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Toxicity and Biological Effects of Some Bacterial Isolates on American Bollworm, *Helicoverpa armigera* (Hubner) and Spiny Bollworm, *Earias insulana* (Boisd.)

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ABSTRACT

The present studies were carried out at Plant Protection Research Institute, Sharkia branch, ARC to study toxic and biological effects of some bacterial isolates; *Pseudomonas plecoglossicida*, *Bacillus endoradicis* and *Bacillus pumilus* which isolated from the contaminated artificial diet, dead pupae of *Helicoverpa armigera* (Hunber) by washing and grinding, respectively against the newly hatched larvae of *H. armigera* and *Earias insulana* (Boisd.). Results showed that all bacterial isolates had toxic effect on the newly hatched larvae of the two insects. Increasing the concentration of each bacteria caused a gradual increase in mortality percentage of the newly hatched larvae of american and spiny bollworms. The newly hatched larvae mortality percentages increased as the days post- treatment increased for the same bacterial isolates. *P. plecoglossicida* had the highest mortality percentage (70.25 & 79.25%) and (68.25 & 77.00%) of the newly hatched larvae of american and spiny bollworms after two and four days from treatment, while the lowest mortality percentage were (39.00 & 61.00%) and (59.5 & 67.00%) recorded with *B. endoradicis*, respectively. These isolates cleared the different effects on all stages of american and spiny bollworm and decreasing pupal weight, pupation & adult emergence percentages, male and female longevity, deposited eggs and hatchability percentages as compared with control. Each strain identified and characterized by Biolog system.

Keywords: *Pseudomonas plecoglossicida, Bacillus endoradicis, Bacillus pumilus*, *Helicoverpa armigera* and *Earias insulana*

INTRODUCTION

The societies in the worldwide are facing with the problem of increasing the use of pesticides against agricultural pests in the absence of their natural enemies or bio-agents also using conventional chemical pesticides has direct and indirect risks to human (Butt *et al*., 2001). On the other hand many insects have developed resistance for many chemical pesticides during the last few years, biologists have turned their attention to the possibility of using other organism as biological control agents and the microbiologists contributing in the development of the efficacy of microbial substances (bacteria, fungi and virus) for the control of many insect pests (Mc Spadden Gardher, 2004). Several studies have been demonstrated the insecticidal properties of different bacterial species such as *Bacillus cereus*, *B. sphaericus*, *Morganella marganii*, *Serratia marcescens*, *Klebsiella* sp. and *B. thurengensis* against different insects (Wright *et al*., 2007 and Wu, 2007). For example; a new strain of *B.laterosporus* showing toxicity against the larva of mosquitoes, black flies, Coleoptera and Lepidoptera (Ruiu*et al*., 2006). While *B. sphaericus* was recorded as effective biological control agent against mosquitoes and black flies (Silva-Filha and Peixota, 2003). Recently, *B. thuringensis* strains are the most common entomopathogenic bacteria against large number of insects such as cotton boll weevil, cotton bollworm and *Acedesaegypti* larvae (Lacey and Arthurs, 2006). Also, Mahfouz and Abou El-Ela (2011) demonstrated the mortality effect of *Bacillus cereus* on *Pectinophora gossypiella (*Saunders).

The entomopathogenic bacteria invade insects through the alimentary canal or penetrate the host exoskeleton (cuticle) (Hajek and St Leger, 1994 and Gillespie *et al*., 1998). Ingress through the cuticle is facilitated by a combination of mechanical force and enzymatic degradation (Goettle *et al*., 1989).

The main objectives of this study was to isolate some bacteria from dead pupae of american bollworm and its contaminated artificial diet also to study the efficiency of these isolates on american and spiny bollworms

MATERIALS AND METHODS

I-Rearing technique:

Colony of american and spiny bollworms used in this study were obtained from the Bollworms Research Department, Plant Protection Research Institute, Sharkia branch and the experiments were carried out in the same laboratory. The experiments were performed at constant temperatures 26± 1°C and 75±5% RH. The diet for maintaining laboratory colony was followed according to the method described by Amer and Elsayed (2015). The colony was away from any contamination with insecticides and microorganisms.

II- Bacteria isolation technique:

The dead pupa was stored slowly in sterilized tightly closed vials at 4^{o}C in refrigerator until needed (Mahfouz and Abou El-Ela, 2011). In order to reveal any microorganisms associated with the subjected insects, each of the refrigerated individuals was examined through 24-72hr. from the time of storage under aseptic conditions. The pupae were surface sterilized by the dipping in 2% sodium hypochlorite for 3-5 minutes. Then the pupae passed through five separate washing with sterile distilled water (Crecchio and Stotzky, 2001). For insuring the appropriate surface sterilization, checks were made by spreading the last washing solution on nutrient agar. Sterilized pupae were dried up between two filter papers (Whattman No. 1), then transferred aseptically into a sterile mortar and macerated with a sterile pestle, diluted and plated on nutrient agar for growth incubating at 30 ^{o} C for 1-5 days. Incubated plates were inspected daily to observe the colonies growth that were then purified and stored on slants of the desired artificial media at 4^{o}C. The isolates were cultured periodically until they had been used in the subsequent experiments. Healthy pupae were subjected to the same procedures of isolation for obtaining the expected dormant pathogens.

III- Mortality effect of the bacterial isolates on *H. armigera* and *E. insulana* newly hatched larvae:

Spores were obtained by washing the old slant of tested bacterial isolates (Dulmage *et al.,* 1971 and Mohd-Salleh and Lewis 1983) then, inoculated 10 ml of nutrient broth for selected bacteria in a 250 ml Erlenmeyer flask

with each suspension. The inoculated broth was incubated at 30 ^{o} C. All isolates were tested for their mortality effect against american and spiny bollworms. One ml from spore suspension (stock, 10^{-2}, 10^{-3} and 10^{-6}) of each isolate was mixed with artificial diet free from microbial agents. The petri dishes were allowed to dryness. Twenty newly hatched larvae of American and spiny bollworms were transferred to each treated and untreated petri dishes and repeated five times. The dishes were examined after two and four days from treatment. Dead larvae were counted and also mortality percentages.

Identification:

The most potent bacterial isolates were preliminary identification using gram staining and biochemical testes to differentiate between isolates. Colonies of bacterial isolates were characterized 24h post incubation with carbon source utilization was determined by using biology GN Micro plates compering outputs to the Micro Log System 2 database (Biolog, CA, USA). Proto type strains used in taxonomic comparisons were obtained from the central laboratory of Biotechnology, Plant Pathology Research Institute, Agricultural Research Center.

IV-Bioassays:

Effect of bacterial isolates on some biological aspects of American and spiny bollworms:

To study effect of the bacterial isolates on some biological aspects of the two insects, the newly hatched larvae of *H. ar*migera and *E. insulana*, were fed on the diet treated with different concentrations of bacterial isolates. Two concentrations from each isolates were prepared (10^{-3} and 10^{-6}). One ml from each concentration was mixed with four gm of diet without antimicrobial agent on petri dishes. While the diet of control mixed with water only. Each treatment was replicated five times. The dishes were left until dryness. Twenty five newly hatched larvae from each insect were transferred to the treated and untreated Petri-dish for each replicate. All treatments were incubated in the electric incubator running at constant conditions 26 ± 1^{o}C and 75 ± 5% RH after two days of treatments, the alive larvae were transferred singly to untreated diet and incubated under the previous conditions. The larval duration, larval mortality, adult emergency, deformed adult, sex ration, male and female longevity and hatchability percentage were recorded.

Data obtained were statically analyzed according to Little and Hills (1975) using Costat computer program Cohort Software. P. O. Box 1149, Berkeley CA 9471 (Costat program methods, 2005).

RESULTS

Identification of bacterial isolates

Data in Table (1) illustrated that the bacteria isolated from contaminated artificial diet of *H. armigera* identified as *Pseudomonas plecoglossicida* with biolog system. Also bacteria isolated from pupae of *H. armigera* (by washing and grinding) identified as *Bacillus endoradici s*and *Bacillus pumilus* with biolog system.

Results in Table (2) showed that increasing the concentration of each bacteria caused a gradual increase in mortality percentage of the newly hatched larvae of American and spiny bollworms. The newly hatched larvae mortality percentages increased as the days post-treatment increased for the same bacterial isolates. The highest mortality percentages were of the newly hatched larvae of American bollworm after two and four days from treatment was 70.25 and 79.25% recorded with *P. plecoglossicida*, while, the lowest mortality percentages were 39.00 and 61.00% recorded with *B. endoradicis* as compared with 0 and 1% for control. On the other hand the highest mortality percentage of the newly hatched larvae of spiny bollworm after two and four days from treatment was 68.25 and 77.00% recorded with *P. plecoglossicida*, while the lowest mortality percentage was 59.5 and 67.00% recorded with *B. endoradicis* as compared with0 and 1% for control.

Table 1. Identification of bacterial isolates

Biolog ID	Source
Pseudomonas plecoglossicida	Contaminated artificial diet of *H. armigera*
Bacillus endoradicis	dead Deadpupae of *H. armigera* (by washing)
Bacillus pumilus	dead Deadpupae of *H. armigera* (by grinding)

Table 2. Mortality percentages of the newly hatched larvae of american and spiny bollworms fed on diet treated by bacteria.

Bacterial isolates	Conc.	Mortality percentages			
		American bollworm		Spiny bollworm	
		After 2 days	After 4 days	After 2 days	After 4 days
P. plecoglossicida	stock	86.00	100.00	80.00	100.00
	10^{-2}	84.00	100.00	77.00	82.00
	10^{-3}	59.00	63.00	70.00	76.00
	10^{-6}	52.00	54.00	46.00	50.00
	Mean	70.25	79.25	68.25	77.00
B. endoradicis	stock	45.00	65.00	72.00	78.00
	10^{-2}	40.00	76.00	70.00	76.00
	10^{-3}	36.00	57.00	61.00	74.00
	10^{-6}	35.00	46.00	35.00	40.00
	Mean	39.00	61.00	59.5	67.00
B. pumilus	stock	75.00	100.00	62.00	84.00
	10^{-2}	44.00	73.00	65.00	77.00
	10^{-3}	20.00	55.00	59.00	75.00
	10^{-6}	18.00	29.00	54.00	60.00
	Mean	39.25	64.25	60.00	74.00
control		0.00	1.00	0.00	1.00

American bollworm

1-Latent effect of bacterial isolates on certain aspects of American bollworm:

a- Larval stages:

Data in Table (3) showed that the *B. endoradicis* at concentration of 10^{-3} caused significantly shortened on *H. armigera* larval duration 15.38 days, the mean larval duration for all treated ranged between 15.38 and 17.75 days compared with 17.12 days for control. *B. pumilus* at concentration of 10^{-3} caused the highest significantly effect on larval mortality percentage (81.16%) followed by *P. plecoglossrations* (63.45%) while the lowest larval mortality was (47.08%) recorded for *B. endoradicis.* Highly significant effect were found between all concentrations of different isolates on larval mortality compared with untreated.

b- Pupal stage:

The obtained results in Table (3) indicated that all isolates caused insignificant reduction in *H. armigera* pupal weight compared with control. The pupal weights were ranged between 0.317 and 0.452g for the tested concentrations compared with 0.3913g for untreated. All

isolates caused insignificant effect in pupal duration of *H. armegera* compared with untreated. All treatments shortened pupal duration compared with untreated. Pupal duration ranged between 11.05 and 13.05 days as compared with 13.32 days for control. *B. pumilus* at concentration 10^{-3} caused highly significantly effect on pupation percentages which reduced to 18.83% as compared with control and all treatments also caused high significant decreased in pupation percentages. The pupation percentages were ranged between 18.83 and 52.91%, as compared with 97.52% in untreated.

Table 3. Latent effect of some bacterial isolate on certain aspects of American bollworm, *H. armigera.*

Treatment	Conc.	Larval Duration (days)	Larval Mortality %	Pupation %	Pupal duration (days)	Pupal Mortality %	Pupal weight (g)	Adult emergence %	Deformed adult %	Female sex ratio	female Long (days)	male Long (days)
B.	10^{-3}	15.38c	62.39b	37.58d	13.05d	39.2c	0.317c	53.81e	6.94	52.00	13.47b	13.05bc
endoradicis	10^{-6}	16.76a	47.08d	52.91b	12.43c	26.74b	0.318c	67.41cd	5.80	47.40	12.93b	12.42c
P.	10^{-3}	15.98cd	63.45b	36.54d	12.98abc	44.69a	0.321c	39.01f	16.28	52.00	13.27b	13.43b
plecoglossicida	10^{-6}	16.37bc	55.78c	44.22c	11.05d	21.21b	0.452a	75.47bc	3.246	54.93	13.06b	13.21bc
B.	10^{-3}	16.75ab	81.16a	18.83e	12.49bc	24.71b	0.391b	60.93de	14.36	51.99	12.98b	13.41b
pumilus	10^{-6}	17.18ab	63.17b	36.81d	12.73abc	13.19c	0.371b	82.98b	3.82	53.68	12.88b	13.33b
Control		17.12ab	2.47e	97.52a	13.32a	0.66d	0.3913b	99.33a	0	49.19	14.93a	14.44a
P		0.01*	0.000***	0.000***	0.000***	0.000***	0.000***	0.000***	Ns	Ns	0.000***	0.0062**
LSD 0.05		1.2478	6.2488	6.2542	0.6052	7.6151	0.028	10.8684			0.6235	0.8876

c- Adult stage:

Pseudomonas plecoglossicida at concentration of 10^{-3} caused highly significantly effect that reduced the adult emergence percentage to 39.01% as compared with 99.33 for control. The highest adult emergence percentage was 82.98 % for *B. pumilus* at concentration 10^{-6}. Highly significant effects were recorded between different concentrations of bacterial isolates and untreated. Insignificant effect was found between isolates. The highest deformed adult percentages were 14.36 % for *P. plecoglossicida* at concentration 10^{-3}as compared with control. Adult deformed percentages ranged between 3.25 and 14.36 % as compared with 0.00 % for control. *B. endoradicis* at concentration of 10^{-6} caused the highest effect on shortened male longevity (12.42 days) of the American bollworm emerged from treated newly hatched larvae as compared with control 14.44 days and all treatments also caused high significant shortened in the male longevity, it ranged between 12.42 and 13.43 days. *B. pumilus* at concentration of 10^{-6}caused the highest shortened female longevity 12.88 days of the american bollworm emerged from treated newly hatched larvae as compared with control 14.93 days and all treatments caused high significant shortened in the female longevity which ranged between 12.88 and 13.47 day. Sex ratio of female moths of the american bollworm resulted from treated newly hatched larvae with all different treatments caused insignificant effect as compared with control.

Spiny bollworm

1-Latent effect of bacterial isolates on certain aspects of spiny bollworm:

a-Larval stage:

Data in Table (4) showed that the *B. endoradicis* at concentration of 10^{-6} caused highly significantly shortened larval duration 13.69 days, while, *P. plecoglossicida* at concentration of 10^{-3} caused elongated the spiny bollworm larval duration compared with untreated. The mean larval duration for all treated ranged between 13.69 and 15.19 days compared with 14.23 days for control. *B. endoradicis* at concentration of 10^{-3} caused highly significantly effect on spiny bollworm larval mortality percentage 86.89%, while at concentration of 10^{-6} caused the lowest larval mortality (40.56%) compared with 2.03% in untreated check.

b- Pupal stage:

Bacillus endoradicis at concentration 10^{-3} caused highly significantly decreased pupation percentages 13.08% as compared with control. All treatments caused high significant decreased in pupation percentages compared with 97.96% in untreated. All treatments caused slightly reduction in pupal weight of the spiny bollworm compared with control. The pupal weights ranged between 0.073 and 0.075gm compared with 0.0758g for control. All treatments caused insignificant effect in pupal duration compared with control which ranged between 9.75 and 10.11 days as compared with 9.78 days for control.

Table 4. Latent effect of some bacterial isolate on some biological aspects of spiny bollworm, *E. insulana*.

Treatment	Conc.	Larval Duration (days)	Larval Mortality %	Pupation %	Pupal duration (days)	Pupal Mortality %	Pupal Weight (g)	Adult emergence %.	Deformed adult %	Sex ratio %
B. endoradicis	10^{-3}	13.83c	86.89a	13.08f	9.75	24.00a	0.070bc	66.00de	10.00ab	50.00
	10^{-6}	13.69c	40.56e	59.43b	10.20	7.54c	0. 074ab	88.69ab	3.76b	50.56
P. plecoglossicida	10^{-3}	15.19a	77.58b	22.42e	10.18	19.86a	0.074abc	73.64cd	6.5b	51.32
	10^{-6}	13.69c	51.66d	48.33d	10.17	11.51bc	0.075a	88.48ab	0.00b	51.78
B. pumilus	10^{-3}	14.01bc	77.37b	22.62e	10.11	21.86a	0.073c	59.93de	18.21a	52.00
	10^{-6}	14.69ab	63.62c	36.36d	10.37	12.15b	0.074bc	84.18cd	3.67b	51.10
Control		14.23bc	2.03f	97.96a	9.78	0.00d	0.075a	100.00a	0.00b	50.05
P		0.0059 **	0.000 ***	0.000 ***	Ns	0.000 ***	0.0115 *	0.000 ***	0.0161 *	Ns
LSD 0.05		0.8365	5.543	5.5476		4.3007	0.001	11.907	10.4113	

b-Adult stage:

Results in Tables (4) and (5) indicated that the *B. pumilus*10^{-3} caused highly significantly reduced adult emergence percentages of the spiny bollworm (59.93%) as compared with control. Adult emergence percentages ranged between 59.93 and 88.69 % as compared with 100.00% in control. *B. pumilus*10^{-3} caused significantly effect on deformed adult percentages recorded 18.21% as

compared with control. Deformed adult percentages ranged between 0.00 and 18.21% as compared with 0.00 % in control. Sex ratio of male and female of the spiny bollworm moths resulted from treated newly hatched larvae with all different concentrations caused insignificant effect as compared with control. All treatments caused insignificant effect on shortened Pre-oviposition period than control. Pre-oviposition period ranged between 3.76 and 4.25 days as compared with 3.5 days in control. Oviposition period of female resulted from treated newly hatched larvae of spiny bollworm with all isolate concentrations were highly significant in shortened oviposition period as compared with control. Statistical analyses of tested treatments were non-significant influence on post oviposion periods of E. insulana female moths as compared with control.

All treatments were insignificant influence on shortened the male longevity of the spiny bollworm male moths resulted from treated newly hatched larvae as compared with control. All treatments were insignificant influence on shortened the female longevity of the spiny bollworm male moths from treated newly hatched larvae as compared with control. The mean number of deposited eggs of female resulted from treated newly hatched larvae with all treatments was highly significant effect as compared with control. The mean numbers of the deposited eggs were 168.00 and 172.00 eggs / female as compared with 193.00 eggs /female in untreated. The all treatments were decreased significantly the mean hatchability percentages of eggs were between 86.44 and 92.27% as compared with 98.45% for control. The sex ratio of male and female of the spiny bollworm moths resulted from treated newly hatched larvae with all different treatments caused insignificant effect as compared with control.

Table 5. Effect of spores of some bacterial isolate on adults of the spiny bollworm

Treatment	Conc.	Preoviposition (days)	Oviposition (days)	Postoviposition (days)	female longevity (days)	male longevity (days)	Eggs no.	Hatchability %
B. endoradicis	10^{-6}	4.20	8.54b	4.26	17.00	15.812ab	170b	92.266b
P. plecoglossicida	10^{-6}	4.25	8.08b	4.45	16.78	16.63ab	172.5b	86.4425c
B. pumilus	10^{-6}	3.76	8.07b	3.92	16.38	15.088b	168b	91.188b
Control		3.5	9.30a	4.46	17.26	17.378a	193a	98.452a
P		Ns	0.001**	Ns	Ns	Ns	0.000***	0.000***
LSD 0.05			0.937				7.1226	3.6439

DISCUSSION

Our study investigated that *P. plecoglossicida*, *B. Endoradicis* and *B. Pumilus*isolated from the contaminated artificial diet, dead pupae of *H. armigera* had toxic effect on newly hatched larvae of *H. armigera* and *E.insulana* after two and four days.These results agreed with several studies that investigated the insecticidal properties of different bacterial species and used as bio control agents such as *Bacillus cereus*, *B. sphaericus* and *B. thurengensis* and against different insects (Wright *et al*., 2007). Results in our works showed that *P. plecoglossicida* caused the highest mortality percentage of newly hatched larvae of american and spiny bollworms. Also El-Sayed, Eman (2014) recorded toxic effect of *Bacillus cereus, B. subtilis* and *P. eruginosa* against *Pectinophora gossypiella*

Our results clearly indicated that the three bacterial isolates caused latent effects on different stages of the american and spiny bollworms and decreasing pupal weight, pupation and adult emergence percentages. Also, increasing male and female longivity, deposited eggs and hatchability percentages as compared with control. The present conclusion was in harmony with El Zoghbey *et al*., (2003) and Wang and Jeal (2005). Also Najlaa *et al.,* 2011 recorded that *B. thuringiernsis* caused considerable toxic effects against 2nd instar larvae of *Muscadomestica* vicina. The obtained results showed that, there was an inverse relationship between the different concentrations under investigation and the pupation percentage, pupal weight Attala *et al*., 2003 and Koja *et al*., 2006. Moreover *B. thuringiernsis* showed an increase in the percentage of malformed adults.

Adult mortality and longevity of both male and female was significantly decreased, these may be due to the latent toxic effects of the tested isolates, and this was agreed with that obtained by Koja *et al*. (2006) and Younes *et al*. (2008). The mean number of deposited eggs per female significantly decreased after the treated 2nd instar larvae of *M. domestica* with tested *B.thuringiernsis* toxin at different concentrations due to the inhibition of protein contents and its synthesis, which is necessary for the nutrition of eggs (El Bandary, 2004). Hatchability percentage (fertility of the deposited eggs decreased after treatments at different concentrations). These results are in harmony with those obtained by Omar (2003) and Narayanan (2004).

Bacterial isolates in this works cause deformation in adult and these malformations may be due to the reduction in proteins, transaminase enzymes, carbohydrate hydrolyzing enzymes and lipids and these results and observation are in agreement with those of Attalla *et al*. (2003).

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التاثيرات السامة والبيولوجية لبعض العزلات البكترية على دودة اللوز الامريكية والشوكية

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الدراسة المقدمة اجريت في معهد بحوث وقاية النباتات فرع الشرقية مركز البحوث الزراعية لدراسة التاثيرات السامة والبيولوجية لبعض العزلات البكترية (سدوميناس بلكتوجلاسديا و باسيلس اندورادیسیس و باسيلس باميلس) المعزولة من البيئة الصناعية الملوثة والعذارى الميتة لدودة اللوز الامريكية بغسلها وطحنها على الترتيب ضد يرقات الفقس الحديث لدودة اللوز الامريكية والشوكية واوضحت النتائج ان كل العزلات البكترية كان لها التأثير السام على يرقات الفقس الحديث للحشرتين وبزيادة التركيز لكل عزلة بكترية سبب زيادة تدريجية في نسب موت يرقات الفقس الحديث بعد المعاملة في كل العزلات وان السيدوموناس بلكتوجلوسيديا سببت اعلى نسب موت للحشرتين كما ارتفعت نسب الموت بزيادة الوقت(70.25&79.25)% ليرقات الفقس الحديث لدودة اللوز المريكية والشوكية بعد يومين واربع ايام من المعاملة. بينما الباسيلس اندورادیسس سببت اقل نسب موت (39.00&61.00)% و (59.50&67.00) على الترتيب وهذه العزلات اظهرت تاثيرات مختلفة على كل الاطوار لدودة اللوز الامريكية والشوكية وقللت وزن العذارى والنسبة المئوية للتعذير ونسب خروج الفراشات بالاضافة الى تأثيرها على عمر الذكور والاناث وعدد البيض ونسبة فقسها.