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Efficiency of Certain Entomopathogenic Bacteria, Fungi with Pesticides for Controlling *Spodoptera littoralis*

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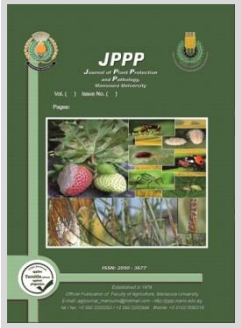

ABSTRACT

This study was conducted to identify the susceptible stages of the cotton leafworm, *Spodoptera littoralis* to the entomopathogens fungus, *Metarhizum anisopliae* and bacterium, *Pseudomonas aeuriginosa.* The mortality percentages recorded after treatment of the 2nd and 4th instar larvae of *S. littoralis* with concentration 1×10^8 spore/ml of *M. anisopliae* were 84.28 and 75.71% after 5 days of treatment. While, LT_{50} were 0.81 and 1.93 days for 2nd and 4th instar larvae of *S. littoralis*, respectively. *P. aeruginosa* concentration 2.3×10^8 spore/ml caused mortality 81.42 and 60% and LT_{50} 3.87 and 4.51 days for 2nd and 4th instar larvae of *S. littoralis*, respectively. Pupae of *S. littoralis* were susceptible for both entomopathogens agents. Moreover adults emergence percentage decreased. Combined treatment of pesticides with *P. aeruginosa* and *M. anisopliae* proved best action causing higher mortality percentages for larvae compared with using pesticides alone.

Keywords: *Spodoptera littoralis, Pseudomonas aeuriginosa, Metarhizum anisopliae*, Pesticides.

INTRODUCTION

Egyptian cotton leafworm, *Spodoptera littoralis* (Lepidoptera : Noctuidae) is one of the key pests that cause great damage to cotton plants as well as other field and vegetables crops. The extensive use of insecticides to control *S. littoralis* larvae has led to several problems and hazards such as development of resistance and residual effects (Frank *et al.*, 1990). Synthetic insecticides are extensively used to control this pest worldwide. However, their indiscriminate application has led to the development of insect resistance, environmental pollution, residual toxicity and serious threats to the non-target organisms including predators, pollinators, fish and human beings (Tonial *et al.,* 2017). Thus, there is a real need to develop new highly selective and biodegradable pesticides to solve the problem of long term toxicity to mammals. Thus, attention was directed to search for alternative control agents with new modes of action. Among these agents, entomopathogenic bacteria as microbial control agents. The bacterium *Bacillus thuringensis* is known to be one of the most pathogenic species of bacteria, which induce larval mortality after a course of infection stages. The interest of using such agent as a microbial bio-insecticide was increased since 1970 (Dulmage and co- operators, 1981). Microbial pesticides are becoming recognized as an important factor in crop and forest protection and in insect vector control. These pesticides consist of microorganisms such as viruses, bacteria, nematodes, protozoa and fungi that infect or intoxicate specific pest groups (Khetan, 2001). However, there is still a need to find new bacterial control agents against this pest since *S. littoralis* is developing resistance to many strains of *B. thuringinsis* (Salama *et al*., 1989). Therefore, the bacterial flora of various harmful insects has been determined in both agriculture and forestry (Osborn *et al*., 2002 and Demir *et al*., 2012). Entomopathogens fungi considered the most important agents in biological control where fungi reach to insect hemolymph through cuticle. Additionally, its ability to penetrate host body and attack all development insect stages increased the importance of using it in pest control. The most important of these microorganisms is *Beauvaria bassiana*. Since these are regarded as natural control agents and also safer for our environment, there is a great interest all over the world for utilizing and manipulating the EPB (Entomopathogenic bacteria) for biological control of insects and mite pests. Moreover the use of biological control agents is economically feasible, environmentally safer and sustainable. It is therefore, need of the time to find some alternate control methods which can be incorporated in the pest management program. A recent study of the author has revealed that the strains evaluated in the current study can be used in combination with some new pesticides. The present study aimed to evaluate pathogenicity of bacterium, *Pseudomonas aeruginosa* and fungus, *Metarhizium anisopliae* in the laboratory against *Spodoptera littoralis* for potentially compatible pesticides in an integrated pest management program.

MATERIALS AND METHODS

Rearing techniques of *Spodoptera littoralis*

The two strains were reared in the laboratory on fresh castor bean leaves under conditions 25± 2ºC and 65±5% R.H. as described by El-Defrawi *et al.* (1964). The first three larval instars were fed on castor bean leaves in 400 ml glass containers covered with muslin. Larger larval instars were transferred to bigger jars provided with the same food till the pre pupal stage. The pre pupae were allowed to pupate in clean jars containing dry sawdust. The resulting pupae were transferred to covered petri dishes containing filter papers and kept in suitable cages and planned for mating. Emerged moths were supplied with a piece of cotton wetted with 10% sugar solution as feeding source. The cages were supplied with leaves of Tafla (*Nerium oleander* L.) that served as oviposition site. Second and fourth instar larvae were used for bioassay in all tests.

Pesticides

1- Trade name: Methomyet 90% SP

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Common name: Methomyl
Recommended concentration: 300 gm/fedan
2- Trade name : Match 5% EC
Common name: Lufenuron
Recommended concentration: 160 cm/fedan
3- Trade name: Pyrodan 50% EC
Common name: Chlorpyrifos-methyl
Recommended concentration: 1 liter/fedan

Entomopathogens

1- Bacterium, *Pseudomonas aeruginosa*
2- Fungus, *Metarhizium anisopliae*

The entomopathogens obtained from the microbiology department, faculty of agriculture, Zagazig University.

Bioassay procedures

The efficiency of biocontrol agents tested were assessed against 2nd and 4th larval instars. Forty larvae of each 2nd and 4th larval instars, (4 replicates with 10 larvae /replicate for each concentration) putted on castor bean leaves in jars that bedded with paper discs and covered with muslin cloth held with rubber band, all replicates sprayed with four concentrations 1×10^5, 1×10^6, 1×10^7 and 1×10^8 spore/ml of fungus, *Metarhizium anisopliae*. 0.02% Tween 80 used for complete solubility and the control sprayed with water that containing 0.02% Tween 80. Another 40 larvae of 2nd and 4th larval instar sprayed with four concentrations 2.3×10^5, 2.3×10^6, 2.3×10^7 and 2.3×10^8 spore/ml of bacterium, *Pseudomonas aeruginosa* 0.02% Tween 80 used for complete solubility. After 24hr. treated leaves were replaced by untreated leaves. The mortality% calculated after 1,2,3,4 and 5 days. To estimate the LT_{50} values the calculate mortality percentage were subjects to probit analysis according to Finney (1952).

Combined application of entomopathogenic, bacterium, fungus and pesticides

Forty larvae of 2nd and 4th larval instars, (4 replicate with 10 larvae per replicate) treated with 1/16 recommended concentrations of pesticides, Methomyl, Lufenuron and Chlorpyrifos-methyl) 24 hr. after treatment of 2nd and 4th larval instars with pesticides all replicates treated with low concentration 1×10^5 spore/ml from fungus. The same protocol followed with bacterium and low concentration 2.3×10^5 spore/ml was used. The two sprays in a sequence were carried out showed combination between pesticides and fungus or bacterium. The applications were performed using spray application by hand atomizers. The mortality percentages were calculated.

Sensitivity test of entomopathogens, *P. aeruginosa* and *M. anisopliae* against *S. littoralis* pupae

The experiment was conducted by using 10 pupae/replicate and 4 replicates/concentration, 1×10^5, 1×10^6, 1×10^7 and 1×10^8 spore/ml of fungus, *Metarhizium anisopliae* and 2.3×10^5, 2.3×10^6, 2.3×10^7 and 2.3×10^8 spore/ml of bacterium, *Pseudomonas aeruginosa.* The newly emerged pupae sprayed with each concentration. The pupae putted in plastic box its high 5.5 cm that covered with muslin cloth for movement till emergence. The control replicates were sprayed with only water and incubated at 25±2ºC. The adult emergence percentages were calculated 10 and 14 days after treatment. The mortality percentages of each treatment was analyzed by analysis of variance (Anova) and Duncan's multiple range test, (1955).

RESULTS AND DISCUSSION

Virulence of entomopathogens against larvae of *Spodoptera littoralis*

Data in table (1) Showed that *S. littoralis* larvae treated with bacterium, *Pseudomonas aeruginosa* resulted in variable mortality percentages, the highest mortality percentage was 81.42% in the second instar and 60% in the 4th instar at concentration 2.3×10^8 spore/ml after 5 days of treatment. There was significant difference between all concentrations that used. Accumulated mortality percentage increased with increasing, both concentrations and time after treatment. The mean mortality percentages can be arranged in descending order at values 47.70, 32.28, 23.99 and 20.85% for 2.3×10^8, 2.3×10^7, 2.3×10^6 and 2.3×10^5 spore/ml on second instar while it were 35.42, 24.82, 18.28 and 10.28% for fourth instar at the same descending order. From the previous results the second instar was more sensitive than fourth instar. LT_{50} values were 6.5, 5.7, 4.52 and 3.87 days for second instar at 2.3×10^5, 2.3×10^6, 2.3×10^7 and 2.3×10^8 spore/ml, respectively while they were 8.4, 6.19, 5.83 and 4.51 days for fourth instar at the same order.

Table 1. Virulence of bacterium, *Pseudomonas aeruginosa* on 2nd and 4th instar larvae of *S. littoralis*

Conc. Spore /ml	Days after treatment 1		2		3		4		5		Mean		LT_{50} (days)	
	2nd	4th	2nd	4th	2nd	4th	2nd	4th	2nd	4th	2nd	4th	2nd	4th
2.3×10^5	7.14 ±0.97	2.85 ±0.86	11.42 ±1.75	4.28 ±1.02	18.57 ±2.11	8.57 ±1.56	28.57 ±2.54	11.42 ±1.94	38.57 ±3.84	24.28 ±2.32	20.85 ±2.11^c	10.28 ±1.75^c	6.5 ±0.80	8.4 ±1.24
2.3×10^6	10.00 ±1.46	8.57 ±1.74	15.71 ±1.91	12.85 ±1.86	24.28 ±2.74	17.14 ±2.07	27.14 ±2.07	17.14 ±2.74	42.85 ±4.01	35.71 ±2.21	23.99 ±2.43^c	18.28 ±2.47^c	5.7 ±0.47	6.19 ±0.94
2.3×10^7	15.71 ±1.97	10.00 ±1.85	22.85 ±2.43	17.14 ±2.45	31.42 ±3.12	27.14 ±2.78	40.00 ±3.78	31.42 ±2.87	51.42 ±3.98	41.42 ±4.02	32.28 ±3.14^b	24.82 ±2.73^b	4.52 ±0.51	5.83 ±0.75
2.3×10^8	28.57 ±2.53	18.57 ±2.42	35.71 ±3.48	22.85 ±2.87	41.42 ±3.78	32.85 ±3.12	51.42 ±4.41	42.85 ±3.45	81.42 ±7.46	60.00 ±5.14	47.70 ±3.86^a	35.42 ±3.84^a	3.87 ±0.24	4.51 ±0.37
Control	0	0	4.28	0	4.28	1.42	4.28	1.42	4.28	1.42	-	-	-	-

Means in column followed by the same letter are not significantly different at 5 % level (Duncan's 1955 multiple range tests).

Data in table (2) showed the toxicity of different concentrations of *Metarhizium anisopliae* against 2nd and 4th instar of *S. littoralis* during 5 days of treatment. Data revealed that mortality percentages tended its increase from 60 to 84.28% for 2nd instar and from 47.14to 75.71% for 4th instar after 5 days of treatment with increasing concentration from 1×10^5 to 1×10^8 spore/ml. There were significant differences between all concentrations after 1,2,3,4 and 5 days of treatment. The mean mortality percentage were 43.71, 50.85, 59.99 and 70.56% for 2nd instar of *S. littoralis* while the mean mortality percentages were 30.28, 41.42, 51.42 and 60.85%for the same order of treatment in 4th instar of *S. Littoralis*. LT_{50} values were 4.79, 2.14, 1.62 and 0.81 days for 2nd instar at 1×10^5, 1×10^6, 1×10^7 and 1×10^8 spore/ml, respectively, while they were 5.17, 4.12, 3.5 and 1.93 days for 4th instar at the same order.

Table 2. Toxicity of fungus, *Metarhizium anisopliae* on 2nd and 4th instar larvae of *S. littoralis*

Conc. Spore /ml	Days after treatment										Mean		LT_{50} (days)	
	1		2		3		4		5					
	2nd	4th	2nd	4th	2nd	4th	2nd	4th	2nd	4th	2nd	4th	2nd	4th
1×10^5	22.85±3.11	15.71 ±2.51	38.57 ±4.00	21.42 ±2.15	48.57 ±3.35	31.42 ±4.11	48.57 ±5.11	35.71 ±3.95	60.00 ±6.29	47.14 ±5.91	43.71 ±4.13^c	30.28 ±2.99^d	4.79 ±0.18	5.17 ±0.90
1×10^6	32.85 ±3.45	24.28 ±2.36	41.42 ±4.00	31.42 ±3.09	51.42 ±4.29	40 ±4.35	61.42 ±6.29	54.28 ±4.43	67.14 ±6.33	61.42 ±5.49	50.85 ±4.58^b	41.42 ±3.65^c	2.14 ±0.12	4.12 ±0.51
1×10^7	48.57 ±3.98	35.71 ±2.49	54.28 ±4.98	44.28 ±3.91	58.57 ±5.16	50.00 ±4.22	65.71 ±6.07	60.00 ±5.15	72.85 ±7.11	67.14 ±5.22	59.99 ±4.93^b	51.42 ±3.75^b	1.62 ±0.30	3.5 ±0.11
1×10^8	54.28 ±9.05	44.28 ±2.77	64.28 ±4.71	51.42 ±3.97	71.42 ±6.22	62.85 ±5.08	78.57±7.29	70.00 ±5.91	84.28 ±7.93	75.71 ±6.02	70.56 ±5.15^a	60.85 ±4.09^a	0.81 ±0.01	1.93 ±0.20
Control	0	0	4.28	0	4.28	1.42	4.28	1.42	4.28	1.42	-	-	-	-

Means in column followed by the same letter are not significantly different at 5 % level (Duncan's 1955 multiple range tests).

Sensitivity of pupae of *S. littoralis* against *Metrhizum anisopliae*

Data in table (3) showed the effect of *M. anisopliae* and *P. aeruginosa* on adult emergency of *S. littoralis*. There were significantly differences between all tested concentrations of fungus and bacteria on adult emergency% of *S. littoralis* after 10 and 14 days from treatment. Adult emergence percentages showed opposite proportions with the concentrations. The least emergence for adult were 25% and37.5% for treated of *M. anisopliae* at concentrations 1×10^8 spore/ml and 17.5% and 27.5% after 10 and 14 days from treatment. With using *P. aeruginosa*.

Table 3. Average adults emergence rate from *S. littoralis* pupae treated with M. *anisopliae* and *P. aeruginosa* concentrations

	Conc. spore/ml	Emergence % after treatment			Conc. spore/ml	Emergence % after treatment	
		10 days	14 day			10 days	14 day
M. anisopliae	1×10^5	67.50±5.13^b	85.00±6.98^b	*P. aeruginosa*	2.8×10^5	55.00±3.98^b	70.00±6.85^b
	1×10^6	50.00±4.01^c	62.50±5.57^c		2.8×10^6	37.50±2.16^c	52.50±4.16^c
	1×10^7	32.50±3.52^d	47.50±3.46^d		2.8×10^7	30.00±2.23^c	40.00±3.98^d
	1×10^8	25.00±3.01^d	37.50±3.11^e		2.8×10^8	17.50±2.04^d	27.50±2.56^e
	Control	82.50±7.11^a	95.00±7.20^a		Control	82.50±6.00^a	95.00±7.16^a

Means in column followed by the same letter are not significantly different at 5 % level (Duncan's 1955 multiple range tests).

Combined effect of pathogenicity and pesticides

A significant differences existed between all treatments whether chemical alone or the chemical followed by the fungus (P< 0.001). On comparing the results obtained 5 days after fungal application *M. anisopliae* (Fig. 1). It's revealed that the combined treatment of Match with the fungus showed highest mortality 71.42% for 2nd instar and 57.14% for 4th instar of *S. littoralis* followed by Methomyet with fungus showed mortality 64.28% and 51.42% for 2nd and 4th instar of *S. littoralis*, respectively. Although the combined treatment of *M. anisopliae* and chemical in a sequential manner showed higher mortality than the chemical alone.

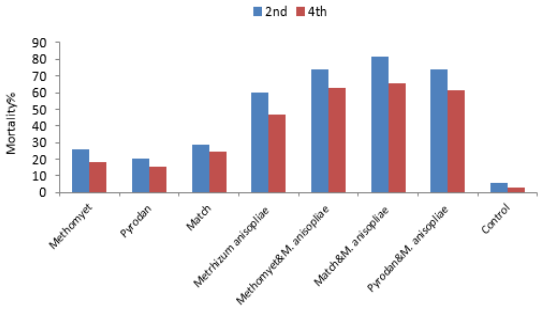

Fig. 1. Efficacy of *M. anisopliae* @ 1×10^5 spore/ ml alone and combined with selective pesticides against 2nd and 4th instar larvae of *S. littoralis*.

Almost similar results were obtained when using the bacterium, *P. aeruginosa* but the mortality was higher than that caused by fungus *M. anisopliae* (Fig.2). Significant differences were observed again 5 days after treatment (P<0.001). 5 days after treatment, Match and *P. aeruginosa* showed the highest mortality.

The obtained results showed that there were increasing in the mortality percentages of *S. littoralis* after using pesticides and *P. aeruginosa* & *M. anisopliae*, while the opposite was observed after using pesticides alone Although the fungus *M. anisolpiae* gave high mortality percentages with Match and Methomyet and low mortality with Pyrodan. On the other hand, using the bacterium, *P. aeruginosa* gave high mortality percentages with the same pesticide, Pyrodan. The obtained results in this study were in agreement with Asi (2012) who observed that the first instar larvae of cotton leafworm was more susceptible than later instar when treated with *B. bassiana* while *S. littoralis* pupae recorded low mortality.

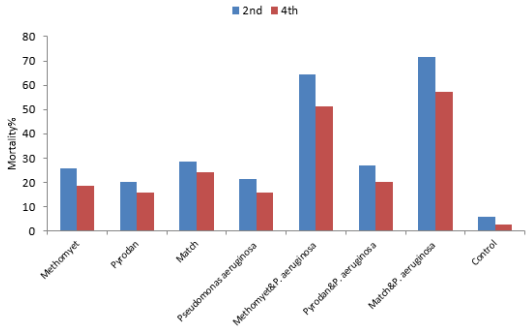

Fig. 2. Efficacy of *P. aeruginosa* @ 2.3 $\times10^5$ spore/ ml alone and combined with selective pesticides against 2nd and 4th instar larvae of *S. littoralis*.

Abou Bakr (1997) reported that mortality percentages and they found that pupae treated with *M. anisopliae* reached to 44.68%. Dulmage and Co-operators (1981) tested the efficacy of bacterium, *Bacillus thuringensis* against *S. litorralis* larvae that recorded high mortality. Kaur *et al*. (2011) revealed that 2nd and 4th larval instar of *S. littoralis* were more affected when treated with *B. bassiana* than pupae stage. Ortiz-vrqwaza and Keyhawi (2013) showed that the role of entomopathogens of fungi against *S. littoralis* occurred when the fungi reached to insect hymoleamph through cuticle while, bacteria and virus penetrate the insect through nervous and digestive system. Ferron (1978) revealed that the entomopathogenic fungi have an important role in controlling the insects through penetrate the

insect body that attack all stages of the insects. Results of this paper agreement with Abou Baker (1997) who showed mortality percentage of *S. littoralis* pupae treated with *M. anisopaliae* 44.68% after 4 weeks of treatment. Kamal *et al*. (2011) revealed that *Pseudomonas sp*. was toxic and act as insecticidal and antifeedant against *Spodoptera littura*. Esa *et al*. (2015) reached to *Bacillus thuringiensis* was toxic against *S. littoralis* larvae and larvae failed to molt into pupa. Kitherian *et al*. (2018) suggested that entomopathogenic *M. anisopliae* caused significantly higher corrected mortality 48% of *S. litura* larvae than *P. fluorescens* that caused mortality 22.3% so *P. fluorescens* and *M. anisopliae* are considered microbial biological control agent of *S. litura*. Funda and Yusuf (2021) conducted a study to investigate the efficacy of *Beauvaria bassiana* and *Metrhizium brunreum* on *S. littoralis* larvae. It was found that *M. brunneum* was more effective than *B. bassiana* against *S. littoralis* that can be considered a potential biological control agent used against *S. littoralis* larvae.

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فاعلية بعض أنواع البكتيريا والفطريات الممرضة مع مبيدات الآفات لمكافحة Spodoptera littoralis

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أجريت هذه الدراسه لتوضيح امكانيه التعاون والتوافق بين المسببات المرضيه الفطريه منها والبكتيريه مع المبيدات فى مكافحه دوده ورق القطن. كان لكل من الفطر *M. anisopliae* وبكتيريا *P. aeruginosa* تأثير سام واضح على العمر الثانى والعمر الرابع لدوده ورق القطن حيث تم تسجيل نسبه الموت وكانت كالتالى 81.42%, 60% ضد يرقات العمر الثانى والرابع لدوده ورق القطن باستخدام البكتيريا بتركيز 2.3×10^{8} جرثومه/مللى كما كان الزمن اللازم لقتل 50% من الأفراد 3.87 و 4.51يوم على يرقات العمر الثانى والرابع على التوالى. كان لأستخدام فطر *M. anisopliae* تأثير فعال على يرقات العمر الثانى والرابع لدوده ورق القطن حيث بلغت نسبه الموت 84.28 و 75.71 % على العمر الثانى والرابع على التوالى بعد 5 يوم من المعامله. أظهرت عذارى دوده ورق القطن حساسيه ضد المسببات المرضيه المستخدمه حيث انخفضت نسبه خروج الحشرات البالغه عند معامله العذارى بتركيزات مختلفه من فطر *M. anisopliae*, وبكتيريا *P. aeruginosa* .كما أجريت دراسه أخرى لأمكانيه استخدام الاتحاد والتعاون بين المبيدات المستخدمه ضد دودة ورق القطن والمسببات المرضيه وذلك باستخدام تركيزات منخفضه وهى 16/1 من التركيز الموصي به لثلاث مبيدات كيماويه (الميثوميت والماتش والبيرودان) حيث تم رش المبيد الكيماوى اولا بالتركيز المستخدم سابقا وبعد 24 ساعه تم رش الفطر والبكتيريا بأقل تركيز استخدم فى هذه الدراسه وأوضحت النتائج أن استخدام المبيدات متحده بالمسببات المرضيه المذكوره والتركيزات الموضحه قد أعطت نتائج موت عاليه بالمقارنه بكلاهما منفرد.