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CONTROL OF THE EGYPTIAN COTTON LEAFWORM *Spodoptera littoralis* (BOISD.) BY USE OF FORMULATED BACTERIA.

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ABSTRACT

The efficacy of two pathogenic bacteria *Bacillus thuringiensis* var. *kurstaki* HD129 and *Serratia marcescens* were evaluated on 3rd and 5th larval instars of *Spodoptera littoralis*. These bacterial products were isolated by the Ain Shams Center of Genetic Engineering and Biotechnology.The commercial product *Bacillus thuringiensis* var. *kurstaki* "Protecto" was considered as a standard for comparison. Although, both two tested bacterial isolates had a high LC_{50} than the commercial product Protecto, both exhibited higher accumulative mortality. This effect was more apparent for *Serratia marcescens* than HD129 i.e. 66.5% and 62% respectively for treatment of 3rd instar. Furthermore, the time required to kill 50% of insects (LT_{50}) was the lowest when *Serratia marcescens* was tested, it was 4 days for both larval instars treated. Meanwhile, this period was 5 and 8 days for 3rd and 5th instar larvae treated with HD129 and it averaged 7 days for both treated instars when Protecto was used. Percentage of malformation was higher and number of larvae pupation was lower when the two tested bacterial isolates were tested than commercial product.

INTRODUCTION

Microbial control is alternatives to chemical control agents to insect pests and is often species specific. Microbial control agents although may not meet the speed of action of chemical insecticides. They have been generally shown to have no negative impacts on plants and mammals or even non-target insects . Bacterial biopesticides are dominated by *Bacillus thuringiensis* strains, meanwhile the virus nuclear polyhedrosis virus (known as NPV) generally plays a significant role.

The Egyptian cotton leaf worm *S. littoralis* is an insect pest of an economic importance with a wide range of host plants. This species has acquired resistance to many insecticides and the use of other control measures is essential to aid in an over all IPM. program Many lepidopteran species have been successfully controlled by microbial agents, e.g control of *S. littoralis* by *B.thuringiensis* [El-Hamaeky *et al.*, 1990; Salama *et al.*, 1993; Salem 1995;EL- Gahr *et al.*1995. ; Salama and Foda 1982; Salama *et al.* 1984] or control by using NPV (Salama *et al.* 1993; Harapaz and Wysoki 1984) .

The present study was conducted to evaluate the control effect of *B. thuringiensis* var. *kurstaki* HD129 and a strain of the bacterium *Serratia marcescens* on larvae of the cotton leaf worm *Spodoptera littoralis*.

MATERIAL AND METHODS

The original colony of the cotton leaf worm *S.littoralis* was obtained from a well-established culture, maintained at the Department of Plant Protection Faculty of Agriculture, Ain Shams University. Insect rearing was conducted in the laboratory as described by Youssef (1991).

Bacterial Cultures: -

The potency of two bacterial isolates were evaluated towards 3rd and 5th instar larvae of *S. littoralis*. The following isolates of bacteria were tested:

(I) *Bacillus thuringiensis* var. *kurstaki* [HD129]

(ii) *Serratia marcescens*.

These two isolates were kindly supplied as slants from Ain Shams Center of Genetic Engineering and Biotechnology to evaluate the efficiency of these two isolates, the commercial product *B. thuringiensis* var. *kurstaki* (Protecto) was used as a standard for comparison this product was obtained as a wettable powder from the Plant Protection Research Institute, Ministry of Agriculture, Cairo.

Maintenance of *B. thuringiensis* var. *kurstaki* (HD129): -

Subcultures from the bacteria *B. thuringiensis* var. *kurstaki* (HD129) were made by inoculation in a defined media of Pepton Yeast Extract as described by Mohammed (2002). The inoculated flasks were incubated at 30± 1^{0} C for 24h on a shaker set at 100-150 rpm. Pepton yeast extract agar plates were streaked by inoculate of the grown bacteria in the cultured test tubes using the streaking dilution method to obtain solitary pure colonies. Plates were incubated for 24h. at 30± 1^{0}C . Solitary colonies grown on the agar surfaces were selected and subcultured on agar slant and kept until needed for the experimental work.

Maintenance of the bacteria *S. marescens*: -

Subcultures from the bacterial samples *S. marcescens* were made by inoculation of Pepton Glycerol media. The inoculated flaskes were incubated at 30± 1^{0} C for24h on shaker (set 100-150 rpm.) to oblain solitary pure colonies Pepton Glycerol agar plates were streaked by inoculate of the grown bacteria in the cultured test tubes and incubated for 24h. at 30± 1^{0} C. Solitary colonies grown on agar surface were selected and subcultured on agar slants and reserved until required for the experimental work.

Bioassay for bacteria: -

One ml. of each of subculture HD129 and *Serratia marcescens* was placed in 100ml distilled water. As descriped by Schlegel (1986), series of dilutions were prepared [1%, .01%, 1x10^{-4}%, 1x10^{-6}%, 1x10^{-8}%, 1x10^{-10}%] from which the number of colony forming unit (cfu) were determined.

The commercial product Protecto (obtained as a wettable powder) series of dilutions were prepared from 1gm of the product [1%, .01%, 1x10^{-}

^{4}%, $1x10^{-6}$%, $1x10^{-8}$%, $1x10^{-10}$%]. Also the number of colony forming unit (cfu) were counted according to Schlegel (1986).

The larvicidal activity of the bacterial strains was evaluated on newly moulted 3rd and 5th instar of *S. littoralis* larvae. Fresh Castor oil leaves were cut in leaf discs, measuring 3 cm indiameter. These discs were immersed in each of the prepared dilution of each tested strain and then left to dry at room temperature before being offered to the 3rd and 5th instar larvae confined in plastic cups. Larvae well fed on contaminated leaf discs for 3 days and then provided with uncontaminated leaf discs for the subsequent duration of the larval instars. Each treatment was comprised 25 larvae and was replicated 5 times. The same numbers of larvae were considered as a control, which was offered castor oil leaves immersed in distilled water. Larval development as well as pupal survival and level of infection were considered, any malformation and sequence of infection were recorded. The sequences of symptoms of infection were recorded as well as larval development. Mortality was calculated daily and an accumulative larval mortality was determined at the end of the larval stage. Mortality percentages were corrected according to Abbott (1925) formula. Results were presented graphicelly as log/probit regression lines and LC_{50} values calculated by computer program Sigma plot for Windows (version 21). Furthermore, any malformation of larvae or pupa was recorded. As the standard commercial product Protecto is of known potency, the LC_{50} of the two tested bioagents HD129 and *Serratia marcescens*. Potency was calculated by the following formula, as described by Salama and Foda (1982).

$$\text{Potency sample (IU\mg)} = \frac{LC_{50}\ \text{standard}}{LC_{50}\ \text{sample}} \times \text{potency of standard (IU\mg).}$$

RESULTS

A range of concentrations was prepared from (HD129) and *S. marcescens*. These preparations were tested on 3rd and 5th instar larvae of *S. littoralis* (Boisd) HD129 and *S. marcescens*. toxicity was exhibited in a dose dependent phenomenon. Generally, the symptoms of the toxins to treated larvae could be summarized in the following sequence: -

(i) Loss of appetite as insect food consumption decreases as denoted by smaller castor oil leafs surface area eaten.
(ii) Decrease response to stimulation.
(iii) Diarrhea and larvae regurgitating vomiting of some fluids.

Furthermore, toxicity of HD129 was exhibited by the appearance of spots on the prolegs that then extend as dark brown on the abdomen then to the entire body. The larvae's body contents were soft to touch and the integument with a firm texture. Meanwhile, infection with *Serratia marcescens* toxins causes the appearance of a reddish pink pigmentation first on the prolegs that then extend on the whole integument, the infected larvae become very soft and the integument ruptures easily. Both tested bioagents lead to subsequent larval paralysis and death. From the plotted regression

lines the LC_{50} values of the tested toxins were determined, results are shown in Fig (1,2). Also LT_{50} and accumulative percentage mortality of larvae are exhibited in Table (1,2).

Table (1): Potency of at LC_{50} values on 3rd instar larvae of *Spodoptera littoralis*.

Bioagents	LC_{50} (cfu)	Slope	Potency (IU \ mg)	LT_{50} (days)	Accumulative % mortality (at the end of larval stage)
Protecto	40*105	0.37273	32000	7	56%
HD129	65*105	0.312715	52000	5	62.1%
S.marcesens	105*107	0.299380	84000	4	66.5%

(F) between treatments= 0.29327 (sign.) LSD=11.724

Table (2): Potency of at LC_{50} values on 5th instar larvae of *Spodoptera littoralis.*

Bioagents	LC_{50} (cfu)	Slope	Potency (IU \ mg)	LT_{50} (days)	Accumulative % mortality (at the end of larval stage)
Protecto	35*107	0.3171522	32000	7	62%
HD129	46*107	0.285920	42057	8	56%
S.marcesens	112*107	0.278338	102400	4	64%

(F)between treatments =1.32071 (sign.) LSD=8.310

Protecto, the commercials *B. thuringiensis* var. *kurstaki* was used as a standard in a range of concentration and LC_{50} determined under conditions of the present work (Fig (3)). It's LT50 and accumulative percentage are shown in Tables (1,2), it was obvious that this commercial product was the most toxic to *S. littoralis* larvae either treated as 3rd or 5th instars. The LC_{50} was $40X10^5$ and $35X10^7$ cfu. respectively HD129 was more toxic than *S. marcescens*. It's LC_{50} was $65X10^5$ and $46X10^7$ cfu for 3rd and 5th larval instars respectively. Meanwhile, for *S. marcescens* these values were $105X10^5$ and $112X10^7$ cfu for the respective mentioned instars. However, the LT_{50} of *S. marcescens* was slightly more rapid than HD129, as 50% of treated 3rd instar larvae died after 4 days approximately.

Meanwhile, LT_{50} was 5 days when HD129 was used, but was extended to 7 days when Protecto was used. As expected 5th instar larvae were much more tolerant than 3rdinstar's. This was evident for the two tested bioagents as well as the standard commercial Protecto. The accumulative percentage mortality (at the termination of the larval stage) was higher when *S. marcescens* was tested than for the use of HD129 or Protecto, (Tables 1,2). It was found to be 66.5 % and 64%for 3rd and 5th instars respectively as compared to 62 % and 56% when HD129 was tested for the respective mentioned larval instars.

The potency sample of *S. marcescens* was much higher than that of HD129, as this potency was 84000 and 102400 IU\mg for the treatment of 3rd and 5th instar larvae respectively, (Table 1,2). This value could be expressed a ratio increase of 1:16 and 1:24 than that of HD129. Some larvae of *S. littoralis* recovered from the toxins up on transfer to control diet.

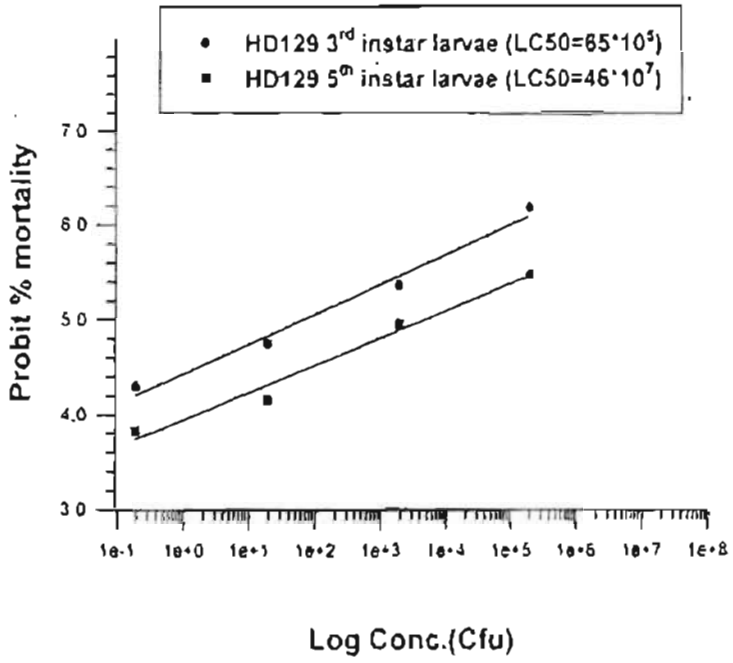

Fig (1) Effect of HD129 on 3rd &5th instar larvae of *S. littoralis*

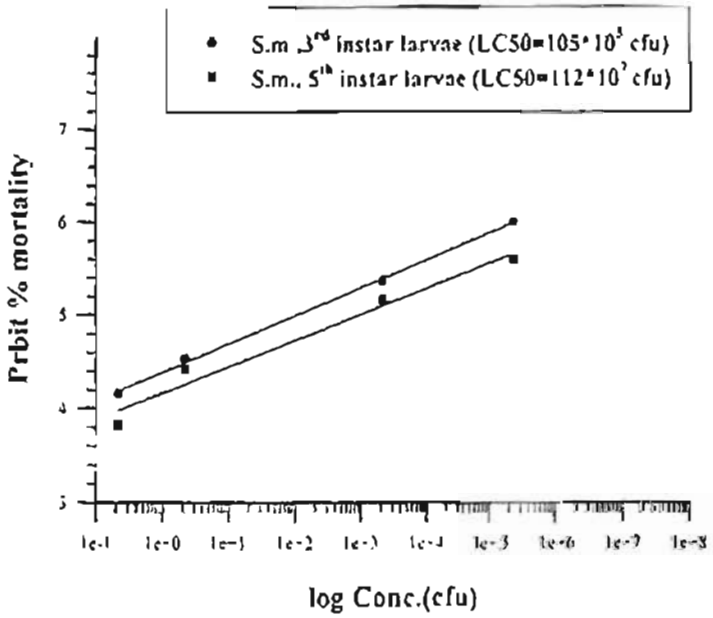

Fig (2) Effect of *S.marcescens* on 3rd &5th instar larvae of *S.littoralis*

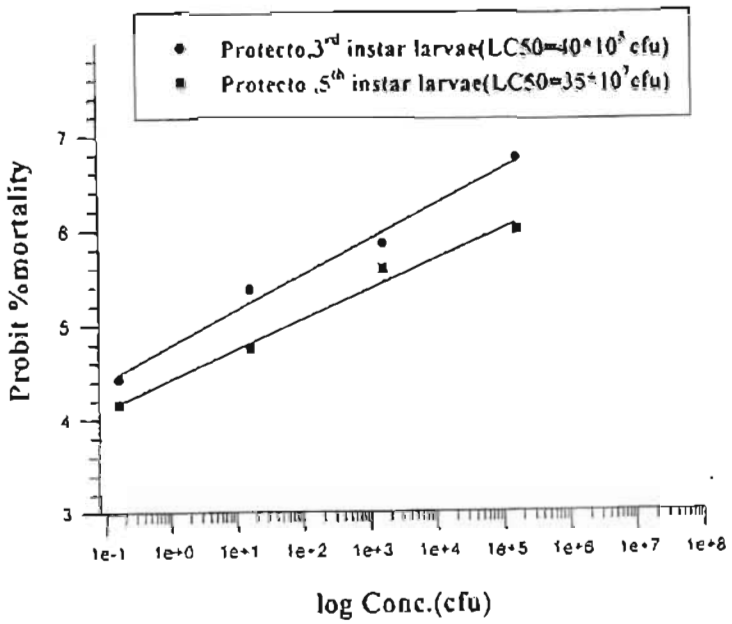

Fig (3) Effect of Protecto on 3rd &5th instar larvae of *S. littoralis*

Meanwhile, other larvae appeared with some abnormalities, which was more evident upon moulting (Fig 4). When 5th instar larvae were infected with LC_{50} of the tested HD129 and *S. marcescens* the duration of the subsequent instars of the larvae that survived was not significantly different than those of the control. Meanwhile, for the treatment of 3rd instar larvae, only the duration of the 6th instar was slightly impaired, the period of this last instar was shortened by 36-48 hours than the control. Following treatment with the two tested bioagents at LC_{50} value, the number of surviving larvae pupating was reduced between 30-36 % for both instars treated. Meanwhile, it was between 26-28 % when Protecto was used. The percentage of malformed pupae was more evident when 5th instar were treated, especially with treatment by LC_{50} of HD129 as it reached 12% as compared to 4 and 8 % when 3rd instar were fed on castor oil leaves contaminated with LC_{50} of *S. marcescens* and Protecto respectively.

Malformation of pupa was mainly observed as shortening of their length and appearance of larvae-pupal intermediates, (Fig 5), in all treatment adult ecdsion was totally inhibited as Insect failed to emerge as moths or died as pupa.

Fig (4): Malformed larvae of *Spodoptera littoralis* following treatment by LC_{50} of HD129 as 5th instar.

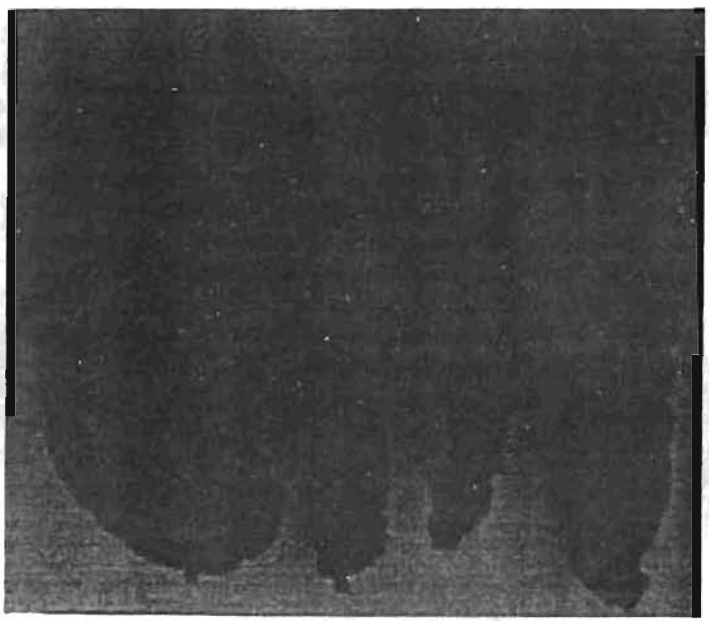

Fig (5): Malformed pupa of *Spodoptera littoralis* following treatment by LC_{50} of HD129 as 5th instar.

DISCUSSION

New isolates of bacteria have to be established so as to avoid the building of resistant of *S. littoralis* to this bacterial bioagents. In the present work a new isolates of *B. thuringiensis* var. *kurstaki* was tested i.e. (HD129) as well as the bacterium *S. marcescens*.

Potency of these two bioagents were compared with a standard commercial *B. thuringiensis* var *kurstaki* product Protecto widely used for the control of many lepidopterous insects [El- Hamaeky *et al.* 1990 , Vandenberg & Shimanuki 1990]. This commercial product proved to have a higher toxic effect than the other two bioagents investigated, as exhibited by it's much lower LC_{50} value. This is somehow expected as it being a commercial product it must has a longer persistence or activity as probable results of the addition of adjuvants or additives to achieve high efficacy. Meanwhile, HD129 and *S. marcescens* are newly prepared isolates. However the potency of the two tested bioagents was quite comparable, LC_{50} and potency sample of HD129 were much lower than that of *S. marcescens* which in contrast exhibited the highest LC_{50} value calculated. Meanwhile, Farrar *et al.* (1998) reported that the bacterial isolate *S. marcescens* killed the corn earworm with a very low oral dose. However, LT_{50} of *S. marcescens* was the lowest exhibiting 4 days for larvae treated either as 3rd or 5th instars. This period was the maximum-recorded i.e. 7 days when Protecto was used. It is a well-known fact that older larvae are usually much more tolerant to the toxic effect of many bioagents [Mohamed *et al.* 2000, Romeilah and Abdel-Megeed 2000].

This site of action of *B. thuringinese* toxin is the insect mid gut epithelium Gill *et al.* (1992) and one of the symptoms of poisoning is gut paralysis Gould and Anderson (1991). The bacterial bioagents do not cause a rapid kill, therefore their effect becomes apparent after a few days as infected insects eat little, later leading to starvation and death. Form the obtained results, it seems that *S. marcescens* caused much more rapid toxic effect, one or more factors are probably responsible for the potency of this toxin. Furthermore, the potency of *Serratia marcescens* when used at LC_{50} values was superior to Protecto and also slightly higher than HD129 to 3rd and 5th instars. This was exhibited in a higher accumulative mortality percentage as well as a higher reduction in larvae entering the pupal stage. Although, with higher LC_{50} value HD129 was more efficient for the control of *S. littoralis* larvae than Protecto. The binding characteristics of HD129 to the mid gut epithelium of the infected larvae could be involved as suggested by Herreo *et al.* (2001) and Gilliand *et al.* (2002).

REFERENCES

Abbott W.S. (1925): A method of computing the effectiveness of an insecticide. J.Econ.Entomol., 18 (2): 256-267.

El-Ghar GESA.; HAS. Radwan ; ZA. ElBermawy and LTM. Zidan (1995). Inhibitory effect of *Bacillus thuringiensis* and abanectin on digestive enzymes and non specific esterases of *Spodoptera littoralis* (Boisd). (Lepidoptera : Noctuidae). Larvae. J. Applied. Entomo., 119(5): 355-359.

El-Hamaeky M.; AF. Refaei ; MA. Heagazy and NM. Hussein (1990). Knock-down and residual activity of certain insecticides *Bacillus thuringiensis* and their binary mixtures against the cotton leaf worm *Spodoptera littoralis* (Boisd) in cotton fields. Mededelingen-van-de-Faculteit-Landbouwweten schappen, Rijks Universiteit. Gent. 55:2b, 593-599.

Farrar R.R .;Martin and R.L.Ridgway (1998).Astrain of Serratia marcescens with high virulence to the corn ear worm .Agric Res. Service. 3:1-2

Gill S.S.; E.A .Cowles and P.V. Pietrantonia (1992). The mode of action of *Bacillus thuringiensis* endotoxins. Annu. Rev. Entomol. 37: 615-636.

Gilliand A.; C.E.. Chambers ; E.J. Bone and D.J .Ellar (2002). Role of *Bacillus thuringiensis* cry10 endotoxin binding in determining potency during lepidopteran larval development. Appl. Environ. Microbial. 68(4): 1509-1515.

Gould F. and A .Anderson (1991). Effects of *Bacillus thuringiensis* and HD73 delta endotoxin on growth, behavior and fitness of susceptible and toxin a dapted s trains o f *H eliothis v irescens* (Lepidoptera: Noctuidae). Environ. Entomol. 20: 30-38.

Harpaz I. and M. Wysoki (1984). Susceptibility of the Carob moth Ectomyelois ceratoniae to *Bacillus thuringiensis*. Phytoparasitica. 12 (3-4): 189-191

Herrero S.; J. Gonzalez-Cabrera ; B.E.. Tabashnik and J. Ferre (2001). Shared binding sites in Lepidoptera for *Bacillus thuringiensis* cry 1J cry 1A toxins. Appl. Environ. Microbial. 67(12): 5729-5734.

Mohamed Manal S. (2002). Genetic improvement of the efficiency of some bacterial biopesticide by gene manipulation techniques. B.Sc. Faculty of Agriculture, Ain Shams University, Shoubra El- Kheima, Cairo, Egypt.

Mohammed S.A.; N.A. Badr and A.A .El-Hafez (2000). Efficacy of two formulations of pathogenic bacteria *Bacillus thuringiensis* against the first instar larvae of *Spodoptera littoralis* (Boisd). and *Agrotis ipsilon* (Hfn.) (Lepidoptera: Noctuidae) Egyptian. J. Agric. Res. 78(3): 1025-1040.

Romeilah M. and M.A .Abdel Megeed (2000). The role of certain bacterial preparations (*Bacillus thuringiensis)* in controlling the cotton leaf worm *Spodoptera littoralis* (Boisd). Egyption. J.Agric. Res. 7 8(5): 1 877-1887.

Salama H.S. and M.S. Foda (1982). A strain of *Bacillus thuringiensis* var *entomocidus* with high potential activity on *Spodoptera littoralis* (Boisd). J. Invert.Path. 39: 110-111.

Salama H.S.; M.S. Foda and A .Sharaby (1984). Novel biochemical avenues for enhancing *Bacillus thuringiensis* endotoxin potency against *Spodoptera littoralis* (Boisd). (Lepidoptera: Noctuidae). Entomophaga, 29 (2): 171-179.

Salama HS.; A .Sharaby and MM. El-Din (1993). Mod of action of *Bacillus thuringiensis* and nuclear polyhedrosis virus in the larvae of *Spodoptera littoralis* (Boisd). Insect Science and it's application 14 (4): 537-543.

Salem S.A. (1995). Integrated biological control of *Spodoptera littoralis* (Boisd) in Soya bean cultivation using six pheromones and *Bacillus thuringiensis*, chemical, physiological and environmental aspects. First International Conference, Ladek-Zdroj, Poland, pp. 323-328.

Schlegel Hans G. (1986). General microbiology. Sixth Edition. PP: 188-191.

Vandenberg J.D. and U . Shimanuki (1990). Application methods for *Bacillus thuringiensis* used to control larvae of the greater wax moth (Lepidoptera: Pyralidae) on stored bee wax combs. J. Econ. Entomol. 83(3): 766-771.

Youssef L. A. (1991). Physiological disorders of the cotton leaf worm *Spodoptera littoralis* (Boisd). fed on certain plants synthetic media. B.Sc. Faculty of Agriculture, Ain Shams University, Shoubra El-Kheima, Cairo, Egypt.

مكافحة دودة ورق القطن المصرية باستخدام مستحضرات بكتيرية

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تم فى هذه الدراسة تقييم اثنان من المستخلصات البكتيرية والتى تم استخلاصها بمركز الهندسة الوراثية كلية الزراعة جامعة عين شمس وهما بكتريا *Bacillus thuringiensis* var. *kurstaki* والمعروفة باسم (HD129) و بكتريا *Serratia marcescens* حيث اجرى التقييم على العمرين الثالث والخامس ليرقات دودة ورق القطن ، كما تم استخدام المركب التجارى البر وتكتو Protecto (*Bacillus thuringiensis* var. *kurstaki*) كمركب قياسى .وقد أوضحت الدراسة زيادة التركيز القاتل ل٥٠% من التعداد الكلى لليرقات لكلا المستخلصين عنه فى المركب القياسى مع زيادة نسبة الموت للتراكمية فى اليرقات المعاملة ببكتريا *Serratia marcescens* عنها فى اليرقات المعاملة ببكتريا (HD129) حيث وصلت الى ٦٦,٥% و ٦٢,١% للعمر الثالث لكلا المستخلصين على التوالى ، اضافة الى ان الفترة اللازمة لقتل ٥٠%من التعداد الكلى لليرقات كانت اقل عند استخدام بكتريا *Serratia marcescens* حيث كانت ٤ أيام لكل من العمرين الثالث والخامس مقارنة بـ ٨ أيام لكل من العمرين الثالث والخامس على التوالى عند استخدام بكتريا (HD129) أما عند استخدام المركب القياسى فكانت هذه الفترة ٧ايام ، كما أوضحت الدراسة أن النسبة المئوية للتشوه فى اليرقات والعذارى كانت اقل عند استخدام بكتريا (HD129) أو بكتريا *Serratia marcescens* عنها عند استخدام المركب القياسى.