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COMPARATIVE EFFICACY OF LOCAL ISOLATES OF ENTOMOPATHOGENIC BACTERIA AND COMMERCIAL PRODUCT OF *Bacillus thurengeinsis* BERL. (AGERIN) ON COTTON BOLLWORMS.

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ABSTRACT

The present study was conducted Aga district El-Dakhlia governorate, throughout 2006 and 2007cotton seasons.

During the course of this study, three strains (I, II and III strains) of entomopathogenic bacteria belonging to *Bacillus thurengeinsis* Berl. were isolated from the pink (PBW), *Pectinophora. gossypiella* (Saund.), spiny (SBW), *Earias. insulana* (Boisd.) and American (ABW), *Helicoverpa. armigera* (Hub.) bollworms. In addition, two bacterial pathogens namely, (*Streptomyces avermecti* and *Micrococcus* sp.) were isolated from the previously bollworm larvae.

The pathogenicity of each pathogen suspension was evaluated under laboratory conditions against the fourth larval instars of ABW, SBW and PBW. Statistical analysis indicated that *B. thurigiensi*s strains (especially strain I) induced the highest pathogenicity to ABW, SBW and PBW larvae, while *S. avermecti* ranked second in the order of activity. *Micrococcus* sp. induced the lowest pathogenicity.

The efficiency of the local strain (I) of *B. thurigiensis* in comparison with the commercial isolate (Agerin) was evaluated under field conditions. The In the first season (2006), the mean reduction percentages of infestation caused by PBW, SBW and ABW were 61.8, 65.5 and 74.2% in cotton fields treated with the local isolate (*B. thurengeinsis* strain I), while, the mean reduction percentages of infestation in Agerin treatment were 48. 5, 56.9 and 66.1%, respectively in comparison with control. In the second season (2007), the mean reduction percentages of infestation were 60.4, 55.6 & 63.5% for local isolate of *B. thurigiensis* and 47.7, 46.0 & 52.4 % for Agerin treatment, respectively.

INTRODUCTION

Cotton bollworms (American bollworm, *Helicoverpa armigera* (Hub.), spiny bollworm, *Earias insulana* (Boisd.) and pink bollworm, *Pectinophora gossypiella* (Saund.)) are important pests of cotton. Bollworms mainly feed on fruiting parts of cotton resulting in considerable quality and quantity losses [Ahmad, 1980] and 20-60% damage and a decrease in market value of fiber [Verma, 1999].

H. armigera tunnels into small squares, terminal buds [Bohmfalk *et al.*, 2001] and large bolls from the base leaving posterior half portion of the body outside the bolls [Munro, 1987]. This may cause abnormal non-reproductive growth [Bohmfalk *et al.*, 2001]. In Egyptian cotton fields, *P. gossypiella* and *E. insulana* cause the greatest part of cotton yield losses (Amin *et al.*, 2001). The larvae of *E. insulana* attack soft and growing tissues especially terminal bud of main stem, flower buds and bolls [Munro, 1987], which ultimately shed [Atwal, 1994].

Cotton bollworms are characterized by their high mobility and fecundity and have great capacity to develop resistance to synthetic insecticides used in their management (Kranthi, 1997). Due to high cost of protecting crops from these pests with chemical pesticides and the increasing resistance and resurgence to many chemical pesticides (Armes *et al.*, 1992; Brewer & Trumble, 1994), there is a growing interest in the use of biological products such as bacterial.

Those insects are attacked by numerous entomopathogenic bacteria such as *Bacillus thuringiensis* (Zidan *et al.*, 1998; Sharma *et al.*, 2001 and Aroonrat *et al.*, 2003), *B. cereus* (Abul-Nasr *et al.*, 1978 and Cheema & Muzaffar, 1979), *B. polymyxa* (Abul-Nasr *et al.*, 1978) and *Streptococcus* sp. (Narayanan *et al.*, 1976 and Cheema & Muzaffar, 1979). *B. thuringiensis* is active against many lepidopterous species and has no adverse effects on natural enemies of target pests (Fadare and Osisanya, 1998). A control program based on selective materials, which would allow survival of beneficial species and cause the mortality of destructive ones is desirable.

The search for new microbial agents for pest control is one of the most pressing needs in the field of biological control. Therefore, isolation of more local *B. thuringiensis* strains that would be more adapted to the local pest hosts and possesses greater insecticidal activities or broader host range (Abd-Elazim *et al.*, 1991 and Osman, 1992) is required. So, isolation of the pathogens from the native ecosystem will help in successful biological control program (Keller, 1998).

The objective of this study aimed to collect, isolate and identify the local entomopathogeneic bacteria associated with cotton bollworms. In addition, the comparative efficacy of the local bacterial isolate and commercial bacterial biocides (Agerin) on cotton bollworms was evaluated under field conditions.

MATERIALS AND METHODS

I- Samples collection and isolation:

The present experiments were conducted at Aga district, in El-Dakhlia Governorate throughout 2006 and 2007 cotton seasons. One feddan was assigned to collect, isolate and identify the local strains of entomopathgenic bacteria associated with the pink [*Pectinophora gossypiella* **(**Saund.)], spiny [*Earias insulana* (Boisd)] and American [*Helicoverpa armigera* (Hub.)] bollworm larvae on Giza 86, cotton cultivar.

Samples were collected weekly during 2006 and 2007 cotton season. Each sample consisted of 250 flower-buds (squares), flowers or bolls which were covered with polyethylene bag in the field and then picked up and transferred to the laboratory for examination.

To collect and isolate pathogeneic bacteria, living and dead *P. gossypiella* , *E. insulana* and *H. armigera* larvae showing primary infection symptoms were collected and put into sterilized tubes throughout the two studied seasons. The tubes were transferred to Microbiology lab, (Microbiology Department, Faculty of Agriculture, Mansoura University) to isolate and identify the bacterial species appearing on the larvae.

The dilution plate method was used for the isolation of the insect microorganisms. The larvae was crushed, then sterile water was added. The suspension was shaked well for 10 min, then diluted to the desired final dilution. One ml of the desired dilution was transferred aseptically into a series of Petri-dishes. Nutrient agar medium (15-20 ml) was used as a bacterial nutrition medium. After incubation at 30±1^{o}C for 3.5 days, plates were examined and the developed colonies were identified according to Holt *et al.* (1974).

II- Pathogenicity of bacterial isolates:

Insect sources : The pink and spiny bollworms were obtained from a colony maintained in the Integrated Pest Management (IPM) laboratory; Bollworms Department, Plant Protection Research Institute. Larvae were reared on a modified diet as described by Abd El-Hafez *et al.* (1982), while American bollworm larvae were collected from the previously mentioned experimental cotton field.

Preparation of inoculums : The bacterial growths on the nutrient agar slants were scraped, using 5 ml sterile tap water, then transferred to a flask containing 50 ml sterile nutrient broth. The resulting cell suspensions (1 X 10^6 cells/ ml.) were used for inoculation

To evaluate the pathogenicity of bacterial isolates on PBW, SPW and ABW larvae, each isolate was dispersed surfically on 5 gm of the artificial medium and left until the diet absorbed the inoculum inside Petri-dishes containing the fourth instar larvae of PBW, SPW and ABW (about 20 larvae/Petri-dish). In each treatment, 0.025 Tween 40 was used. Each bacterial inoculum was replicated five times against each of PBW, SPW and ABW.

After 7 days of treatment, the treated Petri-dishes were examined and the percentage of pathogenicity was estimated. Reisolation for the pathogens has been carried out from the infected PBW ,SPW and ABW individuals.

III – Evaluation the comparative efficacy of local and commercial (Agerin) isolates of *B. thuringiensis* on cotton bollworms:

To report the comparative efficacy of the local isolate of bacterial pathogen (*B. thuringiensis* strain I) and the commercial bacterial biocides (Agerin) on cotton bollworms an area of about 500 m^2 was divided into three plots for planting Giza 86, cotton cultivar on the 2nd of April ,2006 and16th March 2007. In each treatment 0.025 tween 40 was used.

The first plot was treated with bacterial suspension (1 X 10^6 cells/ ml. of the local isolate of *B. thuringiensis* ,strain I).The second plot was treated with the recommended dose of Agerin 6.5 % (BT) 32000 IU/mg. In the first season, the cotton plants were sprayed with bacterial suspension of the local isolate and Agerin on the 27th of July and 24th August. While, in the second season the local isolate and Asgerin were applied on 24th of July and 28th of August 2007. The third experimental plot (as control) was left without treatments. All experimental plots received regular cultural practices and no insecticides were used during flowring and fruting stage against bollworms.

Sampling program: To evaluate the efficiency of local isolate and Agerin against pink , spiny and American bollworms compared to the control

treatment, samples of green bolls or flower buds were collected weekly and examined and the infestation percentages with each cotton bollworm species were estimated as follows:

$$\text{Infestation \%} = \frac{\text{No. of infested bud or bolls}}{\text{Total No. of collected sample}} x100$$

Each sample consists of 100 bolls from each plot.

RESULTS AND DISCUSSION

1. Isolation and identification of entomopathogenic bacteria:

Three strains of entomopathogenic bacteria (strain I, II and III) belonging to *Bacillus thurengeinsis* were isolated from the pink (PBW) (*P. gossypiella*), spiny (SBW) (*E. insulana*) and American (ABW) (*H. armigera*) bollworms. In addition, two bacterial pathogens namely, *Streptomyces avermecti* and *Micrococcus* sp. were isolated from bollworm larvae.

2. Pathogenicity of bacterial isolates to PBW, SBW and ABW larvae:

The pathogenicity of each pathogen suspension was evaluated under laboratory conditions against the fourth larval instar of PBW. As shown in Table (1), the percentages of infection caused by *B. thurigiensis* (strain I, II and III), *S. avermecti* and *Micrococcus* sp. induced (95.2±5.8, 80.4±7.9 and 82.0±10.1%), 78.8±12.1 and 66.2±7.9 infection percentages, respectively, while the control experiment (distilled water) had 1.0±2.2%.

Table (1): Pathogenety of isolated bacteria, *Bacillus thuringeinsis* (strain I, II, III), *Streptomyces avermecti* and *Micrococcus sp*. to the fourth instar larvae of *Pectinophora gossypiella* [L.S.D value = 9.04 (P =5%)].

Pathogen	Infection%
B.thuringeinsis	
Strain I	95.2 ± 5.8 a
Strain II	80.4 ± 7.9 b
Strain III	82.0 ± 10.1 b
S.avermecti	78.8 ± 12.1 b
Micrococcus sp	66.2 ± 7.9 c
(control)	1.0 ± 2.2 d

Statistical analysis revaled that *B. thurigiensis* strain I resulted in the highest pathogenicity to PBW, while *S. avermecti* and *B. thurigiensis* (strain II and III) ranked second in the order of activity. *Micrococcus* sp. Induced the lowest pathogenicity .

In respect to SBW larvae the pathogenicity of bacterial isolates to the fourth larval instar of SBW under laboratory conditions showed that there are significant differences for bacterial infection between the inoculated and control larvae. The percentages of infection caused by *B. thurigiensis* (strain I, II and III), *S. avermecti*. and *Micrococcus* sp. were (100 ± 0.0, 87.2 ± 7.8

and 81.0 ± 4.5%), 73.4 ± 6.9 and 74.6 ± 11.4 %, respectively, while the control experiment (distilled water) had no infection.

Statistical analysis indicated that *B. thurigiensis* had the highest pathogenicity to SBW(Table2). While, the percentages of infection caused by. *S. avermecti*. and *Micrococcus* sp. ranked second in the order of activity with no significant differences between them.

Table (2). Pathogenety of isolatal bacteria, *Bacillus.thuringeinsis* (strain I, II, III), *Streptomyce avermecti* and *Micrococcus sp*. to the fourth instar larvae of *Earias insulana*. [L.S.D value = 6.77 (P =5%)].

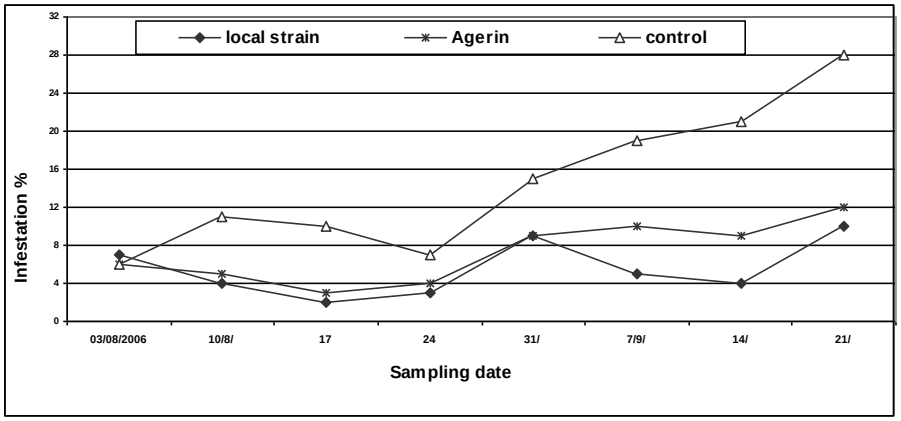
Pathogen	In fection%
B.thuringeinsis	
Strain I	100 ± 0.0 a
Strain II	87.2 ± 7.8 b
Strain III	81.0 ± 4.5 b
S.avermecti	73.4 ± 6.9 bc
Micrococcus sp	74.6 ± 11.4 bc
(control)	0.0 ± 0.0 c

The pathogenicity of each pathogen suspension was evaluated under laboratory conditions against the fourth larval instar of ABW(Table3). The obtained results indicated that there are significant differences were apparent for bacterial infection between the inoculated and control larvae. The percentages of infection caused by *B. thurigiensis* strain I, II and III), *Streptomyces avermecti*. and *Micrococcus* sp. (90.0 ± 6.8 , 78.6 ± 5.5 and 80.0 ± 6.8 %), 76.8 ± 7.2 and 60.0 ± 8.6 infection percentages, respectively, while the control experiment (distilled water) had no infection.

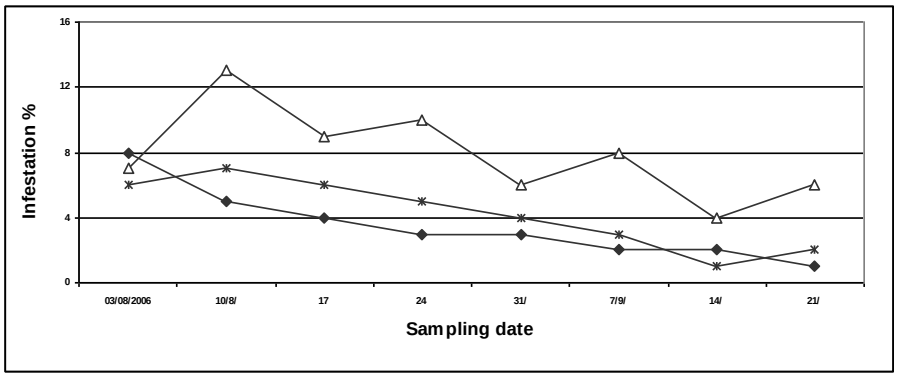
Table (3). Pathogenety of isolated bacteria, *Bacillus thuringeinsis* (strain I, II, III), *Streptomyce.avermecti* and *Micrococcus sp*. to the fourth instar larvae of *Helicovepa armigera* [L.S.D value = 7.07 (P =5%)].

Pathogen	In fection%
B.thuringeinsis	
Strain I	90.0 ± 6.8 a
Strain II	78.6 ± 5.5 b
Strain III	80.0 ± 6.8 b
S.avermecti	76.8 ± 7.2 b
Micrococcus sp	60.0 ± 8.6 c
(control)	0.0 ± 0.0 d

Statistical analysis indicated that *B. thurigiensis* had the highest pathogenicity to ABW, while *B.thuringeinsis* (strain I, II, III) and *S. avermecti* ranked second in the order of activity. *Micrococcus* sp. were the lowest entomopathogenic bacterium (Table 3).



(A)



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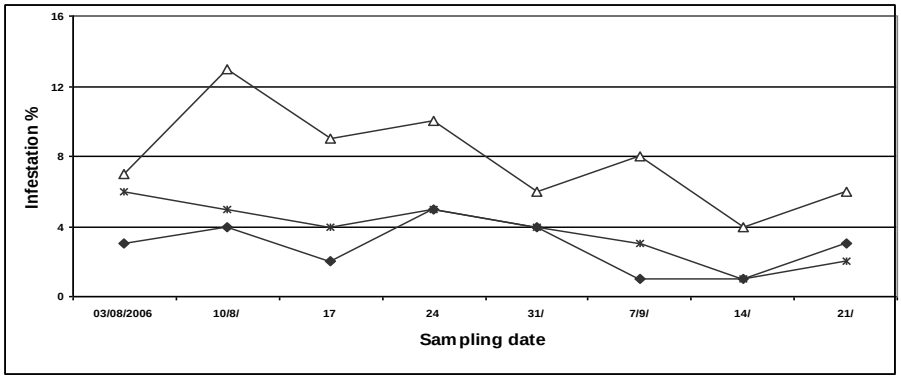
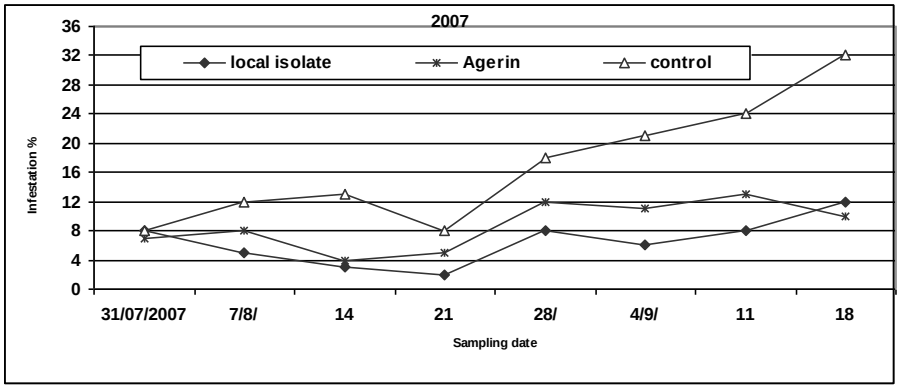
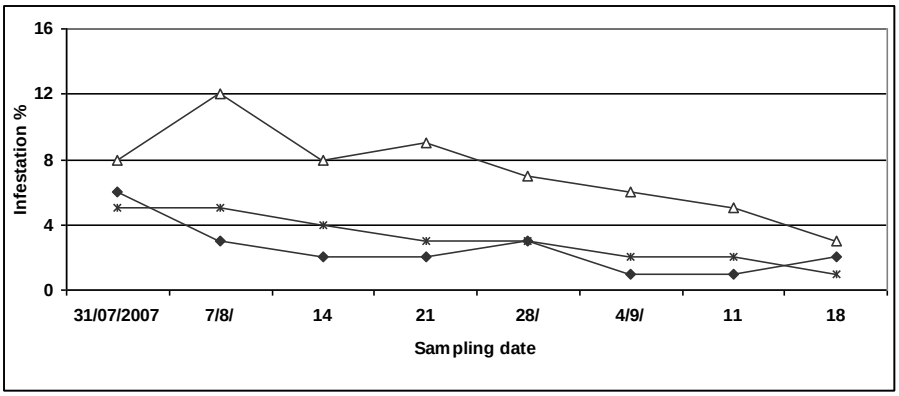


(C)

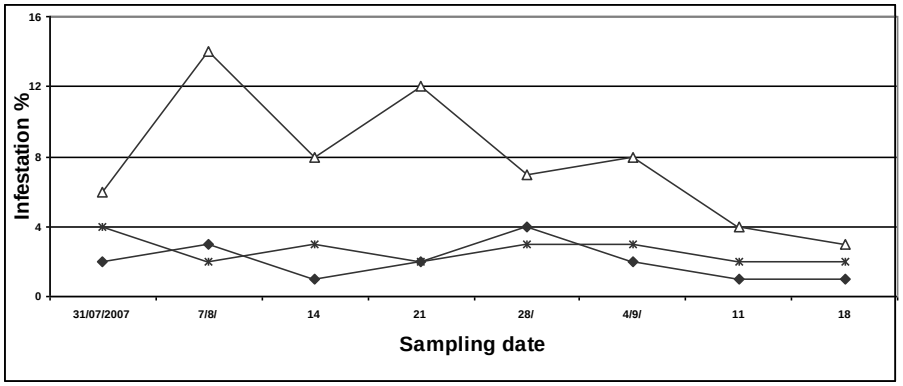
Figure (1). Weekly infestation percentages caused by *P. gossypiella* (A), *E. insulana* (B) and *H. armigera* (C) in cotton plots treated with local isolate of *Bacilus thuringiensis* and Agerin treatments during 2006 seasons.



(A)



(B)



(c)

Figure (2). Weekly infestation percentages caused by *P. gossypiella* (A), *E. insulana* (B) and *H. armigera* (C) in cotton plots treated with local isolate of *Bacilus thuringiensis* and Agerin treatments during 2007 seasons.

3. Comparative efficacy of local and commercial (Agerin) isolates of *B. thuringiensis* on cotton bollworms:

The effectiveness of local isolate of *B. thuringiensis* strain I as compared with recommended commercial isolate(Agerin) was evaluated under field conditions against *P. gossypiella*, *E. insulana* and *H. armigera* during two successive cotton seasons. The obtained data are summarized and illustrated in Figures (1 and 2).

As shown in Figures 1 and 2 the infestation percentages with all cotton bollworms (*P. gossypiella*, *E. insulana* and *H. armigera*) were low in treated field with the local isolate of *B. thuringiensis* strain I in comparison with those in Agerin and untreated field during seasons 2006 (Figure, 1) and 2007(Figure, 2).

On the other hand,the obtained results indicated that in the first season (2006) the mean reduction percentages of infestation caused by PBW , SBW and ABW were 61.8, 65.5 and 74.2 % in cotton fields treated with the local isolate of *B. thurengeinsis* strain I(figure 3). While, the mean redaction percentages of infestation in Agerin treatment were 48. 5, 56.9 and 66.1 %, respectively in comparison with control. In the second season (2007) the mean reduction percentages of infestation were 60.4, 55.6 & 63.5 % (for local isolate of *B. thurigiensis*) and 47.7, 46.0 and 52.4 % (for Agerin treatment), respectively.

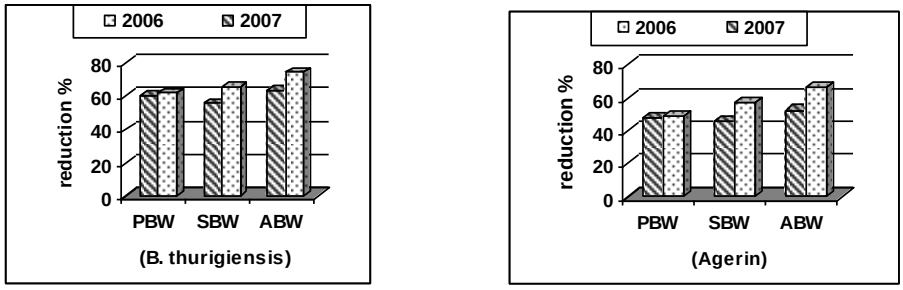


Figure (3) :The mean redaction percentage of infestation caused by pink (PBW), spiny (SBW) and American (ABW) bollworms in cotton field treated with *B. thurengeinsis* strain I and Agerin in comparison with control field during 2006 and 2007 seasons.

DISCUSSION

During the course of this study, three strains of entomopathogenic bacteria(strain I, II and III) belonging to *Bacillus thurengeinsis* were isolated from the pink (*P. gossypiella*) , spiny (*E. insulana*) and American bollworms (*H. armigera*. In addition, two bacterial pathogens (*Striptomyces avermecti* and *Micrococcus* sp.) were isolated from the bollworm larvae. Also, *B. thringiensis*, *S. avermecti* and *Micrococcus* sp. were isolated from cotton bollworm larvae (Abul-Nasr *et al.*, 1979 , Bekheit *et al.*, 1995 and El-Barbary, 2006).

Laboratory experiments indicated that pathogenic bacteria, *B. thurigiensis* especially strain I had the highest pathogenicity to the pink , spiny and American bollworms, while *S. avermecti* ranked second in the order of activity. *Micrococcus* sp. exhibited the lowest pathogenicity to the tested larvae. Similar results were obtained by El-Barbary (2006). He mentioned that *B. thurengeinsis* exhibited the highest pathogenecity to pink and spiny bollworms in comparison with *S. avermecti* and *Micrococcus* sp.. Also, Abdel-Mageed (2005) recorded that *B. thurigiensis* was the most effective entomopathogen on seychellarum mealybugs followed by *Micrococcus* sp. and *S. avermecti*.

According to El-Hamady (1997), *B. thuringensis* is the most widely exploited pathogen, as its activity is attributed to endotoxin contained in the para sporal body (crystal). The mode of action of the tested bacterial species could be attributed to secretion of exochitinase activity when grown in a medium containing chitin. Allosamidin, a specific chitinase inhibitor secreted from certain *B. thurengeinsis* strains (Graham, 1998).

The effectiveness of the local isolate of *B. thuringiensis* (strain I) as compared with recommended commercial biocide (Agerin) a *B. thuringiensis* (strain I)gainst *P. gossypiella* , *E. insulana* and *H. armigera* was evaluated during two successive cotton seasons. The obtained data showed that Agerin had less efficiency for control the Pink , Spiny and American bollworms as compared with the local isolate , where the local isolate of *B. thuringiensis* (strain I) highly reduced infestation percentages with bollworms larvae in comparison with Agerin during the cotton seasons. Also, Abdel-Halim et al., 2002 reported that Agerin showed less efficiency against cotton bollworms. So, isolation of the pathogens from the native ecosystem can help in successful biological control program (Keller, 1998).

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مقارنة فاعلية العزلات البكتيرية المحلية بالتجهيزات البكتيرية التجارية (الاجرين) على ديدان اللوز

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[١] قسم الحشرات الاقتصادية – كلية الزراعة – جامعة المنصورة – مصر .

[٢] مركز البحوث الزراعية – وزارة الزراعة – مصر .

أجريت الدراسة الحالية في منطقة أجا بمحافظة الدقهلية على محصول القطن خلال موسمي ٢٠٠٦ و ٢٠٠٧ لجمع وعزل وتعريف المسببات المرضية المصاحبة لديدان اللوز (القرنفلية والشوكية والأمريكية) ومقارنة كفاءة هذه العزلات المحلية بالمستحضر التجاري (الأجرين ٦,٥ %) على هذه الحشرات.

ويمكن تلخيص النتائج المتحصل عليها فيما يلي :

1- تم عزل وتعريف ثلاث سلالات من بكتريا الـ *Bacillus thurengeinsis* بالإضافة إلى نوعين آخرين هما *Micrococcus* sp , *Streptomyces avermecti* ، وذلك من يرقات ديدان اللوز القرنفلية والشوكية والامريكية .

2- أثبتت السلالة رقم (١) من بكتريا *B.thurengeinsis* كفاءة عالية في إحداث العدوى لأنواع ديدان اللوز الثلاثة بالمقارنة بالسلالات والأنواع الأخرى المعزولة.

3- أجريت دراسة حقلية لمقارنة كفاءة العزلة المحلية المعزولة *B. thurengeinsis* (strain I) والمستحضر التجاري(الأجرين ٦,٥) على ديدان اللوز الثلاثة : أوضحت النتائج أن العزلة المحلية *B. thurengeinsis* (strain1) قد أحدثت خفضاً كبيراً فى نسبة الإصابة بديدان اللوز الثلاثة فى الموسم الأول والثاني بالمقارنة بالكنترول.

- فقد بلغت نسبة الخفض فى نسبة الإصابة بدودة اللوز الشوكية والأمريكية والقرنفلية ٦١,٨ ، ٦٥,٥ و ٧٤.٢% فى الموسم الأول بينما كانت ٦٠,٤ ، ٥٥,٦ و ٦٣,٥% فى الموسم الثانى.
- بينما فى معاملة الأجرين فقد بلغت نسبة الخفض لنسب الإصابة ٤٨,٥ ، ٥٦,٩ و ٦٦,١ %(فى العام الأول) و ٤٧,٧ ، ٤٦ ، ٥٢,٤ %(فى الموسم الثانى) على التوالى .