EFFICACY OF NATIVE ENTOMOPATHOGENIC FUNGI ISOLATES AND BIOLOGICAL STUDIES ON *SPODOPTERA LITTORALIS* (BOISD.)

SAHAR S. ALI and MARWA M.A.EL-SABAGH

Plant Protection Research Institute, ARC, Dokki, Giza, Egypt

(Manuscript received 29 September 2015)

Abstract

Virulence of three local isolates of entomopathogenic fungi ; *Beauveria bassiana* (AUMC 9896), *Lecanicillium antillanum* (=*Verticillium antillanum*) (AUMC 9905) and *Paecilomyces lilacinus* (AUMC 9897), as well as, *Metarhizium anisopliae* which isolated in Bio-insectcide Production Unit, Plant Protection Research Institute were evaluated against *Spodoptera littoralis* larvae under laboratory conditions. Five concentrations of spores suspension from each of the four fungal isolates (1×10^5, 1×10^6, 1×10^7, 1×10^8 and 1×10^9 conidia / ml) were used against newly hatched larvae of *S. littoralis* pest. The results showed that the percentage mortality of the larvae increased with increasing concentrations of tested pathogens. The median time of mortality decreased as the spores concentration increased. Also, the isolate *B. bassiana* was the most effective against *S. littoralis* larvae at different tested concentrations. *B. bassiana* at high concentration of $1.0x10^9$ revealed 88.5 % mortality within mean time to larval mortality 5.1 ± 1.9 days. In biological Studies all tested fungi decreased the mean larval duration about 24 hours than untreated larvae and decreased of pupation. *L. antillanum* recorded the least Pupation percentage 45%. All tested fungi showed significantly shortening in the mean adult longevity for both males and females. All tested fungi significantly decreased the mean number of eggs laid and hatched /female.

INTRODUCTION

The Egyptian cotton leafworm, *S. littoralis* is one of the most destructive polyphagous insect, not only to cotton, but also to other field crops and vegetables in Egypt (Kandil *et al*., 2003). Due to extensive using of insecticide groups, many populations of *S. littoralis* have acquired resistance towards most of them (Alford, 2000). The problems and hazards that have arisen as a result of using conventional insecticides were incentives for the search of alternative control agents. Microbial control agents are a primary means of biological control for insect pests. The use of microbial control agents is targeted for a particular pest species. The entomopathogens that have most been used in biological control include representatives of bacteria, fungi, viruses, nematodes, protozoa and insect growth regulator (Dent, 2000).

The entomopathogenic fungi are the promising agents that are used against insect pests for several decades. These organisms include taxa of several fungal groups like Hypocreales of Ascomycota that *B. bassiana* and *M. anisopliae* are the two most recognized species (Vincent *et al*., 2007). *B. bassiana* and *M. anisopliae* grow naturally throughout the world and acts as parasites of many arthropod species causing white and green muscardine diseases due to the color of their spores . Besides entomopathogenic fungi cause natural mortality on insects, these agents are environmentally safe, so there is a worldwide interest of their using and improvement for biological control of insects (Vincent *et al*., 2007). Aim of the present investigation is to evaluate the virulence of four entomopathogenic fungi against *S. littoralis* (Boisd.).

MATERIALS AND METHODS

A-Fungus culture:

Different isolates of the entomopathogenic fungi; *B. bassiana* (AUMC 9896), *L. antillanum* (=*V. antillanum*) (AUMC 9905), *P. lilacinus* (AUMC9897) and *M. anisopliae* were isolated in Bio-insectcide Production Unit, Plant Protection Research Institute and were identified in Mycological Center, Faculty of Science, Assiut University. Sahar and Moharram (2014), these entomopathogenic fungi were cultured on potato dextrose agar medium, incubated at 27±1°C for 15 days. The conidia were harvested by scraping the surface of 14-15 days old culture gently with inoculation needle. The conidia were suspended in distilled water containing 0.1% Tween-80. The mixture was stirred with a magnetic shaker for ten minutes.

B-Rearing of the *S. littoralis* (Boisd):

Egg masses of a sensitive strain of the cotton leaf worm, *S. littoralis* (Boisd.) were incubated under laboratory condition at 27±2^{O}C, 60 ± 5% RH and 8:16 LD photoperiod. The original insect culture was obtained from the Research Division of the Cotton Leaf worm, Plant Protection Research Institute. Newly hatched larvae were transferred to clean glass jars covered with muslin cloth held in position with rubber bands. They were fed on castor bean leaves, *Ricinus communis* (L.) and examined daily. Upon pupation, pupae were collected; sexed and emerged moths were placed in pairs in breeding glass jars, supplied with leaves of tafla, *Nerium oleander* (L.) as an oviposition site. Each jar was provided with cotton piece soaked in 10 % honey solution and placed in small plastic cup for moths feeding. The honey solution was renewed daily to avoid fermentation and growth of microorganisms.

C-Bioassay:

Virulence of the fungi isolates against newly hatched *S. littoralis* larvae was performed by testing five concentrations of spores suspension from each of the four fungal isolates (1×10^5 , 1×10^6, 1×10^7, 1×10^8 and 1×10^9 conidia / replicate). Fresh of castor leaves (2 leaves) were sprayed with each concentration and distilled water as control. After air drying, the treated leaves were transferred into a plastic container (15 cm diameter) were contaminated with each concentration and provided with 20 larvae each replicate (5 replicate); then were covered with muslin cloth for aeration **(Hicks *et al.*, 2001).** Larvae were maintained in an incubator at 27.0 ± 1.0 °C with adjusted relative humidity of 70.0 ± 5.0% RH. Dead larvae were counted daily and mortality was calculated. Also, the median lethal time (LT_{50}) of mortality was calculated. The percentage reduction in mortality of larvae was calculated and corrected according to Abbott's formula (1925). The experiment was replicated 4 times (20 larvae / replicate). (El-Hawary and Abd El-Salam, 2009).

D-Biological studies:

Newly hatched larvae from the maintained insect colony were collected and offered daily with castor bean leaves that treated with 1×10^7 conidia / ml as the recommended dose (Lin *et al.*, 2007). Treated instars larvae were examined daily in order to study the following parameters: larval and pupal duration of each instar and percentage of pupation. Pupae were sexed and then placed in 3 pairs in the glass jars of the following combinations: treated male x treated female and for a control untreated male x untreated female. Subsequently, percentage of adult emergence, longevity of moths and the fecundity and fertility of eggs/female, were determined.

RESULTS AND DISCUSSION

A-Virulence of fungi isolates by bioassay:

The data in Table (1) indicate that the percentage mortality of the larvae was increased with increasing concentrations of tested pathogens. However, median lethal time (LT_{50}) decreased as the spores concentration increased. Also, the isolate *B. bassiana* was the most effective against *S. littoralis* larvae. *B. bassiana* at high concentration of $1.0x10^9$ achieved 88.5 % mortality within mean time to larval mortality 5.1 ± 1.9 days, flowed by the concentrations $1.0\ x10^8$, $1.0\ x10^{7,}$ $1.0\ x10^6$ and $1.0\ x10^5$ which recorded 66.5 %, 59.5%, 47.5% and 45.0% mortality within 5.6±1.6, 5.8±1.1, 9.2 ±1.0 and 12.1±1.2 days, respectively (Table 1).

While *L. antillanum* at the high concentration $1.0x10^9$ resulted in 62.5% mortality within 7.1±1.4 days. Followed by the concentrations $1.0\ x10^8$, $1.0\ x10^{7,}$ 1.0

$x10^6$ and 1.0 $x10^5$ achieved 55.5%, 41.0%, 22.0% and 21.5% mortality within 9.3± 1.6, 10.3±1.0, 10.5±2.1 and 11.0±1.6 days, respectively (Table 1).

Also, in concentration $1.0x10^9$, of *P. lilacinus* gave 67.5 % mortality within 6.6±2.1days.Wherer as the concentrations 1.0 $x10^8$, 1.0 $x10^{7,}$ 1.0 $x10^6$ and 1.0 $x10^5$ achieved 56.5%, 45.5%, and 35.0% and 33.5% mortality within 7.1±2.2, 7.5±1.5, 9.0±1.2 and 10.3±1.1 days, respectively. Finally, *M. anisopliae* at the high concentration $1.0x10^9$ achieved 60.5% mortality within 6.2±2.3 days. Followed the concentrations 1.0 $x10^8$, 1.0 $x10^{7,}$ 1.0 $x10^6$ and 1.0 $x10^5$ resulted in 50.5%, 31.5%, 25.5% and 19.5% mortality within 7.5± 1.9, 8.8±2.6, and 10.1±2.8 and 11.8±1.8 days, respectevly.

Results are agree with those obtained by El-Hawary and Abd El-Salam (2009) who found that percentage mortalities of treated third instar larvae of *S. littoralis* with (i.e. Bio- Power, *B. bassiana*) was87.5%. However, Hassani *et al*., (2000) stated that *P. fumosoroseus* is highly virulent against these three important cotton pests (*S. littoralis Helicoverpa armigera and Aphis gossypii*).

Quesada-Moraga and Vey, (2004) found that injection of Bassiacridin (*B. bassiana* metabolite) at a dose of 2.8 µg/g to fourth instar nymphs of locusts *Locusta migratoria*, *Schistocerca gregaria* and *Dociostaurus maroccanus*, and to fifth instar larvae of the lepidopterans *Galleria mellonella* and *S. 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.

B-Biological Studies:

The obtained results in Table (2) clarified the effect of the tested fungi on the mean larval duration, pupation, and pupal duration. Treatment of the newly hatched *S. littoralis* instars larvae with 1×10^7 conidia / ml of fungi decreased the mean larval duration about 0.7-1.7 day than untreated larvae. And this was in agreement with Abd El-Kareem, (2012) who found that the treatment of 2^{nd} and 4^{th} instars larvae of *S. littoralis* with Bioranza has reduced the mean larval duration while that treated with Protecto, Viruset, and Profect has prolonged the duration of the treated instars larvae. Meanwhile, the pupation were decrease in all isolates and the pupal stage of instar larvae treated with *B. bassiana, L. antillanum, P. lilacinus* and *M. anisopliae* were decreased (13.2, 12.3, 13.6 and 11.3 days) respectively, than untreated larvae (14.6 days).

Instars larvae treated with *L. antillanum* have recorded the least Pupation percentage (45%); than that recorded with treated larvae with *B. bassiana*, *P lilacinus* and *M. anisopliae* (48.8% 48.3% and 46.5%) respectively. These results were agreed with those of Hafez *et al.* (1997) who treated *Phthorimae aoperculella* larvae with *Beauveria bassiana*.

Observed reduction in the adult emergence was recorded in all treatments. Also, all tested fungi showed significantly shortening in the mean adult longevity for both males and females (Table 3). Some of emerged adults were also malformed with reduced wings or reduced body size. They were unable to fly and died without mating. It has been investigated that pupae treated with fungal pathogens often result reduction in the adult emergence (Ekesi *et al.*, 2002), increase in pupal duration and malformed adults (Hafez *et al.*, 1997). Our findings are contradictory to findings of *S. littoralis*.

That was in concides with Abd El-Kareem (2012) who noticed decrease in the percentage of adult emergenceand mean adult longevity of treated larvae of *S. littoralis* with Bioranza. Dubois *et al.* (2004) studied the effect of two commercially products of *B. bassiana* and *B. brongniartii,* a reduction in the adult longevity of the beetle *Anoplophora glabripennis* was found, same findings recorded with Abd El-Kareem (2007) who treated larvae of *Ostrinia nubilalis* with *A. flavus*.

Table (4) showed the latent effect of the treated *S. littoralis* with 1×10^7 conidia / replicate of tested fungi on the mean number of laid and hatched eggs/female. All tested fungi significantly decreased the mean number of eggs laid/female. *M. anisopliae* was the most effective fungus, followed by *B. bassiana, L. antillanum* and *P. lilacinus* On the other hand, significantly reduction in the mean number of hatched eggs/female was observed when treated instar larvae with recommended dose of all tested (Table 4). That was in agreement with Noma and Strickler (2000) who investigated the effects of *B. bassiana* infection on ovipositional behavior of *Lygus hesperus caused.*

The decrease in reproductive potential of *S. littoralis* and decrease egg formation and number of deposited eggs in treated moth with fungi was explained by Santiago-Alvarez and Osuna (1988) who found that males of *S. littolaris* infected with bioagents are not able to mate with un treated females and produced the normal egg percentage like un treated male in addition to reduced the percentage of the egg hatchability also. Aldeebis *et al.,* 1993 clarified that was due to inability of the sperms to transfer to the females during copulation, which was suggested also by Ismail (1980). Same findings was discussed by (Hassan, 2004; Hatem, 2006; Abdel-Aziz, 2007 and El-Khateeb and El-Sabagh, 2008).

From the present studies is could be concluded that the isolate *B. bassiana* was more potent against *S. littoralis* and that all biological stages of *S. lituralis* were affected to varying degrees to infection by the entomopathogenic fungi isolates.

Table 1. Corrected accumulative mortality percentages and mean time to larval mortality of the larval stage of *S. littolaris* treated with different concentrations of entomopathogenic fungi isolates.

Tested isolate								
*Conc .	*B. bassiana*		*L. antillanum*		*P. lilacinus*		*M. anisopliae*	
	mortality %	LT_{50}	mortality %	LT_{50}	mortality %	LT_{50}	mortality %	LT_{50}
1×10^5	45.0	12.1 ± 1.2	21.5	11.0 ± 1.6	33.5	10.3 ± 1.1	19.5	11.8 ± 1.8
1×10^6	47.5	9.2 ± 1.0	22.0	10.5 ± 2.1	35.0	9.0 ± 1.2	25.5	10.1 ± 2.8
1×10^7	59.5	5.8 ± 1.1	41.0	10.3 ± 1.0	45.5	7.5 ± 1.5	31.5	8.8 ± 2.6
1×10^8	66.5	5.6 ± 1.6	55.5	9.3 ± 1.6	56.5	7.1 ± 2.2	50.5	7.5 ± 1.9
1×10^9	88.5	5.1 ± 1.9	62.5	7.1 ± 1.4	67.5	6.6 ± 2.1	60.5	6.2 ± 2.3

*Conc., Concentrations of conidiospores.

LT50, medial lethal time (days) ±SE.

Table 2. Effect of entomopathogenic fungi isolates on larval duration, pupation rate and duration of *S. littoralis* treated as new hatch instars.

Fungal isolates	Mean larval duration (days) ± S. E.	%Pupation	Mean pupal duration (days) ± S. E.
B. bassiana	15.0±0.3*	48.8	13.2±0.1*
L. antillanum	14.3±0.5*	45	12.3±0.5*
P. lilacinus	15.3±0.4*	48.3	13.6±0.6*
M. anisopliae	14.6±0.1*	46.5	11.3±0.5**
Control	16.0±0.2	100	14.6±0.5

*: Significant at P> 0.05 **: highly significant at P> 0.01

Table 3. Effect of entomopathogenic fungi isolates on adult emergence percentage and adult longevity of *S. littoralis* treated as new hatch instars.

Fungal isolates	Adult emergence %	Mean adult longevity (days) ± S. E.	
		♂	♀
B. bassiana	91.00	12.3±0.4*	11±0.28*
L. antillanum	95.00	9.0±1.0**	10.3±0.57*
P. lilacinus	90.25	12.3±0.48*	13.7±0.4*
M. anisopliae	94.40	8.3±1.15**	9.3±0.57**
Control	100.00	13.6±1.15	14.6±0.58

*: Significant at P> 0.05 **: highly significant at P> 0.01

Table 4. Effect of entomopathogenic fungi isolates on fecundity of *S. littoralis* treated as new hatch instars.

Fungal isolates	Mean no. of eggs/female ± S.E.	Mean no. hatched eggs/female ± S.E.
B. bassiana	598±9.1***	459±3.6***
L. antillanum	897±16.8***	545±8.5***
P. lilacinus	623±10.2***	413±4.9***
M. anisopliae	499±12.5***	382±4.7***
Control	2250±60.6	2203±4.04

***: Very highly significant at P> 0.001.

REFERENCES

1. Abbott, W.S. 1925. A method for computing the effectiveness of an insecticide. J. Econ. Entomol. 18: 265- 277
2. Abd El-Kareem, S. M. 2007. Biological and histopathological studies on the effect of certain entomopathogenic microorganisms on the European corn borer, *Ostrinia nubilalis* Hübner (Lepidoptera: Pyralidae) M. Sc. Thesis, Fac. Sci., Ain Shams Univ.
3. Abd El-Kareem, S. M. I. 2012. Effect of some entomopathogens and insecticides against the cotton leafworm, *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae). Ph. D. thesis, Fac. of Science, Ain Shams University, Cairo, Egypt.185 Pp.
4. Abdel-Aziz, M. M. M. 2007. Controlling of the cotton leafworm, *Spodoptera littoralis* (Boisd.), by using environmentally safe (nontraditional) methods. M.Sc. Thesis, Inst. Environmental Studies and Research, Ain Shams Univ.

5. Aldebis, H. K.; E.V. Osama and C. Santiago Alvarez. 1993. Development of the male reproductive system *in Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae) treated as larvae with nuclear polyhedrosis virus (Baculovi- ridae). Boletin de Sanidad Vegetal, Plagas. 19 (1): 3 – 9.
6. Alford, D. V. 2000. Pest and disease management hand book British crop protection council, Blackwell Sci., Oxoford, 615pp.
7. Dent, D. 2000. Biological control. In: Insect Pest Management. 2nd edition. London. CABI publishing. Ch. 6: 180-234 pp.
8. Dubois, T.; Z. Li; H. Jiafu; and E. Hajek. 2004. Efficacy of fiber bands impregnated with *Beauveria brongniartii* cultures against the Asian long horned beetle, *Anoplophora glabripennis* (Coleoptera: Cerambycidae). Biol.Control, 31: 320-328.
9. Ekesi, S.; Maniania, N.K. and Lux, S.A. 2002. Mortality in three African tephritid fly puparia and pupa caused by entomopathogenic fungi, *Metarhizium anisopliae* and *Beauveria bassiana*. Biocontrol Sci. Technol., 12(1): 7-17.
10. El-Hawary, F.M. and A.M.E. Abd El-Salam. 2009. Laboratory bioassay of some entomopathogenic fungi on *Spodoptera littoralis* (Boisd.) and *Agrotis ipsilon* (Hüfn.) larvae (Lepidoptera: Noctuidae). Egypt. Acad.J. biol. Sci., 2 (2): 1- 4.
11. El-Khateeb, S. M. and M. M. El-Sabagh. 2008. Toxicological and biological effects of *Aspergillus flavus* (Link) on the cotton leafworm, *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae). Egyptian J. Agric. Res., 86 (1): 205-214.
12. Hafez, M.; F. N. Zaki; A. Moursy; and M. Sabbour. 1997. Biological effects of the entomopathogenic fungus, *Beauveria bassiana* on the potato tuber moth, *Phthorimaea operculella* (Seller). J. Pest. Sci., 70(8): 158-159.
13. Hassan, M .M. 2004. New approach for controlling the cotton leaf worm, *Spodoptera littoralis*. M.Sc. Thesis, Fac. Science, Mansoura Univ., Egypt.
14. Hassani, M.; G. Zimmermann and S. Vidal. 2000. *Paecilomyces fumosoroseus*: efficacy against the cotton pests *Spodoptera littoralis* (Lepidoptera: Noctuidae), *Helicoverpa armigera* (Lepidoptera: Noctuidae) and *Aphis gossypii* (Homoptera: Aphididae) and effect of temperature on germination and mycelial growth. Bulletin – OILB/ SROP.23 (2):217-221.
15. Hatem, A.E. 2006. Comparacion de los efectos de insecticidas selectivos sobre el desarralloy repoduccion de *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae). Thesis Dextoral Universided de Cardoba.240.
16. Hicks, B. J.; A. D. Watt and D. Cosens. 2001. The potential of *Beauveria bassiana* (Hyphomycetes: Moniliales) as a biological control agent against the pine beauty moth, *Panolis ammea* (Lepidoptera: Noctuidae). Forest Ecol. & Management, 149: 275-281.

17. Ismail, I. E. 1980. Physiological studies on the effect of juvenile hormone analogues upon the cotton leaf worm, *Spodoptera littoralis* (Boisd.). Ph. D. Thesis, Cairo Univ.
18. Kandil, M. A.; N.F. Abdel-Aziz and E.A. Sammour. 2003. Comparative toxicity of chlorofluazron and leufenuron against cotton leaf worm, *Spodoptera littoralis* (Boisd.). Egyp. J. Agric. Res., 2:645-661.
19. Lin, H.P., X.J. Yang, Y.B. Gao and S.G. Li. 2007. Pathogenicity of several fungal species on *Spodoptera litura*. Chinse J. Appl. Ecol., 18(4): 937-940.
20. Noma, T. and K. Strickler. 2000. Effects of *Beauveria bassiana* on *Lygus hesperus* (Hemiptera: Miridae) feeding and oviposition. Environ. Entomol.; 29(2): 394-402.
21. Quesada- Moraga, M. E. and A. Vey. 2004. Bassiacridin, a protein toxic for locusts secreted by the entomopathogenic fungus *Beauveria bassiana*. Mycol. Res. 108 (4): 441- 452.
22. Sahar, S. A. and A. M. Moharram. 2014. Biodiversity and enzymatic profile of some entomopathogenic fungi. Egypt. Acad. J. Biol. Sci., 6(1): 73-80.
23. Santiago-Alvarez, C. and E.V. Osuna. 1988. Reduction of reproductive capacity of *Spodoptera littoralis* males by a *nuclear polyhedrosis virus* (NPV). J. Invert. Pathol. 52: (1): 142- 146.
24. Vincent, C.; M.S. Goettel and G. Lazarovits. 2007. Biological control, a global perspective. CABI publishing. Oxfordshire, United Kingdom, 2007.

فعالية عزلات فطرية محلية ممرضة للحشرات ودراسات بيولوجية علي دودة ورق القطن

سحر سيد علي ، مروة محمد محمود الصباغ

معهد بحوث وقاية النباتات – مركز البحوث الزراعية – دقي – جيزة – مصر.

تـم عمـل إختبـار مقارنة بين اربـع عزلات فطرية ممرضة للحشرات وهي *Beauveria bassiana* و *Lecanicillium antillanum* (=*Verticillium antillanum*) و *Paecilomyces lilacinus* و*Metarhizium anisopliae* والتي سبق عزلهم بوحدة انتاج المبيدات الحيوية بمعهد بحوث وقاية النباتات وذلك لمعرفة شدة إلإصابة بإستخدام الجراثيم الكونيدية بتركيزات ($10x1^5$ و$10x1^6$ و $10x1^7$ و $10x1^8$ و $10x1^9$ جرثومة/ مل) عـــــلي يرقات دودة ورق القطن. وقد أعطت العزلة *B. bassiana* أعلي نسبة موت لليرقات بلغت 88.5 % عند اعلي تركيز . وبدراسة التغيرات البيولوجية للحشرة والتي سبق معاملتها بتركيز $10x1^7$ جرثومة/ مل في بداية الطور اليرقي ومتابعة التطور البيولوجي بالمقارنة بالحشرات غير المعاملة بالفطر وجد حدوث انخفاض في طول الطور اليرقي للعزلات الاربع وانخفاض في نسبة التعذير والتي بلغت 45 %من نسبة التعذير للحشرات المتبقية في فطر *L. antillanum* وكذلك انخفضت فترة الطور العذري في جميع المعاملات بالمقارنة بالحشرات غير المعاملة ثم انخفضت نسبة ظهور الفراشات في جميع المعاملات وقصرت فترة بقاء الفراشات الاناث والذكور علي حد سواء في جميع المعاملات واخيرا حدث خفض كبير في اعداد البيض التي وضعتها الفراشات في جميع المعاملات وكذلك خفض كبير في متوسط اعداد الفقس .