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NEW ISOLATED *BACILLUS* SPP. AGAINST THE COTTON LEAF-WORM, *SPODOPTERA LITTORALIS* (BOISD.) (LEPIDOPTERA: NOCTUIDAE)

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Abstract

The efficiency of five isolates of *Bacillus* spp. isolated from the most important agricultural pests in Alexandria and Beheira regions,Egypt were evaluaed, for the first time to conrol the 4th larval instar of the cotton leafworm, *Spodopter littoralis* Boisd. under laboratory conditions. The results indicated that *Bacillus thuringiensis* var. *Kurstaki* (H3a, 3b) was more effective on the larvae of *S.littoralis* than the other species.At the highest concentration (12 x 10^7 spores/ml), percentages of mortality reached 90, 86.66, 76.66, 70 and 53.33 with *B.thuringiensis var. Kurstaki, B.sphaericus, B.pumilus, B.megaterium andB.coagulans*, respectively after 7 days from treatment. Whereas, the LC50 value for *B.thuringiensis* var. *Kurstaki* (1.10 x 10^7 v.s./ml) was less than that of *B.coagulans* (9.00 x 10^7 v.s./ml). So, this bacterium *B.thuringiensis* var. *Kurstaki* was more effective on the death of the 4th larval instar of *S.littoralis*.

Therefore, the pathogenicity of these bacterial strains can be used as microbial control agent against *S.littoralis*.

INTRODUCTION

In Egypt, the cotton leafworm, *Spodoptera littoralis* Boisd., is the most common and widespread inscet pest on cotton, vegetables and forage crops. The use of chemicals is still having an important role in controlling of insect pests.

Biochemical and serological studies of twenty-four separate isolates of crystalliferous *B.thuringiensis* was carried out by de Barjac and Bonnefoi (1962). A further study was done by de Barjac and Frachon (1990) individualized 27 serotypes from 1,600 isolates. In effect, *B.thuringiensis* is a *B.cereus* with the capacity to synthesize a protein "crystal" which enables the bacterium to kill larvae of various insecs in the Lepidopteran, Dipteran and Coleopteran orders.

About 50 strains of *B.thuringiensis* have been isolated from different insects and classified into 12 groups by esterase patterns and by serological and biochemical tests. *B.thuringiensis* and its relative have been tested against many insect species, mainly Lepidoptera, many of them were found highly suceptible to these bacteria (Burges, 1981).

Microbial control agents are receiving now a great deal of consideration, as a possible safer substitues and as a mean of reducing chemical insecticides (Hamed, 1991 and Zaki, 1991).

The present investigation aimed to clarify the efficiency of isolated *Bacillus* spp. against the cotton leafworm larvae in laboratory.

MATERIALS AND METHODS

Isolation and identification of bacteria: A first record of five bacterial strains of *Bacillus* spp. were isolated from diseased individuals of the most important agricultural pests in Alexandria and Beheira regions, Egypt. These strains were given code numbers as indicated in Table 1.

1. Nas. 1, *Bacillus thuringiensis var. Kurstaki* (H3a, 3b).
2. Nas. 2, *B.coagulans* (HB/AC n^{o} 86/59).
3. Nas. I + II, *B.megaterium* (HdB / MFB 90/564)
4. Nas 11 + 12, *B.pumilus* (HdB/BFB 91/.700).
5. Nas. 25, *B.sphaericus* (Mayer and Neide).

Isolated bacteria were identified by Prof. Dr. H. De Barjac at the Biological control, Pasteur Institute; a Collaborating Centre for Entomopothogenic *Bacillus* in Paris, France. Isolation and purification of bacteria were carried out according to the methods of Mortignoni and Steinhaus (1961),. The bacteria were cultured on Glycerol Agar Medium (G.A.M.), then incubated at 28^{o}C for 48 hrs. Plate count technique was used to determine the number of viable spores of *Bacillus* spp. per ml to prepare the concentrations.

Test insect: Laboratory strain of Egyptian cotton leafworm, *S.littoralis* (Boisd.) was maintained and reared on a semi-artificial diet was prepared according to Hegazi *et al.*, (1976) at Agricultural Research Station, Sabahia, Alexandria, Egypt.

Bioassay tests: Bioassay tests were carried out on the fourth larval instar of

Table 1. List of five *Bacillus* spp. isolated from some important agricultural pests in Alexandria and Beheira governorates, Egypt.

Insect					
Order and Family	Species	Isolated Bacillus spp.	Code number	Location	Host plant
1- Coleoptra Curculionidae	*Hypera brunneipennis* Boh. (Larvae and adults)	1-*B.thuringiensis var. Kurstaki* (H3a,3b)	Nas 1	Alexandria governorate (Abis)	Egyptian clover *Tri-folium alexandrinum*
		2- *B.coagulans*	Nas 2		
2- Lepidoptera Tortricidae	*Lobesia botrana* (Den. & Schiff.) (Larvae)	3- *B.megaterium*	Nas I +II	Beheira govemorate (Jinaklies and Borg-Elarab)	Grape vine *Vitis vinefera*
3- Hemiptera Lygaeidae	*Oxycarenus hyalinipennis* Costa (Nymphs & Adults)	4- *B.pumilus*	Nas 11+12	Alexandria governorate (Abis)	Okra *Hibiscus esculentus*
4- Isoptera Kalotermitidae	*Kalotermes flavicollis* Fab., (Nymphs)	5- *B.sphaericus*	Nas 25	Alexandria governorate (Abis)	Swamp oak *Casuarina glauca*

S.littoralis under laboratory conditions of 27 ± 1°C and70 ± 2% R.H. in plastic cups (5.5 cm in diameter and 6.5 cm hight).All tested individuals were starved for about 24 hours, then introduced to the cups provided with semi artificial diet treated with bacterial suspension. Five concentrations of the isolated bacteria (12 x 10^7,9 x 10^7, 6 x 10^7,3 x 10^7 and 1.2 x 10^7 spores/ml) were used. All treatments, including the untreated check, were replicated three times and 10 larvae were used for each replicate. Only distilled water was used in the untreaed check.

Mortality counts were recorded after 2,5 and 7 day of treatment and data were corrected according to Abbott's formula (Abbot, 1925). Percentage of mortalities (transformed by using angular transformation) were subjected to analysis of variance (ANOVA) and means were compared by L.S.D. test at 0.05 level. LC50 values and slope were estimated by using the method of Litchfield and Wilcoxon (1949) for susceptibility of the 4th larval instar of *S.littoralis* to five *Bacillus* spp. after 7 days of treatment.

RESULTS AND DISCUSSION

The isolated bacterial strains, *B.thuringiensis var. Kurstaki* (H3a, 3b), *B.coagulans, B.megaterium, B.pumilus* and *B.sphaericus* recorded in Egypt for the first time. These five strains isolated from four important pests in four families belong to four different orders.

The susceptibility of the fourth larval instar of *S.littoralis* to these five strains of *Bacillus* spp. after certain days of treatment were recorded and shown in Table 2. *B.thuringiensis var. Kurstaki* at the lowest concentration of 1.2 x 10^7 v.s./ml revealed 0, 36.66 and 56.66% mortalities after 2,5 and 7 days, respectively. At the same concentration, *B.sphaericus, B.pumillus, B.megaterium* and *B.coagulans* the percentage of mortalities after 2,5 and 7 days of treatment were (0,30,53.33%), (0,23.33,40%), (0,20,36.66%) and (0,10,20%),respectively.

At the highest concentration of 12 x 10^7 v.s./ml, the percentage mortalities after 2,5 and 7 days of treatment were (20, 83.33,90%), (16.66,73.33, 86.66%), (10,66.66,76.66%), (6.66, 63.33, 70%) and (3.33, 46.66, 53.33%) for *B.thuringiensis var.Kurstaki, B.sphaericus, B. pumilus, B.megaterium* and *B.coagulans*, respecively.

The statistical analysis proved significant differences between : (1) the percentage mortalities and the concentrations of five strains of *Bacillus* spp., (2) the

Table 2. Effect of five *Bacillus* spp. on the susceptibility of the fourth larval instar of *S.littoralis* after 2, 5 and 7 days of treatment.

Bacteria	Concentration (spores/ml)	Mortality % at indicated days			Means
		2	5	7	
B.t.var.Kurstaki	$1.2x10^7$	0.00	36.66	56.66	31.11d
	$3x10^7$	6.66	46.66	70.00	41.11cd
	$6x10^7$	10.00	56.66	73.33	46.66bc
	$9x10^7$	13.33	63.33	83.33	53.33b
	$12x10^7$	20.00	83.33	90.00	64.44a
	$\overline{X}$	9.99c	57.33b	74.66a	47.33A
B.sphaericus	$1.2x10^7$	0.00	30.00	53.33	27.78d
	$3x10^7$	3.33	43.33	66.66	37.78c
	$6x10^7$	6.66	50.00	70.00	42.22c
	$9x10^7$	10.00	60.00	76.66	48.89b
	$12x10^7$	16.66	73.33	86.66	58.88a
	$\overline{X}$	7.33c	51.33b	70.66a	43.11B
B.pumilus	$1.2x10^7$	0.00	23.33	40.00	21.11d
	$3x10^7$	3.00	40.00	53.33	32.22c
	$6x10^7$	6.66	46.66	66.66	39.99b
	$9x10^7$	6.66	56.66	70.00	44.44b
	$12x10^7$	10.00	66.66	76.66	51.11a
	$\overline{X}$	5.33c	46.66b	61.33a	37.77C
B.megaterium	$1.2x10^7$	0.00	20.00	36.66	18.89d
	$3x10^7$	0.00	36.66	50.00	28.89c
	$6x10^7$	3.33	40.00	60.00	34.44b
	$9x10^7$	6.66	53.33	66.66	42.23a
	$12x10^7$	6.66	63.33	70.00	46.66a
	$\overline{X}$	3.33c	42.66b	56.66a	34.22D
B.coagulans	$1.2x10^7$	0.00	10.00	20.00	10.00e
	$3x10^7$	0.00	16.66	30.00	15.55d
	$6x10^7$	0.00	26.66	43.33	23.33c
	$9x10^7$	0.00	40.00	50.00	30.00b
	$12x10^7$	2.33	46.66	53.33	34.44a
	$\overline{X}$	0.67c	27.99b	39.33a	22.66E

Means followed by the same letter are not significantly different at 0.05 level by L.S.D. test.

mortality percentages and the days after treatment (2, 5 and 7 days), and (3) the mortality percentages and the *Bacillus* treatments.

The results revealed that *B.thuringiensis var. Kurstaki* was more effective on *S.littoralis* larvae than the other used bacteria. So, the efficiency of the five strains of *Bacillus* on the 4th instar larvae was arranged descendingly as follows: *B.thuringiensis var. Kurstaki, B.sphaericus, B.pumilus, B.megaterium* and *B.coagulans.*

The LC50 and slope values for the 4th larval instar of *S.littoralis* after 7 days of treatment by using the five *Bacillus* spp. are recorded in Table 3. The LC50 value for *B.thuringiensis var. Kurstaki* (1.10 x 10^7 v.s./ml) was less than that of *B.coagulans* (9.00 x 10^7 v.s./ml). Therefore, *B.t.var. Kurstaki* was more effective against *S.littoralis* larvae.

Table 3. Parameters determined according to the statistical method of analysis adapted by (Litchfield and Wilcoxon, 1949) for susceptibility of the 4th larval instar of *S.littoralis* to five *Bacillus* spp. after 7 days of treatment.

Bacillus spp.	LC50 (v.s./ml)	Slope (S)	Confidence limits of LC50
B.t.var. Kurstaki	1.10 x 10^7	6.41	1.71x10^7 : 0.71 x 10^7
B.sphaericus	1.40 x 10^7	6.89	2.24 x 10^7 : 0.88 x 10^7
B.pumilus	2.70 x 10^7	7.85	4.46 x 10^7 : 1.64 x 10^7
B.megaterium	3.40 x 10^7	9.48	6.09 x 10^7 : 1.89 x 10^7
B.coagulans	9.00 x 10^7	7.08	14.4 x 10^7 : 5.63 x 10^7

Reviewing the above-mentioned results it could be concluded that the efficiency of the isolated *Bacillus* spp. on the 4th larval instar of *S.littoralis* after certain days of treatment, agreed with Zaki (1991) who mentioned that the entomopathogenic bacteria *B.T.* represent a good example of biological control. The successful use of *B.thuringiensis* for conrolling some insect pest specially the cotton leafworm, *S.littoralis*, the black cutworm *Agrotis ypsilon*, the lesser cotton leafworm, *S.exigua* and other lepidoperous insects was repeatedly demonstrated in field experiments.

In Egypt, Hamed (1991) mentioned that the results of his experiments indicated that *B.t.var. Kurstaki* and *var. aizawai* can be used against *S.littoralis* on cotton and forage crops.

Table 4. Effect of isolated *Bacillus* spp. on different pests species.

Bacillus spp.	Treated pests	Host	Location of rsearch	Concentration	% Mortality		References
					Laboratory	Laborat	
B.thuringiensis	1- *Hypera bruneipernis*	Alfalfa	Nubaria (new reclaimed	10.0%			
Kurstak	larvae.	*Meducago sativa* L.	area in Egypt).		99.33	--	Nasr and Zarif (1996)
(H3a,3b)	2- *Leuzera pyrina*	Pear orchards	El-Tabia, Alexandria,	12x107v.s./ml	100.00	92.00	Shehata et al., (1995)
	larvae.		Governorate				
	3- *Hypoborus ficus*	Fig trees	El-Agami district and Agri-	74.70x106v.s/ml	55.90	60.80	
	larvae.		cultural Experimental sta-			57.70	Shehata et al., (1993)
	4- *Oxycarenus hyalinipen-*		tion, El-Sabahia, A.G.				
	nis Nymphs	Okra plants	El-Sabahia Horticultural	82.80x105 v.s./m	51.30	57.58	
	adults		Experimental Station, A.G.				
	5- *Sesania cretica larvae.*	Maize plants	Abis, Alexandria, G.E.	12x107 v.s./ml	- -	54.86	Nasr et al., (1992)
B.sphaericus	1- *Kalotermes flavicollis*	Casuarina trees	El-Sabahia Research Sta-	84 x 107 v.s./ml	85.00	--	Nasr and Ibrahim (1997)
	Nymph		tion, Alxandria		86.60	--	
	2- *Gyptotermes brevis*		Agricultural Experimental	12x107 v.s./ml	65.00	--	Nasr and Moein (1992)
	Nymph		Station, El-Sabahia,	12x107 v.s/ml	20.00	--	Shehata et al., (1995)
	3- *Z.pyrina* larvae	Pear orchards	Abis, Alexandria, G.E.		83.30	--	Nasr and Ibrahim
	4- *S.cretica* larvae	Maize plants		78x106 v.s/ml	87.50	--	(1997)
	1- *Lobesia botrana* larvae.		Agricultural Experimental		57.50	--	
B.megaterium	2- *Crptoblabis gnidiella*	Grape vine	Station, El-Sabahia, A.E.	12x107 v.s./ml	60.00	--	Nasr ans Rashed (1992)
	3- *Spodoptera littoralis*				33.80	32.80	
	larvae.			78x107 v.s./ml	30.50	30.50	Shehata et al., (1993)
	4- *H.ficus* larvae.	Fig trees	El-Agami district and A.E.S.				
	5- *Z.pyrina larvae.*		El-Sabahia, A.E.	16.90x105 v.s./m	-	--	Shehata et al., (1995)
	6- *Ceratitis capitata* adult	Pear occhards	El-Agami district and				
B.pumilus	1- *O.hyalinipennis*	50% sugar solution	A.E.S., El-Sabahia, A.G.	16.90x105 v.s./m	-	--	Nasr and Rashed (1992)
	Nymphs adult	Okra plants	Agricultural Experimental				
	2- *C.apitata* adults		Station, El-Sabahia.	12x107 v.s./ml	75.84	71.90	
	3- *H.ficus larvae* adults	50% sugar solution	Agricult Experimental Sta-		78.37	73.00	Shehata et al., (1993)
	4- *Z.pynna* larvae	Fig trees	tion, El-Sabahia.	12x107 v.s./ml	- -		Shehata et al., (1995)
B.coagulans	1- *S.cretica* larvae	Pear orchards	El-Sabahia Horticultural			--	Nasr and Ibrahim
	2- *Z.pyrina* larvae	Maize plants	Experimental Station, A.G.				(1997)
		Pear orchards	Agricultural Experimental	12x107 v.s./ml	- -		
			Station, El-Sabahia		0.00	--	Shehata et al., (1995)

Also, These results support the findings shown in Table 4 which showed the effects of isolated *Bacillus* spp. on some important pests in Alexandria, Egypt. The results revealed that *B.t.var. Kurstaki* was the most effective on larvae of *H.brunneipennis*. The highest mortality percentage was 93.33 at the highest concentration (10.0%) after 7 days of treatment (Nasr and Zarif, 1996). While *B.t. var. Kurstaki* at concentration of 12 x 10^7 v.s./ml represented the most effective for controlling *Zeuzera pyrina* larvae, 100% and 92% mortality under laboratory and field conditions. On contrast, *B.coagulans*, *B.pumilus* and *B.megaterium* do not affect the same larvae. (Shehata *et al.*, 1995). It is clear from Table 4 that *B.t.* var. *Kurstaki* and *B.sphaericus* at the highest concentration of 12x10^7 v.s./ml, the percentage mortalities were 86.60 and 83.30%, respectively for the 2nd larval instar of the greater sugar-cane borer, *S.cretica* after 7 days of treatment under laboratory conditions, (Nasr and Ibrahim 1977).

On the other hand, Table 4 showed larvae of *L.botrana* were more susceptible to *B.megaterium* than both *C.gnidiella* and *S.littoralis* larvae. The percentages of mortality at 78 x 10^6 v.s./ml were 87.5,60 and 57.50% for *L.botrana, S.littoralis* and *C.gnidiella*, respectively after 7 days of treatment (Nasr and Rashed 1992).

Also, the results indicated that the nymphs of *K.flavicollis* were more susceptible to *B.sphaericus* than the nymphs of *C.brevis*. The percentages of mortality after 14 days at the concentration of 84 x 10^7 v.s./ ml of *B.sphaericus* were 87.5 and 65 for both termites, respectively (Nasr and Moein, 1992).

In conclusion, mortalities were depending on the concentration used of *Bacillus* spp. The results proved that *S.littoralis* was more susceptible to *B.t.var. Kurstaki* and *B.sphaericus* than to *B.pumilus*, *B.megaterium* and *B.coagulans.*

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عزلات جديدة من جنس .Bacillus spp لمكافحة دودة ورق القطن

فيبي نبية نصر

معهد بحوث وقاية النباتات ، مركز البحوث الزراعية - - قسم بحوث المكافحة الحيويه - الصبحيه - الإسكندرية.

تناول هذا البحث تقدير فاعليه خمس سلالات بكتيرية من جنس *Bacillus*معزوله من بعض الآفات الزراعية الهامة في محافظتي الإسكندرية والبحيرة. وهذه السلالات تم تعريفها وتسميتها في معهد باستير في فرنسا. ولقد قيمت لأول مرة لمكافحة العمر اليرقي الرابع لدودة ورق القطن المرباه علي بيئة صناعية تحت الظروف المعملية.

وقد أظهرت النتائج أن أفضل السلالات البكتيرية تقليلا للإصابة بدودة ورق القطن هي *Bacillus thuringiensis var. Kuristaki (H3a, 3b)* عن السلالات البكتيرية الأخري. و تشير النتائج أن نسب الموت ليرقات دودة ورق القطن بعد سبعة أيام من المعاملة بأعلي تركيز مستخدم (١٢x $١٠^٧$ جرثومه/مل) بالسلالات البكتيرية *B.thuringiensis var. Kuristaki (H3a,3b), B.sphaericus, B.pumiles, B.megaterium, B.coagulans* هي٩٠ و ٨٦,٦ و ٧٦,٦٦ و ٧٠ و ٥٣,٣٣ ٪ علي التوالي وأتضح من النتائج أن زيادة معدل التركيز للأنواع البكتيرية السابقة أدي إلي خفض أكبر لدرجة الإصابة بدودة ورق القطن. ويمكن ترتيب هذه البكتيريا ترتيبا تنازليا علي أساس خفض الإصابة كالاتي :

B.thuringiensis var.Kuristaki, (H3a,3b),B.sphaericus, B.pumilus, B.mgaterium, B.coagulans.

كذلك أظهرت النتائج أن قيمة التركيز المسبب لموت ٥٠٪ من اليرقات المعاملة بالبكتيريا *B.thuringiensis var. Kuristaki (H3a, 3b).* بتركيز (١,١ x $١٠^٧$ / مل) أقل بكثير من قيمة التركيز المسبب لموت ٥٠٪ من اليرقات المعاملة بالبكتيريا *B.coagulans* بتركيز (٩ x $١٠^٧$ جرثومه/مل).