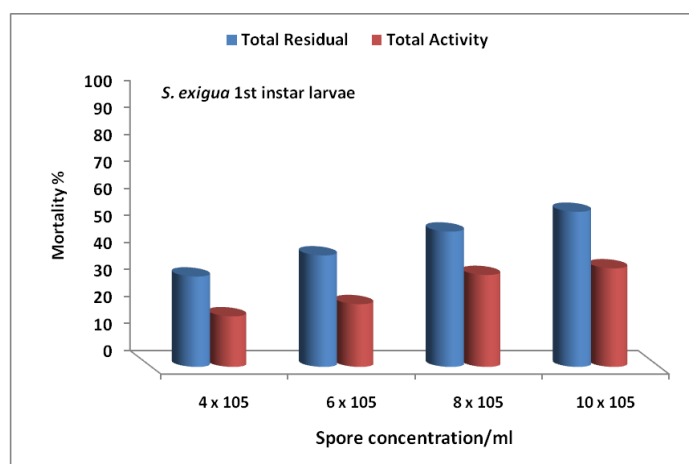


Fig. 8: Pathogenicity of *Metarhizium anisopliae* against *S. exigua* 1st instar larvae treatment by four spore concentrations of under lab conditions *in vitro*.

Discussion:

One of the practical means of increasing crop production is to minimize the pest-associated losses and damage. Therefore, the actual losses have been estimated and varied according to the insect species, types of crops and date of infestation (Sharma *et al.*, 2001). Without pesticides, an estimated two-thirds, the production of all crops would be lost, depriving millions of people from their daily foods (Deedat, 1994). As a consequence of heavy reliance on insecticides, a number of ecological problems including development of insecticide resistance in most of insect pests.

Today, certain of entomopathogenic fungi are important as bio-control agent and/or as natural regulators of pest populations and have potential as myco-insecticide agents against diverse insect pests in agro-ecosystem (Abdel-Baky, *et al.*, 1998). These fungi infect their hosts by penetrating through the cuticle, gaining access to the hemolymph, producing toxins, and grow by utilizing nutrients present in the haemocoel to avoid insect immune responses (Hajek and St. Leger, 1994). Consequently, these entomopathogenic fungi may be applied in the form of conidia or mycelium which sporulates after application (Abdel-Baky and Abdel-Salam, 2003).

Data in tables 1 and 3 confirmed the results that got by Studdert and Kaya (1990), who found that more soybean caterpillars, *S. exigua* readily infected by *B. bassiana* when exposed to drier soils compared with wetter soils containing residues of insecticides. This also explain that the success of *B. bassiana* and *M. anisopliae* (tables 1, 2, 3 and 4) depends on conidial viability and number of spores/ml (Bidochka and Hajek, 1998; Olivera and Neves, 2004), where the beginning of epizootics is conditioned to the capacity of these structures to germinate on the host. Moreover, the results obtained in this study shows that, although the initial killing was zero, the epizootics occurred within 15 days and reached their maximum levels after 3 weeks of treatment (tables 1, 2, 3 and 4).

B. bassiana was more infective at any spore concentration than *M. anisopliae* and resulted in maximum average percent mortality among eggs and 1st instar larvae of *S. exigua* under investigation. Based on earlier reports, we presume that the pathogenic potential of *B. bassiana* and *M. anisopliae* could be due to either a cumulative effect of the following factors: (a) the presence of adhesions on the surface of conidia for attachment to the cuticle of insects (Wang and Leger 2007, Anand *et al.*, 2009), (b) one or more virulence factors such as chitinases, Pr1 and Pr2 proteases, etc. (Freimoser *et al.* 2003; Anand *et al.*, 2009) and (c) the presence of collagenous protective coat which enables fungi to overcome the innate immunity of insects when the fungus comes into contact with hemolymph (Wang and Leger 2006; Anand *et al.*, 2009; Freed *et al.*, 2012).

These findings are in line with the finding of Yoon *et al.* (1999); Sabbour and Sahab (2005) and Freed *et al.* (2012) who reported that both of *B. bassiana* and *M. anisopliae* can be used as microbial control agent against *S. exigua* eggs and larvae in greenhouses and in field conditions. According to the findings of laboratory bioassay, we suggest that the four fungal concentrations infected eggs and the 1st instar larvae of *S. exigua* in a dose-dependent manner. It is clear from the data that the use of different concentrations of both entomopathogenic fungi resulted in different mortality depending on the pathogenic species, fungal concentrations used and the target stages of insect pests (Figs 1-8, and tables 1-4). This is in agreement with Balasubramaniyam and Sundaresan, (2009), who's found that *B. bassiana* at different concentrations of 10³, 10⁴, 10⁵, 10⁶, 10⁷ and 10⁸ mL⁻¹ showed insecticidal activity of 5-33% on *H. hampei*. Jayanthi and Padmavathamma (2001) studied the individual and combined larvicidal activity of *B. bassiana* with other pathogenic agents' against *S. litura*. They noticed that the maximum larvicidal activity of 46.67% was observed with *B. bassiana* and also their combination had 70% in NPV+ *B. bassiana*, NPV+ *B. thuringiensis* showed 90% and *B. bassiana* + *B.*

thuringiensis had 80% larval mortality. On the other hand, *B. bassiana* and *Nomuraea rileyi* showed more than 90% larval mortality against *H. armigera* (Malarvannan *et al.*, 2003). Hung and Bouicas (1992) reported that *B. bassiana* reduced the population of *S. exigua*.

From these results, it could be concluded that *B. bassiana* and *M. anisopliae* contains some virulence characters that can make them suitable to be effective bio-control agent against *S. exigua*. However, more work is needed to be addressed for determine the efficiency and the characters of those fungi against other life stages of *S. exigua* in both greenhouses and open field strains.

This study also showed that the 1st larval stage was more sensitive to entomopathogenic infection than eggs which showed resistance to the entomopathogenic infection. These results are consistent with the study of Tanada and Kaya (1993) which mentioned that during the life cycle of an insect, the larvae and adults are more susceptible to attack by entomopathogenic those eggs and pupae. However, there are reports indicating *M. anisopliae* as a potential pathogen of insect eggs, such as undecimpunctata Diabrotica (Coleoptera: Chrysomelidae) and Blissus antillus (Hemiptera: Lygaeidae) (Tallamy *et al.*, 1998; Coracini and Samuels, 2002). Rupert and Kellner (2002) noted that the susceptibility of eggs to pathogen attack is because they are immobile, and nutritional resources supplied to neonate larvae until hatching, are also a good substrate for microorganisms.

However, the use of *B. bassiana* and *M. anisopliae* to control eggs of *T. absolute* had not been reported, probably because the control was achieved by the integration of other agencies such as egg parasitoids of the genus *Trichogramma* or bacteria larvicides such as *B. thuringiensis* (Theoduloz *et al.*, 1997). The interaction between *B. bassiana* and *M. anisopliae* and their potential hosts of insects are not less complex since their entomopathogenicity had apparently evolved independently in many major taxa of fungi. This may explain the variation in degree of specificity and total or partial reliance on the presence of an appropriate host or its suitable stages for their survival. In any case, once encountering a suitable host, an intricate relationship commences between the insect and the fungus, beginning with invasion, then evasion, culminating in proliferation and dispersion of conidia to new potential hosts. Such convergent evolution may also be the reason that evasion from host defense by the mode of avoiding recognition as “non-self,” a strategy employed by many entomopathogenic fungi, is based on variable characteristics amongst the various species. Finally, this sequence of events may be affected, directly or indirectly, by an array of biotic and abiotic factors, including the insect's host plant.

This study indicates the possibility of using *B. bassiana* and *M. anisopliae* to: (i) a major reduction in pesticidal usage; (ii) reduced exposure of non-target organisms to pesticides; (iii) increased activities of natural enemies; (iv) reduced amounts of pesticide residues in food; and (v) a safer environment to live.

REFERENCES

- Abbott, W.S., 1925. A method of computing the effectiveness of an insecticide. J. Econ. Entomol., 18: 265-267. <http://www.bcin.ca/Interface/openbcin.cgi?submit=submit&Chinkey=99430>.
- Abdel Rahman, K.M., M. Barta and L. Cag  n, 2010. Effects of combining *Beauveria bassiana* and *Nosema pyrausta* on the mortality of *Ostrinia nubilalis*. Cent. Eur. J. Biol., 5(4): 472-480. DOI: 10.2478/s11535-010-0035-z
- Abdel-Baky, N.F. and A.H. Abdel-Salam, 2003. Natural incidence of *Cladosporium* spp. as a bio-control agent against whiteflies and aphids in Egypt. J. Appl. Entomol., 127(4): 228-235. <http://www.springerlink.com/index/D723R98630741172.pdf>
- Abdel-Baky, N.F., N.S. Arafat and A.H. Abdel-Salam, 1998. Three *Cladosporium* spp. as promising biological control candidates for controlling whiteflies (*Bemisia* spp.) in Egypt. Pakistan J. Biological Sci., 1(3): 188-195. <http://www.ansijournals.com/pjbs/1998/188-195.pdf>.
- Anand, A., B. Prasad and B.T. Tiwary, 2009. Relative susceptibility of *Spodoptera litura* pupae to selected entomopathogenic fungi. Bio. Control, 54: 85-92. DOI 10.1007/s10526-008-9157-x.
- Baskar, K., G. Antony Raj, P. Murali Mohan, S. Lingathurai, T. Ambrose and C. Muthu, 2012. Larvicidal and Growth Inhibitory Activities of Entomopathogenic Fungus, *Beauveria bassiana* against Asian Army Worm, *Spodoptera litura* Fab. (Lepidoptera: Noctuidae). Journal of Entomology, 9: 155-162. DOI: 10.3923/je.2012.155.162
- Bidochka, M.J. and A.E. Hajeck, 1998. A nonpermissive entomophthoralean fungal infection increases activation of insect prophenoloxidase. Journal of Invertebrate Pathology, 72: 231-238.
- Brewer, M.J., J.T. Trumble, B. Alvarado-Rodrigues and W.E. Chaney, 1990. Beet armyworm (Lepidoptera: Noctuidae) adult and larval susceptibility to three insecticides in managed habitats and relationship to laboratory selection for resistance. J. Econ. Entomol., 83: 2136-2146.
- Butt, T.M., C.W. Jackson and N. Magan, 2001. Fungi as Biocontrol Agents: Progress, Problems and Potential. CABI Publishing, Wallingford, Oxon, United Kingdom. <http://bookshop.cabi.org/Uploads/Books/PDF/9780851993560/9780851993560.pdf>

Cobb, P.P. and M.H. Bass, 1975. Beet armyworm: dosage mortality studies on California and Florida strains. *J. Econ. Entomol.* 68: 813-814.

Coracini, D. and R. Samuels, 2002. Natural enemies of the Chinch Bug, *Blissus antillus* Leonard (Hemiptera: Lygaeidae: Blissinae), pasture pest in Rio de Janeiro State, Brazil. *Neotropical Entomol.*, 31: 165-167. DOI: 10.2478/s11535-010-0035-z.

Deedat, Y.D., 1994. Problems associated with the use of pesticides: An overview. *Insect Science and its Application*, 15(3): 247-251. DOI: 10.1303/jjaez 41.17.

Feng, H.Q., K.M. Wu, D.F. Cheng and Y.Y. Guo, 2003. Radar observations of the autumn migration of the beet armyworm *Spodoptera exigua* (Lepidoptera: Noctuidae) and other moths in northern China. *Bull. Entomol. Res.*, 93: 115-124.

Feng, Z., R.I. Carruthers, D.W. Roberts, D.S. Robson, 1985. Age-specific dose-mortality effects of *Beauveria bassiana* (Deuteromycotina: Hyphomycetes) on the European corn borer, *Ostrinia nubilalis* (Lepidoptera: Pyralidae), *J. Invertebr. Pathol.*, 46: 259-264.

Freed, S., M.A. Saleem, M.B. Khan and M. Naeem, 2012. Prevalence and Effectiveness of *Metarhizium anisopliae* Against *Spodoptera exigua* (Lepidoptera: Noctuidae) in Southern Punjab, Pakistan. *Pakistan Journal of Zoology*, 44(3): 753.

Freimoser, F.M., S. Screen, S. Bagga, G. Hu and S. Leger, 2003. Expressed sequence tag (EST) analysis of two subspecies of *Metarhizium anisopliae* reveals a plethora of secreted proteins with potential activity in insect hosts. *Microbiology*, 149: 239-247.

Goettel, M.S. and D.L. Johnson, 1992. Environmental Impact and Safety of Fungal Biocontrol Agents. In: *Biological Control of Locusts and Grasshoppers*, Lomer, C.J. and C. Prior (Eds.). CAB International, Wallingford, USA., pp: 356-361.

Grennburg, S.M., T.W. Sappington, J.R. Legaspi, B.C. Liu and M. Setamou, 2001. Feeding and Life History of *Spodoptera exigua* (Lepidoptera: Noctuidae) on Different Host Plants. *Ann. Entomol. Soc. Am.*, 94(4): 566-575.

Hajeck, A.E. and St. R. Leger, 1994. Interactions between fungal pathogens and insect hosts. *Annu. Rev. Entomol.*, 39: 293-322. DOI: 10.1146/annurev.en.39.010194.001453.

Han, Q., B.S. Hansson and S. Anton, 2005. Interactions of mechanical stimuli and sex pheromone information in antennal lobe neurons of a male moth, *Spodoptera littoralis*. *J. Comp. Physiol.*, A191: 521-528. DOI 10.1007/s00359-005-0618-8.

http://oar.icrisat.org/2096/1/Genetic_transformation_of_crop_plants.pdf.

Hung, S.Y. and D.G. Boucias, 1992. Influence of *Beauveria bassiana* of the cellular defense response of the beet armyworm *Spodoptera exigua*. *J. Invert. Pathol.*, 60: 152-158.

Jayanthi, P.D.K. and K. Padmavathamma, 2001. Joint action of microbial and chemical insecticides on *Spodoptera litura* (Fab.) (Lepidoptera: Noctuidae). *J. Trop. Agric.*, 39: 142-144.

Lacey, L.A., S.P. Arthurs, A.L. Kinght and J. Huber, 2007. Microbial control of lepidopteran pests of apple orchards. IN: (Lacey, L. A. and Kaya, H.K.; eds.: *Field manual of techniques in Invertabrate pathology*), pp: 527-546. DOI: 1007/978-1-4020-5933-9_25.

Lai, T. and J. Su, 2011. Effects of chlorantraniliprole on development and reproduction of beet armyworm, *Spodoptera exigua* (Hübner). *Journal of Pest Science*, 84(3): 381-386. DOI: 10.1007/s10340-011-0366-1.

Lee, W.W., T.Y. Shin, S.H. Ko, J.B. Choi, S.M. Bae, S.D. Woo, 2012. Entomopathogenic Fungus *Nomuraea rileyi* for the Microbial Control of *Spodoptera exigua* (Lepidoptera: Noctuidae). *The Korean Journal of Microbiology*, 48(4): 284-292. DOI: 10.7845/kjm.2012.044.

Leland, J.E., M.R. McGuire, J.A. Grace, S.T. Jaronski, M. Ulloa and Y.H. Park, 2005. Strain selection of a fungal entomopathogen, *Beauveria bassiana*, for control of plant bugs (*Lygus* spp.) (Heteroptera: Miridae). *Biol. Control.*, 35: 104-114.

Mitchell, E.R., 1979. Monitoring adult populations of the fall armyworm (Lepidoptera: Noctuidae). *Florida Entomol.*, 62: 91-98.

Olivera, R.C. and P.M.O.J. Neves, 2004. Biological control compatibility of *Beauveria bassiana* with acaricides. *Neotropical Entomology*, 33: 353-358.

Pearson, A.C., 1982. Biology, population dynamics, and pest status of the beet armyworm (*Spodoptera exigua*) in the Imperial Valley of California. Ph.D. dissertation, University of California, Riverside. *Proc Natl Acad Sci USA*, 103: 6647-6652.

Pendland, J.C., S. Hung and D. Boucias, 1995. *In vivo* development of the entomogeneous hyphomycete *Paecilomyces farinosus* in host *Spodoptera exigua* (beet armyworm) larvae. *Mycopathologia*, 130(3): 151-158.

Rupert, L. and L. Kellner, 2002. The role of microorganisms for eggs and progeny, pp: 149-164. In M. Hilker and T. Meiners (eds.). *Chemoecology of insect eggs and egg deposition*. Blackwell Publishing Ltd., Berlin, Germany.

Saeed, S., A.H. Sayyed and I. Ahmad, 2010. Effect of host plants on life-history traits of *Spodoptera exigua* (Lepidoptera: Noctuidae). *Journal of Pest Science*, 83(2): 165-172. DOI: 10.1007/s10340-009-0283-8.

Shah, P.A. and J.K. Pell, 2003. Entomopathogenic fungi as biological control agents. Appl. Microbiol. Biotech., 61: 413-423. DOI: 10.1007/s00253-003-1240-8

Sharma, H.C., K.K. Sharma, N. Seetharama and R. Ortiz, 2001. Genetic transformation of crop plants: Risk and opportunities for the rural poor. Current Science, 80(12): 1495-1508.

Showler, A.T., 2001. *Spodoptera exigua* Oviposition and Larval Feeding Preferences for Pigweed, *Amaranthus hybridus*, over Squaring Cotton, *Gossypium hirsutum*, and a Comparison of Free Amino Acids in Each Host Plant. Journal of Chemical Ecology, 27(10): 2013-2028. DOI: 10.1023/A:1012238803311.

Studdert, J.P. and H.K. Kaya, 1990. Water potential, temperature, and clay coating of *Beauveria bassiana* conidia: Effect on *Spodoptera exigua* pupal mortality in two soil types. Journal Invertebrate Pathology, 56: 327-336. DOI:10.1016/0022-2011(90)90119-Q.

Suenaga, H. and A. Tanaka, 1997. Occurrence of beet armyworm, *Spodoptera exigua* (Hubner) (Lepidoptera: Noctuidae) on young growing stage of garden pea, *Pisum sativum* L. Jp. J. Appl. Entomol. Zool., 41: 17-25.

Tanada, Y. and H. Kaya, 1993. Fungal Infections. p. 318-366. In Y. Tanada and H. Kaya (eds.). Insect pathology. Academic Press, Inc., San Diego, California, USA.

Theoduloz, C., P. Roman, J. Bravo, C. Padilla, C. Vasquez, L. Meza Zepeda and L. Meza Basso, 1997. Relative toxicity of native Chilean *Bacillus thuringiensis* strains against *Scrobipalpus absolutus* (Lepidoptera: Gelechiidae). J. Appl. Microbiol., 82: 462-468. DOI: 10.1046/j.1365-2672.1997.00137.x

Tong, H., Q. Su, X. Zhou and L. Bai, 2013. Field resistance of *Spodoptera litura* (Lepidoptera: Noctuidae) to organophosphates, pyrethroids, carbamates and four newer chemistry insecticides in Hunan, China. J. Pest. Sci., 86: 599-609. DOI 10.1007/s10340-013-0505-y.

Vega, F.E., F. Posada, M.C. Aime, M. Pava-Ripoll, F. Infante, M.C. Rehner, 2008. Entomopathogenic fungal endophytes. Biological Control, 46: 72-82. doi:10.1016/j.biocontrol.2008.01.008.

Wang, C. and S. Leger, 2007. The MAD1 adhesin of *Metarhizium anisopliae* links adhesion with blastospore production and virulence to insects; the MAD2 adhesin enables attachment to plants. Eukaryot Cell, 6: 808-816. doi: 10.1128/EC.00409-06.

Wang, C. and S. Leger, 2006. A collagenous protective coat enables *Metarhizium anisopliae* to evade insect immune responses. PNAS, 103(17): 6647-6652. doi: 10.1073/pnas.0601951103.

Zaki, F.N., M.A. Abdel-Raheem, 2010. Use of entomopathogenic fungi and insecticide against some insect pests attacking peanuts and sugarbeet in Egypt. Archives of Phytopathology and Plant Protection, 43(18): 1819-1828(10). <http://dx.doi.org/10.1080/03235400902830838>.

Zheng, X., P. Wang, C. Lei, W. Lu, Z. Xian and X. Wang, 2013. Effect of soil moisture on overwintering pupae in *Spodoptera exigua* (Lepidoptera: Noctuidae). Applied Entomology and Zoology, 48(3): 365-371. DOI: 10.1007/s13355-013-0196-0.

Zhou, C., Y. Liu, W. Yu, Z. Deng, M. Gao, F. Liu and W. Mu, 2011. Resistance of *Spodoptera exigua* to ten insecticides in Shandong, China. Phytoparasitica, 39(4): 315-324. DOI: 10.1007/s12600-011-0157-5.

Zimmerman, G., 2007a. Review on safety of the entomopathogenic fungi *Beauveria bassiana* and *Beauveria brongniartii*. Biocontrol Sci. Tech., 17: 553-596. DOI:10.1080/09583150701309006

Zimmerman, G., 2007b. Review on safety of the entomopathogenic fungus *Metarhizium anisopliae*. Biocontrol Sci. Tech., 17: 879-920. DOI: <http://dx.doi.org/10.1080/09583150701593963>.