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A Laboratory Study on the Efficacy of *Metarhizium anisopliae* (Metsch.) Sorokin and *Beauveria bassiana* (Bals.) Vuill Against *Spodoptera exigua* (Hb.) (Lepidoptera: Noctuidae)

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ABSTRACT

The efficiency of the entomopathogenic fungi, *Metarhizium anisopliae* (Metsch.) Sorokin and *Beauveria bassiana* (Bals.) Vuill against 2nd instar larvae of *Spodoptera exigua* (Hb.) was investigated under laboratory conditions of 27º ±1ºC and 65% ±10 % R.H. Both of the tested entomopathogenic fungi proved to be effective on larvae of *S. exigua*, the mortality percentage increasing with increase in spore concentration. Results revealed marked changes in the duration of the larval and pupal stages and in the different biological aspects in general. There was a decrease in percentage of pupation and adult emergence in addition to appearance of deformities.

Key words: *Beauveria bassiana*, Bioassay, Entomopathogenic fungi, Lesser cotton leafworm, *Metarhizium anisopliae*, *Spodoptera exigua*.

INTRODUCTION

The lesser cotton leafworm *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae) is a polyphagous insect pest affecting various economically important crops (Agrawal *et al*., 2002). Its importance as a pest is due to its wide host range. It is considered a major pest of cotton causing serious damage to the cotton plant both in quality and in yield.

The chemical control of *Spodoptera exigua* as in the case for other pests, causes environmental pollution. In addition, chemical control programs against this pest have been complicated by its propensity to develop insecticide resistance (Brewer *et al.*, 1990).

Safer methods for control are the use of entomopathogenic organisms as biological control agents. Entomopathogenic fungi are considered successful control agents against several pests (Dent, 2000). They have been shown to have activity against a range of insect pests and thus used as biocontrol agents **(**Gillespie and Claydon, 1988). *Beauveria bassiana* (Bals.) Vuill and *Metarhizium anisopliae* (Metsch.) Sorokin are two species of entomopathogenic fungi, belonging to the Hyphomycetes group, that are natural inhabitants of soil, where they are found infecting a wide range of insect species. They are also found in agricultural crops as epizooties on defoliator lepidopteran larval populations (Toledo *et al.*, 2006).

The present study investigates the effectiveness of the two entomopathogenic fungi *Metarhizium anisopliae* (Metsch.) Sorokin and *Beauveria bassiana* (Bals.) Vuill as biological control agents with the aim of controlling this pest.

MATERIALS AND METHODS

Rearing technique

Larvae of *Spodoptera exigua* were collected from Kaha region Qualubia Governorate, Egypt. Larvae were kept in glass jars with castor bean leaves, *Ricinus communis* as a source of feed. Third or fourth instar larvae were reared separately to avoid cannibalism and infection. Pupae were placed in glass jars until adult emergence. Couples of female and male moths were kept in glass jars covered with muslin and provided with strips of paper for oviposition. Cotton pads soaked with 10% honey solution were provided on a daily bases. Eggs were collected and kept in clean glass jars till hatching.

Isolation and cultivation of entomopathogenic fungi

Metarhizium anisopliae and *Beauveria bassiana* were isolated from dead and abnormal larvae of Family Noctuidae related in features to *S. exigua* (Fargues *et al*. 1997). The technique used for isolation from dead and abnormal larvae was according to Campbell and Roberts (1971). Hyphal tips were taken from the surface of the body of infected larvae by a sterile loop and streaked on plates containing Dox agar medium for *M. anisopliae* (Patel *et al.*, 1990) and Sabouraud agar medium for *B. bassiana* (El-zoghby, 2003). The Petri dishes were incubated for 2 weeks at 25°C and 50-60% RH (Nada, 1999).

Purification of entomopathogenic fungi

To purify the strains of *M. anisopliae* and *B. bassiana*, a loop of pure fungal colony was cultured in conical flasks containing 50 ml of Dox medium in case of *M. anisopliae*, and Sabouraud medium for *B. bassiana*. The cultures were incubated for 2 weeks at 25°C and 50-60% RH.

Spore count

The conidia were harvested from the growing culture medium by scraping with a sterile solution of 0.01% Tween 80. A suspension of solution was made to determine the concentration using a hemocytometer ($6.32x10^8$ conidia/ml for *M. anisopliae* and $1.6x10^8$ conidia/ml for *B. bassiana*).

Preparation of the spore suspension concentration

Five concentrations of the fungal spores of each fungus were prepared. *M. anisopliae* was prepared at

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$6.32x10^2$ - $6.32x10^4$ - $6.32x10^6$- $6.32x10^8$ - $6.32x10^{10}$ conidia/ml. *B. bassiana* was prepared at $1.6x10^2$- $1.6x10^4$ - $1.6x10^6$ - $1.6x10^8$ - $1.6x10^{10}$ conidia/ml by using sterile solution of 0.01% Tween 80 and distilled water.

Pathogenicity tests

Castor bean leaves were treated with each concentration of fungal spore suspension. Dipping technique was adopted. Three replicates were prepared for each concentration each containing ten 2nd instar larvae. Mortality was recorded after 48h. Surviving larvae were fed on fresh *castor bean leaves* and data were recorded daily till pupation. The control was sprayed with 0.01% Tween 80 in distilled water.

Calculation of the lethal concentration values

Lethal concentration values were calculated as log/probit regression lines according to Finney (1971). The computer program Sigma Plots for Windows (version 2) and the slope of the probit line were determined.

Statistical analysis

All data obtained from the above experiments were analyzed statistically using complete randomized blocks design. Student t-test as statistical analysis of the obtained data was used (COSTAT program, for Windows).

RESULTS AND DISCUSSION

Evaluation of the efficacy of *Metarhizium anisopliae* against *Spodoptera exigua*

laboratory bioassay

The results of accumulative percentage mortalities for 2nd instar larvae of *S. exigua* treated with the different concentrations of *Metarhizium anisopliae* appear in table (1). Results show a gradual increase with increase in the time elapsed after treatment, ranging between 26.91-74.95% after 9 days post treatment.

Table (1): Effect of *M. anisopliae* on the accumulated mortalities of *S. exigua.*

Conc. (spore/ml)	Mortality % (days post treatment)					Total mortality%
	1st	3rd	5th	7th	9th	
$6.32 x10^2$	3.46	6.79	10.25	23.58	26.91	26.91
$6.32 x10^4$	6.9	22.67	26.13	26.13	36.13	36.13
$6.32 x10^6$	13.8	23.8	34.15	47.48	54.15	54.15
$6.32 x10^8$	20.69	37.36	54.6	61.27	61.27	61.27
$6.32 x10^{10}$	27.59	27.59	48.28	74.95	74.95	74.95

For studying the toxicity of *Metarhizium anisopliae* an aqueous suspension was used. Mortalities were found to increase in values with increase in the concentration of *M. anisopliae*. Data in table (1) show the mortality percentages which increased from 3.46 to 27.59% after 24 hours of treatment and from 6.79 to 27.59% after three days post treatment.

It ranged from 10.25 to 48.28% five days post treatment, 23.58 to 68.28% seven days post treatment and 26.91 to 74.95% nine days post treatment at the concentrations $6.32x10^2$, $6.32x10^4$, $6.32x10^6$, $6.32x10^8$, and $6.32x10^{10}$spore/ml, respectively.

Metarhizium anisopliae proved to be effective on larvae as depicted by the calculated LC values. The LC_{50} and LC_{90} values for 2nd instars were $1.86x10^5$ and $2.02x10^{12}$spore/ml, respectively (Table2). The slope values were 1.53 for 2nd instars larvae, proving the homogeneity of the tested individuals (Fig. 1).

The present studies show that increasing the concentrations applied to 2nd instar larvae of *S. exigua* gave an increase in the mortality percentage. Chienyan *et al.* (1998) stated that *Metarhizium anisopliae* var. *anisopliae* showed high virulence to *Spodoptera exigua*. They obtained 86.7% mortality for 3rd instar larvae after 24 hours of infection, and 95% mortality after 2 days. They recorded an LC_{50} of $1.41x10^5$ ppm, $7.07x10^4$ ppm, and 5.1 x104 ppm after 1 day and 3days of infection, re-

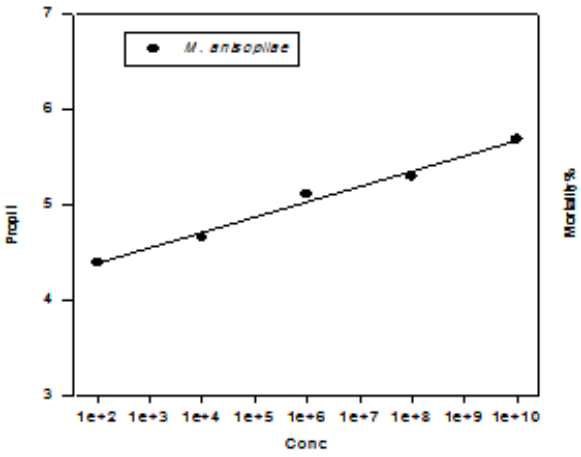

Figure (1): Toxicity regression lines for *M. anisopliae* against 2nd instar larvae of *S. exigua.*

spectively.

Effect of Metarhizium anisopliae on the biological aspects of Spodoptera exigua (Hb.)

Application of LC_{50} ($1.86x10^5$spore/ml) and LC_{90} ($2.02x10^{12}$ spore/ml) of *M. anisopliae*, resulted in a mean larval duration of 2nd instars of 8.00 and 8.67 days respectively (table 3). The mean pupal duration of 2nd instar of *S. exigua* treated with *M. anisopliae* was 13.67 and 14.00 days, for the LC_{50} and $LC_{90,}$ respectively

(table 3). The results show an increase in pupal duration when applying the LC_{50} and LC_{90} of *M. anisopliae*.

There was a marked decrease in the percentage of 2nd instar larvae of *S. exigua* which succeeded in reaching pupation (48.33% and 20% for LC_{50} and $LC_{90,}$ respectively) in comparison to the control group (93.33%) (table3).

Table (2): The LC_{50} and LC_{90} values of *M. anisopliae* against 2nd instars of *S.exigu*

	Value	95%(fiducial limit)		Slope
		Lower:	Upper:	
LC_{50}	$1.86x10^{5}$	$6.59x10^{5}$	$2.88x10^{6}$	1.53
LC_{90}	$2.02x10^{12}$	$1.09x10^{10}$	$1.1x10^{18}$	

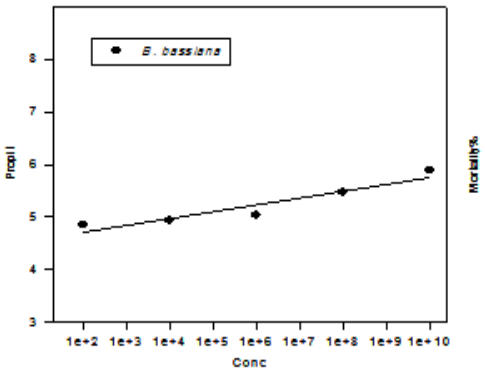

Figure (2): Toxicity regression lines for *B. bassiana* against 2nd instar larvae of *S. exigua.*

Table 3: Effect of LC_{50} andLC_{90} of *M. anisopliae* on larval duration, pupal duration, pupation %, adult emergence % and deformation % of *S. exigua.*

LC values	Larval duration (Days±S.E)	Pupal duration (Days±S.E)	Pupation%	Adult emergence%	Deformation%
LC_{50}	$8.00±0.58^{ns}$	$13.67±0.89^{ns}$	48.33	79.9	9.41
LC_{90}	$8.67±0.33^{ns}$	$14.00±0.58^{ns}$	20	50	4.17
Control	7.33±0.33	12.33±0.33	93.33	100	-

°Pupation % based on the number of treated larvae, •Deformation was larval-pupal deformation, ns**:** not significant,(student-t test).

It is clear from table (3) that the percentage of adult emergence after treatment of 2nd instar larvae of *S. exigua* with LC_{50} and LC_{90} of *M. anisopliae* was 79.9 and 50% respectively, while the percentage of adult emergence of untreated pupae was 100%. Treating 2nd instar larvae of *S. exigua* by *M. anisopliae* resulted in a relatively low pupal deformation percentage of 9.41and 4.17% respectively, while there were no deformities in case of the control group (table 3).

Filho *et al.* (2002) screened for the Pathogenicity of *Metarhizium anisopliae* and *Beauveria bassiana* isolates against *Alabama argillaceae* in Brazil at 27 ± 2°C, RH 70 ± 5%. They found that mortalities caused by the *M. anisopliae* isolates at the different concentrations ranged from 4.5 to 91.2%.

Evaluation of the efficacy of *Beauveria bassiana* (Bals.) against *Spodoptera exigua* Hb.

Laboratory bioassay

Accumulative mortalities of 2nd instars of *S. exigua* were calculated for treatment with the different concentrations of *B. bassiana*. The data in table (4) show a gradual increase with increasing the time elapsed after treatment, ranging between 43.92-81.27% 9 days post treatment.

Data indicate that the mortality percentage was 3.46 to 24.14% after 24 hours of treatment, 13.46 to 37.47% three days post treatment, 27.26 to 51.27% 5 days post treatment, 40.59 to 71.27% seven days post treatment and 43.92 to 81.27% nine days post treatment at the concentrations $6.32x10^{2}$, $6.32x10^{4}$, $6.32x10^{6}$, $6.32x10^{8}$, and $6.32x10^{10}$spore/ml respectively (table4).

B. bassiana proved to be effective on larvae of *S. exigua* which is evident from the calculated LC values. The LC_{50} and LC_{90} values for 2nd instar larvae were $2.26x10^{6}$and $3.22x10^{11}$spore/ml, respectively (Table5).

The slope values were 1.58 for 2nd instar larvae, proving the homogeneity of the tested individuals (Fig. 2). The results show an increase in mortality percentage with increase in spore concentration. Aly (2002) conducted laboratory bioassay on the effect of different concentrations of *Beauveria bassiana* on various stages of *Agrotis ipsilon.* The fungus proved virulent against eggs and larval stage of the insect. LC_{50} values were 2.01×10^{3} spores/ml for eggs and 4.74×10^{3} spores/ml for larvae. Filho *et al.* (2002) evaluated the pathogenicity of *Beauveria bassiana* isolates against *Alabama argillaceae* in Brazil at 27 ± 2°C, RH 70 ± 5%. The lowest lethal time obtained was 4.1 days for the isolate 483.

Effect of Beauveria bassiana on the biological aspects of Spodoptera exigua (Hb.)

Based on the LC_{50} concentration ($2.26x10^{6}$ spore/ml) and LC_{90} ($3.22x10^{11}$ spore/ml) of *B. bassiana*, gave a mean larval duration for 2nd instar larvae of 8.33 and 8.67 days, respectively (table 6).

Malarvannan *et al*., (2010) in a laboratory (2010) in a Laboratory evaluation of *Beauveria bassiana* against *Spodoptera litura*, obtained a larval duration of 7.3 days at a spore concentration of 2.4×10^6 compared to the untreated (7.9 days).

Table 4: Effect of *Beauveria bassiana* on the accumulated mortalities of *S. Exigua*

Conc. (spore/ml)	Mortality% (days post treatment)					Total mortality%
	1st	3rd	5th	7th	9th	
$6.32x10^2$	3.46	13.46	27.26	40.59	43.92	43.92
$6.32x10^4$	10.35	17.02	23.92	40.59	47.26	47.26
$6.32x10^6$	13.8	23.8	41.04	47.71	51.04	51.04
$6.32x10^8$	20.69	37.36	47.71	61.04	67.71	67.71
$6.32x10^{10}$	24.14	37.47	51.27	71.27	81.27	81.27

Table 5: The LC_{50} and LC_{90} values of *B. bassiana* against 2nd instars of *S.exigu.*

	value	95% (fiducial limit)		Slope
		Lower	Upper	
LC_{50}	$2.26x10^6$	$2.96x10^5$	$3.39x10^5$	1.58
LC_{90}	$3.22x10^{11}$	$2.46x10^9$	$8.1x10^{16}$	

Table 6: Effect of LC_{50} and LC_{90} of *B. bassiana* on larval duration, pupal duration, pupation %, adult emergence % and deformation % of *S. Exigua.*

LC Values	Larval duration (Days±S.E)	Pupal duration (Days±S.E)	Pupation%	Adult emergence%	Deformation%
LC_{50}	$8.33±0.67^{ns}$	$14±0.58^{ns}$	43.33	76.92	17.65
LC_{90}	$8.67±0.33^{ns}$	$13.33±0.33^{ns}$	13.33	50	15.39
Control	7.33±0.33	12.67±0.33	93.33	100	-

°Pupation % based on the number of treated larvae, •Deformation was larval-pupal deformation, ns: not significant, (student-t test).

Data in table (6) show the percentage of pupation of 2nd instar larvae of *S. exigua* treated with *B. bassiana*. The results were 43.33%, and 13.33% for LC_{50} ($2.26x10^6$spore/ml) and LC_{90} ($3.22x10^{11}$spore/ml), respectively, which is clearly lower than the control group (93.33%).

It is clear that the percentage of adult emergence after treatment of the 2nd instars of *S. exigua* with LC_{50} and LC_{90} of *B. bassiana* was 76.92% and 50%, respectively, while the percentage of adult emergence of untreated pupae in the control was 100% (table 6). Angel-Sahagún *et al*. (2005) found a reduction in adult emergence of *Haematobia irritans* when treating eggs and pupae with *Metarhizium anisopliae*, *Paecilomyces fumosoroseus* and *Beauveria bassiana*. Similarly, Sabbour (1996) obtained a progressive decrease in emergence of *Phthorimaea operculella* with increase in concentration of *B. bassiana*. Exposing the 2nd instar larvae of *S. exigua* to LC_{50} and LC_{90} concentration of *B. bassiana* resulted in a deformation percentage of 17.65% and 15.39% respectively, while no deformities appeared in the control group (table6). Hafez *et al*. (1997) similarly recorded adults exhibiting malformed characteristics when treating *Phthorimaea operculella* with *Beauveria bassiana.* In general, results reveal marked changes in the different biological aspects of *Spodoptera exigua* (Hb.) due to treatment of the 2nd instar larvae with *Metarhizium anisopliae* and *Beauveria bassiana*.

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دراسة معملية على كفاءة فطر *ميتارهيزيوم آنيسوبلايا* وفطر *بيوفاريا باسيانا* ضد دودة ورق القطن الصغرى

الملخص العربى

تمت دراسة كفاءة إستخدام الفطريات المسببة للأمراض الحشرية (*ميتارهيزيوم آنيسوبلايا* و *بيوفاريا باسيانا*) و التى تم عزلها من اليرقات المصابة بها على العمر اليرقى الثانى لدودة ورق القطن الصغرى تحت ظروف معملية (1º C ± 27 درجة مئوية و رطوبة نسبية 10% ± 65).

وقد أثبتت النتائج فاعلية الفطريات ضد الطور اليرقى حيث زادت نسبة الوفيات بزيادة التركيز. أظهرت النتائج ايضاً تغيرات فى طول الطور اليرقى و العذرى للحشرة وأيضاً بالنسبة للجوانب البيولوجية المختلفة بالمقارنة بالضابط. و قد قلت نسبة التعذر و نسبة خروج الطور اليافع من العذارى مع ظهور تشوهات.