

Laboratory bioassay of some entomopathogenic fungi on *Spodoptera littoralis* (Boisd.) and *Agrotis ipsilon* (Hufn.) larvae (Lepidoptera: Noctuidae).

El-Hawary, F.M and Abd El-Salam, A.M.E

Pests and Plant Protection Department, National Research Centre,
El-Tahrir St., Dokki, Cairo, Egypt

ABSTRACT

Efficacy of entomopathogenic fungi products (i.e. Bio- Power (*Beauveria bassiana*), Bio-Catch (*Lecanicillium lecanii*) and Priority (*Paecilomyces fumosoroseus*)) against *Spodoptera littoralis* and *Agrotis ipsilon* larvae were evaluated under laboratory conditions. Four different concentrations i.e. 0.125×10^9 , 0.25×10^9 , 0.5×10^9 and 1×10^9 spores/ 1000ml D.W of each formulation were used against each pest under investigation and compared with control insects. The results obtained show that Bio- Power was the most effective product followed by Bio-Catch and Priority against *S. littoralis* 3rd instar larvae, whereas, the LC_{50,90} values were 0.2×10^9 and 1.5×10^9 , 0.22×10^9 and 4.6×10^9 , 0.44×10^9 and 4.7×10^9 , respectively. While in case of *A. ipsilon*, Priority was the most potent product followed by Bio- Power and Bio-Catch, whereas, the LC_{50,90} values were 0.14×10^9 and 0.6×10^9 , 0.2×10^9 and 0.9×10^9 , 0.4×10^9 and 9.7×10^9 , respectively. Also, The results obtained show that percentage mortalities of treated third instar larvae of *S. littoralis* with Bio- Power, Bio-Catch and Priority were 87.5, 72.5 and 67.5 %, respectively, within 6.3, 8.2 and 7.4 days at the highest concentration used (1×10^9 spores). However, The treated third instar larvae of *A. ipsilon* with Priority, Bio- Power and Bio-Catch achieved 100, 90.0 and 62.5 % mortality, respectively, within 5.3, 7.1 and 3.9 days at concentration of 1×10^9 spores. The different formulations had delayed effects, the percentage of pupae and adults emerged were significantly decreased with an increase in concentrations.

Key words: *Spodoptera littoralis*; *Agrotis ipsilon*; Entomopathogenic fungi; *Beauveria bassiana*; *Lecanicillium lecanii*; *Paecilomyces fumosoroseus*.

INTRODUCTION

Over-reliance on broad-spectrum pesticides had come under severe criticism from different parts of the world after the publication of Silent spring in 1962 by Rachel Carson. Since then, an alternative ecofriendly strategy for the management of noxious insect pests has been searched to reduce harmful effects of chemical insecticides on humanity. Studies of biodiversity in agroecosystems and the delivery of ecosystem services to agricultural production have usually ignored the contribution of entomopathogens in the regulation of pest populations (Altieri, 1999; Gurr *et al.*, 2003; Tschamntke *et al.*, 2005). In recent years, crop protection based on biological

control of crop pests with microbial pathogens like virus, bacteria, fungi and nematodes has been recognized as a valuable tool in pest management (Bhattacharya *et al.*, 2003). The appropriate use of environment-friendly microbial pesticides can play a significant role in sustainable crop production by providing a stable pest management program. Among the fungi, several asexual fungi are associated with Arthropods, especially with insects. Entomopathogenic fungi that parasitize insects are valuable weapons for biocontrol and play an important role in promoting integrated pest management. To date, various strains of entomopathogenic

fungi such as *Lecanicillium* (previous name, *Verticillium*) sp. (Jackson *et al.*, 1985; Steenberg and Humber, 1999; Jung *et al.*, 2006), *Beauveria bassiana* (Quesada *et al.*, 2006), and *Paecilomyces* (Shia and Feng, 2004), have been used to control aphids, lepidopteran larva and other pests.

Therefore, for successful establishment of *B. bassiana*, *L. lecanii*, *P.*

MATERIALS AND METHODS

Commercial Formulations:

A number of commercial formulations of the entomopathogenic formulation were liquid products, Bio-Power (containing 1×10^9 *Beauveria bassiana* spores/ml), Bio-Catch (containing 1×10^9 *Lecanicillium lecanii* spores/ml) and Priority (containing 1×10^9 *Paecilomyces fumosoroseus* spores/ml) were obtained from T. Stanes Company limited, India.

Insects used

Newly moulted 3rd instar larvae of *Spodoptera littoralis* and *Agrotis ipsilon* were obtained from sensitive culture reared for several generations on castor leaves under laboratory conditions (27.0 ± 1.0 °C & $70.0 \pm 5.0\%$ RH).

Experiments:

Mortality Bioassay

Serial dilutions were prepared in 1000 ml distilled water for each formulation (0.125×10^9 , 0.25×10^9 , 0.5×10^9 , 1×10^9 spores / 1000ml D.W). Ten 3rd instar larvae for *S. littoralis* or *A. ipsilon* in a Petri dish (9cm diameter) lined with a filter paper were sprayed with 2.0 ml from each concentration per each formulation using a hand atomizer or hand sprayer (JSGW, Ambala, India). After air drying, the treated larvae were carefully transferred to individual sterile round plastic vials (4.5×12.0 cm²) containing fresh pieces of castor leaves (larva / vial). The vials had screw caps having provision for proper aeration. Larvae were maintained in an incubator at 27.0 ± 1.0 °C and adjusting the relative humidity to $70.0 \pm 5.0\%$ RH. Another group, 10 larvae sprayed with distilled water and reared under the above mentioned conditions, served as control. Mortality was counted daily and the lethal concentration of 50 and 90 % from treated larvae was calculated by Probit analysis

fumosoroseus and to reduce insecticidal treatment in IPM program, the role of these fungi is very important. The present study was conducted for evaluating some Entomopathogenic fungi products against *Spodoptera littoralis* and *Agrotis ipsilon* as polyphagous insect pests.

(Finney, 1971). Also, the mean number of days for larval mortality was calculated as shown below:

$$\text{Mean time to death (days)} = \frac{X_1y_1 + X_2y_2 + X_ny_n}{\text{Total } x \text{ mortality}}$$

where x is the number of larvae that mortality occur on a given day and y is the number of days from when the trial was initiated; 1, 2 and n are the first, second and last day of observation, respectively (Edde and Amatobi (2003). The percentage reduction in mortality of larvae were calculated and corrected according to Abbott's formula (1925). The experiment was replicated 4 times (10 larvae / replicate). Also, the percentage pupation and adult emergence were recorded for each formulation and concentrations used.

RESULTS AND DISCUSSION

Toxic effect of entomopathogenic fungi against *S. littoralis* and *A. ipsilon* 3rd instar larvae.

The results in Table (1) show that *S. littoralis* 3rd instar larvae that treated with Bio-Power were highly affected whereas, the fifty and ninety lethal concentrations values were 0.2×10^9 and 1.5×10^9 spores/1000ml. while Bio-Catch achieved 0.22×10^9 and 4.6×10^9 spores/1000ml, respectively for LC_{50,90} values. However, Priority was the least toxic products. On the other hand, *A. ipsilon* 3rd instar larvae that treated with Priority was highly affected where LC_{50,90} values were 0.14×10^9 , 0.6×10^9 , respectively. The other two products tested were less effective. Sabbour and Sahab, (2007) showed that the Lc₅₀s of the entomopathogenic fungi *Beauveria bassiana*, *Metarhizium anisopliae* and *Verticillium lecanii*, when applied to greasy cutworm, *A. ipsilon* were 137, 156, and 178 spores/ml, respectively.

Hassani *et al.*, (2000) found that 3rd instar larvae of *S. littoralis* treated with *P. fumosoroseus* spores at 1×10^8 achieved 88.5% mortality during 7.6 days.

Table (1): Lethal concentrations of some entomopathogenic fungi against *S. littoralis* and *A. ipsilon* larvae

Formulations	<i>S. littoralis</i>					<i>A. ipsilon</i>						
	Spores/1000ml D.W*				Slope	X ²	Spores/1000ml D.W				Slope	X ²
	LC ₅₀		Lower limit	Upper limit			LC ₅₀		Lower limit	Upper limit		
Bio-Power	LC ₅₀	0.2X10 ⁹	0.13X10 ⁹	0.3X10 ⁹	1.5	1.0	LC ₅₀	0.2X10 ⁹	0.15X10 ⁹	0.3X10 ⁹	2.0	0.4
	LC ₉₀	1.5X10 ⁹	0.9X10 ⁹	5.1X10 ⁹			LC ₉₀	0.9x10 ⁹	0.65X10 ⁹	1.8X10 ⁹		
Bio-Catch	LC ₅₀	0.22X10 ⁹	0.1X10 ⁹	0.38X10 ⁹	0.99	0.09	LC ₅₀	0.4X10 ⁹	0.3X10 ⁹	1.0X10 ⁹	0.9	0.2
	LC ₉₀	4.6X10 ⁹	1.6X10 ⁹	4.9X10 ⁹			LC ₉₀	9.7X10 ⁹	2.6X10 ⁹	20.0X10 ⁹		
Priority	LC ₅₀	0.44X10 ⁹	0.3X10 ⁹	0.7X10 ⁹	1.24	0.75	LC ₅₀	0.14X10 ⁹	0.1X10 ⁹	0.2X10 ⁹	2.01	1.5
	LC ₉₀	4.7X10 ⁹	1.9X10 ⁹	5.4X10 ⁹			LC ₉₀	0.6X10 ⁹	0.4X10 ⁹	1.1X10 ⁹		

*D.W= distilled water

Toxic effect and mean time to death.

Data in Table (2) indicate that the percentage mortality of the larvae increased with increasing concentrations of both pathogens however, the mean time to death decreased as the spores concentration increased. Also, Bio-Power was the most effective against *S. littoralis* 3rd instar larvae. While, in case of *A. ipsilon* 3rd instar larvae, Priority was the most effective. Bio-Power at high concentration (1.0×10^9) achieved 87.5 %

mortality for *S. littoralis* within 6.3 days while Priority at high concentration (1.0×10^9) achieved 100% mortality for *A. ipsilon* within 5.3 days. Tounou *et al.*, (2008) found that when *M. anisopliae* applied at 1×10^3 and 1×10^4 spores/*Schistocerca gregaria* nymph the Median survival time estimates for these treatments were 18.8 ± 0.6 and 12.3 ± 0.9 days, respectively. Mortality increased with increasing concentrations of both pathogens and with the time interval between the two treatments.

Table (2): Toxic effect of some Entomopathogenic fungi against *Spodoptera littoralis* and *Agrotis ipsilon* 3rd instar larvae.

Formulations	Conc. spores/1000ml D.W*	<i>Spodoptera littoralis</i>		<i>Agrotis ipsilon</i>	
		% mortality	Mean time to larval mortality (days) ±SE	% mortality	Mean time to larval mortality (days) ±SE
Bio-Power	0.125x10 ⁹	40.0	12.1±1.2	32.5	11.0±1.9
	0.25x10 ⁹	50.0	10.2±2.3	52.5	9.2±2.9
	0.5x10 ⁹	67.5	7.1±1.5	80.0	8.5±3.3
	1.0x10 ⁹	87.5	6.3±2.5	90.0	7.1±1.2
Bio-Catch	0.125x10 ⁹	37.5	15.1±1.6	27.5	7.9±1.6
	0.25x10 ⁹	52.5	13.2±2.15	42.5	6.1±1.7
	0.5x10 ⁹	62.5	11.5±2.64	50.0	5.4±2.5
	1.0x10 ⁹	72.5	8.2±2.7	62.5	3.9±2.1
Priority	0.125x10 ⁹	27.5	10.6±1.8	50.0	11.0±2.2
	0.25x10 ⁹	32.5	7.8±2.41	62.5	9.1±1.4
	0.5x10 ⁹	55.0	5.6±1.98	87.5	7.2±3.1
	1.0x10 ⁹	67.5	7.4±2.2	100	5.3±2.2

Quesada-Moraga and Vey, (2004) found that injection of

Bassiacridin (*B. bassiana* content) at a dose of 2.8 µg/g to fourth instar nymphs of the locusts *Locusta migratoria*, *Schistocerca gregaria* and *Doclostaurus maroccanus*, and to fifth instar larvae of the lepidopterans *Galleria mellonella* and *Spodoptera littoralis*, and of the coleopteran *Tenebrio molitor*. Bassiacridin was not toxic to *S. littoralis* and *T. molitor*,

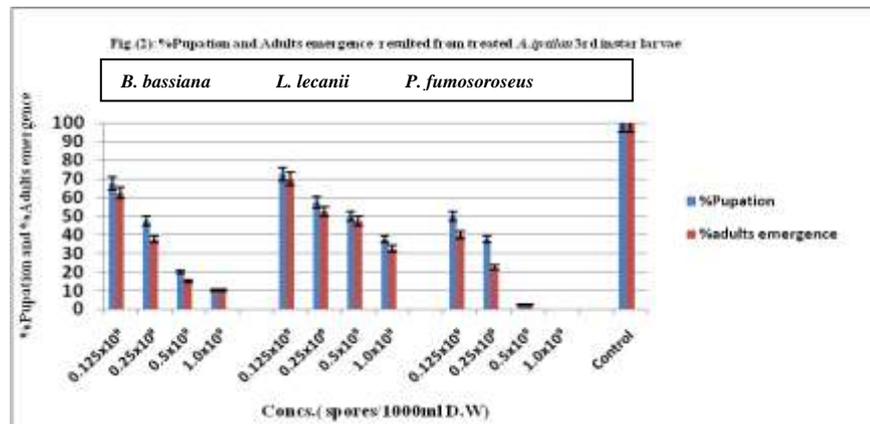
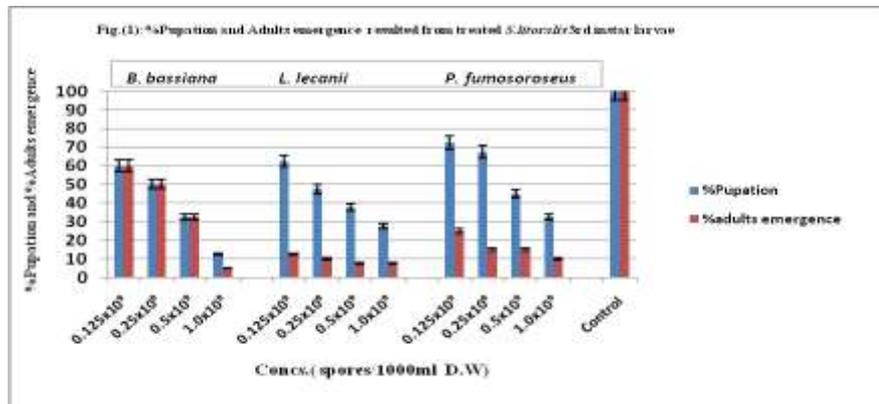
whereas it was slightly toxic to *G. mellonella* causing 16.6% corrected mortality. In contrast, it was equally toxic to the migratory locusts *L. migratoria* and to the desert locust *S. gregaria*, with mortality rates of 42.5 and 38.3% respectively, and slightly more toxic to the Moroccan locust *D. maroccanus*, causing 49.2% mortality. Hassani *et al.*, (2000) stated that *P. fumosoroseus* is highly virulent against these three important cotton pests (*Spodoptera littoralis*,

Helicoverpa armigera and *Aphis gossypii*).

Delayed effects of the entomopathogenic fungi on the resulted stages

The entomopathogenic fungi *Beauveria bassiana* (Bio-Power) had

delayed effect on the tested pests. The percentage pupation from treated *S. littoralis* larvae were 32.5 and 12.5% at concentration of 0.5×10^9 and 1.0×10^9 spores/1000ml.



Figs. (1&2): The percentage pupation and the adults emergence of *S. littoralis* and *A. ipsilon* 3rd instar larvae treated with entomopathogenic fungi products (Bio- Power (*Beauveria bassiana*), Bio-Catch (*Lecanicillium lecanii*) and Priority (*Paecilomyces fumosoroseus*)). Means (\pm SE).

The percentage adults obtained decreased to 5.0% at 1.0×10^9 spores/1000ml but there is no effect at 0.5×10^9 (32.5% pupae continued to adults Fig.(1). *S. littoralis* that treated with *L. lecanii* spores (Bio-Catch) at all concentrations used cause delaying effects, whereas, each survived larvae that pupated failed to emerge to adults i.e. at concentration of 1.0×10^9 , the percentage pupation was 27.5% decreased 7.5% adults emergence. Also, *P. fumosoroseus* spores (Priority) affected the percentage of adults emergence Fig. (1). In case of treated larvae of *A. ipsilon* with the fungi

of *P. fumosoroseus* (Priority), the percentage pupation decreased to 2.5% at concentration of 0.5×10^9 Fig. (2).

The results were in agreement with the findings of Altre and Vandenberg (2001) who found that *Paecilomyces fumosoroseus* penetrated the cuticle and proliferation in hemolymph of diamondback moth, *Plutella xylostella* and fall armyworm, *Spodoptera frugiperda* larvae within 22 h after inoculation at the concentration of 4×10^5 spores.

The percentage mortality was 91.0 and 54.0% of *Plutella xylostella* and *Spodoptera frugiperda*, respectively.

Hicks *et al.*, (2001) stated that the mean time to death decreased as the conidial *B. bassiana* dose increased when treated fifth instar larvae of *Panolis flammea*. The concentrations were 4.8×10^3 , 4.8×10^4 and 4.8×10^5 caused mortality within 11, 8 and 5 days, respectively. Shaw *et al.*, (2002) found that three isolates of *M. anisopliae*, one of *V. lecanii*, and one of *B. bassiana* killed 100% of *Varroa destructor* within 7 days at a conidial concentration of 1×10^8 ml/l. One isolate of *P. fumosoroseus* also killed 97% of *V. destructor* within 7 days at a conidial concentration of 1×10^8 ml/l. Smith *et al.*, (2006) found that 90.0% mortality of *Prostephanus truncatus* was observed after 1 week and 100% mortality after 2 weeks in maize treated with the highest dose of conidia of *Beauveria bassiana* with ash (0.2 g/g).

From the results obtained from the present studies it could be concluded that *Beauveria bassiana* (Bio-Power) was more potent against *S. littoralis*. However, *P. fumosoroseus* (Priority) was more potent against *A. ipsilon*.

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ARABIC SUMMARY

التقييم الحيوى لبعض الفطريات الممرضة للحشرات ضد الطور اليرقى لدوده ورق القطن *Spodoptera littoralis* والدوده القارضه *Agrotis ipsilon*

فاطمه محمد على الهوارى – أحمد محمد عزت عبد السلام

قسم آفات ووقايه النبات – المركز القومى للبحوث – شارع التحرير- الدقى – القايره – مصر

تم في هذه الدراسه تقييم كفاءه بعض المستحضرات الفطريه الممرضة للحشرات مثل مركب بيبوباور (فطر *Beauveria bassiana*)، مركب بيوكاتش (فطر *Lecanicillium lecanii*)، مركب بريوريتى (فطر *Paecilomyces fumosoroseus*) ضد العمر اليرقى الثالث لدوده ورق القطن والدوده القارضه وذلك تحت الظروف المعملية. وقد تم تقييم فعاليه 4 تركيبات مختلفه ($10 \times 0,125$ ، $10 \times 0,25$ ، $10 \times 0,5$ ، 10×1) جرثومه /1000 مللى ماء مقطر) من كل مستحضر ضد كل حشره على حده ، بالاضافه الى معامله اخرى تمت ككنترول بالماء المقطر فقط. وقد اظهرت النتائج المتحصل عليها ان مركب بيبوباور كان اكثر المركبات المستخدمه فعاليه ضد العمر اليرقى الثالث لدوده ورق القطن تلاه فى الفعاليه مركب بيوكاتش ومركب بريوريتى حيث وجد ان التركيز المميت ل 50، 90% من اليرقات المعامله ($10 \times 0,2$ ، $10 \times 0,5$ ، 10×1) جرثومه)، ($10 \times 0,22$ ، $10 \times 0,6$ ، 10×1) جرثومه)، ($10 \times 0,44$ ، $10 \times 0,7$) جرثومه) على التوالي. بينما اكثر المركبات فعاليه على العمر اليرقى الثالث للدوده القارضه كان مركب بريوريتى (الفطر *P. fumosoroseus*) وتلاه مركب بيبوباور (فطر *B. bassiana*)، مركب بيوكاتش (فطر *L. lecanii*) حيث وجد ان التركيز المميت ل 50، 90% لليرقات المعامله هو ($10 \times 0,14$ ، $10 \times 0,6$) جرثومه)، ($10 \times 0,9$ ، $10 \times 0,4$) جرثومه)، ($10 \times 0,9$ ، $10 \times 0,4$) جرثومه) على التوالي. ايضا اوضحت النتائج ان يرقات دوده ورق القطن التى عوملت بالمركبات السابقه الذكر والتي تحتوى على الفطريات *B. bassiana* ، *L. lecanii* ، *P. fumosoroseus* بتركيز 10×1 جرثومه نتج عنها نسبة موت وصلت 5، 87، 72، 67% على التوالي وذلك خلال 3، 2، 8، 7، 4 يوم . على الجانب الاخر وجد ان يرقات الدوده القارضه التى عوملت بنفس التركيز 10×1 جرثومه لفطريات *P. fumosoroseus* ، *B. bassiana* ، *L. lecanii* للمركبات المستخدمه قد نتج عنها نسبة موت 100، 90، 62% على التوالي خلال 3، 5، 7، 9، 3 يوم. كما وقد وجد ان الحشرات المعده بهذه الفطريات الممرضة ظهر عليها تأثيرات متأخره ظهرت من خلال النقص الذى حدث فى نسبة التعذير والحشرات الكامله الناتجه سواء لدوده ورق القطن او الدوده القارضه.