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Efficacy of UV radiation on *Bacillus thuringiensis* mutants against Lepidopterous Insects

A.S. Abdel-Razek [a*], F. N. Talkhan[b] and Marwa M. Azzam[b].
Department of Pests and Plant Protection, Agriculture and Biological Research Division [a];
Department of Genetics and Cytology [b], National Research Centre, Cairo, Egypt;

ABSTRACT

The ability of some isolates of Bacillus thuringiensis to produce dark brown pigment was measured as an indicator to UV resistance. M5 as an indigenous Egyptian isolate was used as wild type to improve its resistance to UV. It was exposed to UV irradiation for different periods ranging between 1 and 10 h. The induced mutants were examined morphologically by phase contrast microscope. One hundred and forty four mutants were obtained; 10 of them were selected and tested for their toxicity against Spodoptera littoralis. The results showed that mutants 62, 65 and 85 were the most toxic ones. These three mutants and the wild type were examined by transmission electron microscope. Crystal proteins with bipyramidal shape and active against Lepidopteran insects were detected in all the selected mutants.

Key words: entomopathogenic bacteria; mutation; melanin pigment; bioassay; cotton leafworm; mortality.

INTRODUCTION

Recently, commercial bio-insecticides especially the bacteria, have been used in insect biological control as an alternative to synthetic chemical insecticides. These *B. thuringiensis* products facing major problems when used in the field. The parasporal crystal proteins and spores, which are the primary toxic substances to insects, are inactivated quickly after their exposure to sunlight. The proteins are cross-linked by hydroxyl radicals (OH) and hydrogen peroxide (H_2O_2) when exposed to UV and /or ionizing radiation [1], which reduced their dissolution in insect midgut. Since the discovery of *B.thuringiensis* in 1901, many investigations were used to improve their ability and activity in the field. Despite all its advantages, acceptance of insecticides formulated with *B. thuringiensis* has been disappointing, primarily because they have not proven as effective under field conditions as laboratory tests have predicted [2]. The use of conventional *B. thuringiensis* as insecticides is however limited because the spores and toxins are inactivated by solar radiation [3-6]. Various formulations are not sufficiently stable under field conditions and rapidly lose their biological activities [7&8]. Attempts to protect *B. thuringiensis* toxicity from damaging by UV radiation under field conditions have yielded limited success. Different formulations were developed with addition of variety of screens [9-11], some substances with different optical features were selected in order to test their protective quality against inactivation of *B. thuringiensis* spores by UV [12]. In the last few years, there has been a growing interest in melanin research as some important functions have been recognized. Many reports focus on the ability of melanin to protect against radiation damage. Melanin absorbs light at all wavelengths and reaches its maximum absorbance in the UV range. In particular, melanin's ability to increase UV resistance and preserve the insecticidal activity of *B. thuringiensis*

products has been reported with [13-15]. A novel mutant of *B. thuringiensis* which produces a dark-brown diffusible pigment, which is characterized as melanin, a natural UV screen was reported by [14]. This mutant has increased UV resistance as well as increased toxicity against *Plutella xylostella* (diamondback moth). Another mutant of *B. thuringiensis* producing melanin was described by [15]. They mentioned that characterization of such mutant with increased UV resistance might contribute to develop stable formulations for field application. Being a natural product, melanin is easily biodegradable and, thus, will not pose any threat to the environment. On the other hand, [16] isolated a UV resistant wild type strain of *B. thuringiensis* subsp. *dendrolimus* L-7601 producing a dark brown pigment in a general nutrition-abundant medium, which had no L-tyrosine.These studies aim to identify some melanin pigment based on chemical testing.

MATERIALS AND METHODS

Materials:

Ten indigenous Egyptian *B. thuringiensis* isolates were used in this study and isolate M5 was selected according to its ability to produce pigment (melanin) and used as a wild type.

Media:

LB medium (per 1 liter): Yeast extract 5gm, Tryptone 10gm, NaCl 10gm, PH7.. **NCM Medium (per liter):** Tryptone 5gm, Yeast extract 3gm, 0.7 mM CaCl2, 0.05 mM MnCl2, 1 mM MgCl2, and pH7.2. This medium was solidified with 2 per cent agar

UV experiments:

Samples preparations:

M_5 isolate was selected and used to enhance its ability to UV resistance. Under sterilized conditions, 7 ml of one week culture (spores and crystals) of the selected isolate were centrifuged at 4000 rpm, supernatant was discarded, pellets were washed with 5 ml of water then centrifuged, 5 ml of saline (NaCl 0.85%) were added after discarding water then sprayed onto a 3 cm diameter sterilized Petri dish (with a magnetic bar) to be ready for the UV irradiation treatment. The volume of saline were increased to be 10 ml instead of 5 ml when UV irradiation was used for long times (4, 5, 6, 7, 8, 9 and 10 hrs) to equalize the evaporation and heating of the suspension of spores and crystal.

In a dark room, a UV lamp (30 W. Philips T-UV lamp type No. 57413 P/40) was used with a 20 cm distance away from the opened Petri dish that was put on a magnetic stirrer for slow stirring during the irradiation period. Irradiation periods were 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 hrs. After irradiation, solutions were left under dark cover for at least an hour to prevent photo-reactivation repair. Then, under sterilized conditions, 500 µl of irradiated solutions were added to4.5 ml of sterilized distilled water in a 9 cm test tube with and a serial of dilutions were carried out. After that, a 500 µl of diluted solutions were added to LB media in 10 cm Petri dishes and incubated at 28^{o}C for 24-48 hrs then counted and studied for its UV survival.

Mutants' characterization:

Mutants were studied as colony morphology (Cry^+ and Cry^-) that was described by [17] where colonies were selected randomly. Then, these colonies were studied using microscopic examination for the presence or absence of spores and crystal proteins.

Screening for dark brown pigment:

To study the ability of wild type strain(M5) to produce dark brown pigments and according to [18], components of 1/4 liter of NCM medium were dissolved in 200 ml distilled water and 0.25 gm L-tyrosine were dissolved in 50 ml distilled water, then autoclaved separately. Upon cooling both were mixed well then flowed into Petri dishes and inoculated with bacteria the incubated at 42^{o}C for 48 hrs followed by incubation at 37^{o}C for 48 hrs. The ability of the UV mutants to produce the dark brown pigment was detected using the NCM broth medium and L-tyrosine. Broth was inoculated with the mutants and incubated at 42^{o}C for 48 hrs then incubated at 37^{o}C for 5 days. Growth and pigments were noticed and registered after 3 days and after 7 days. After 7 days, broth were centrifuged and pigment was measured by Spectrophotometer (Model Shimadzu, Graphicord) at 400, 500, 600 nm according to [16].

RESULTS

Melanin experiments for UV resistance:

The tested 10 isolates of *B. thuringiensis* were varied in their ability to produce melanin (Figs.1 and 2) in spite of the suitable conditions for that (L-tyrosine and incubation at 42^{o}C). Some isolates produced dark brown pigment, like J, M_5, M_1 at different levels; other isolates didn't (Table 1).

Formation of this pigment depended on the presence of L-tyrosine in the culture medium. Therefore, melanin is formed by the action of tyrosinase [19]. Furthermore, [18] observed the presence of L-DOPA, which is subsequently polymerized to melanin via a series of nonenzymatic reactions, in the supernatant of *B. thuringiensis* cultured at 42°C and confirmed a hypothesis that the black pigment produced by *B. thuringiensis* was synthesized via L-DOPA.

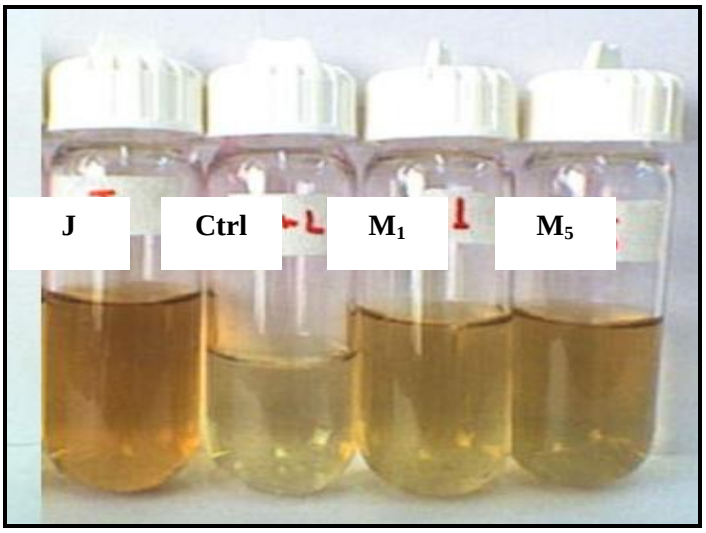

Fig.(1): The ability of *B. thuringiensis* isolates to produce dark pigments

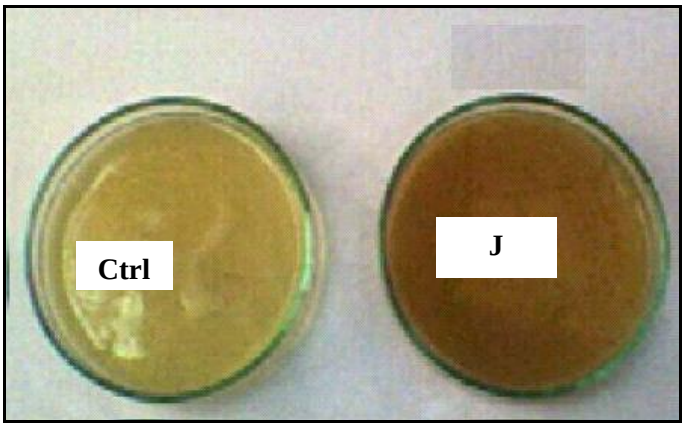

Fig. (2): Pattern of melanin production of (J) isolate, the highest, on a NCM plate containing 0.1% L-tyrosine at 42°C and a plate without any growth as a control.

Table (1): Ability of *B. thuringiensis* isolates, under study, to produce dark pigments

Isolates	Pigment
J	+++++
M_5	++
M_1	++
M_6	---------
M_8	---------
C	---------
ATCF	---------
M_{13}	---------
M_{12}	---------

Table (1) showed that the isolate (J) gave a high production of dark pigment. Isolates M_5 and M_1 were lower than (J) in their pigment productivity, while other isolates; (M_6, M_8, C, ATCF, M_{12} and M_{13}) did not give any.

UV experiments:

UV radiation almost is used as a mutagenesis agent to enhance the productivity in some microorganisms of some important byproduct substances (enzymes, proteins, antibiotics, alcohols…etc). Spores of the selected isolate (M_5) that was used as a wild type strain were exposed to UV irradiation for different exposure periods (1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 hrs). UV irradiation was used for long periods of time (1, 2 and 3 hours) to get mutants able to produce dark brown pigment and subsequently able to resist the damage caused by UV [18]. Table (2), showed results of the UV experiments where at dilution factor 10^{-10}; the average number of colonies in the control reached 243 ones. This number was subsequently decreased with increasing periods of exposure to UV irradiation. With the dilution factor 10^{-1}, the average number of colonies were at 1 hr of irradiation was 300, 45.6, 35, 29.3, 14.3, 11.3, 9.3, 8.6, 8 and 7.3 at 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 hrs, respectively.

Most of grown mutants' colony was examined according to [17], who mentioned that colonies phenotype of *B. thuringiensis* strains that lost their plasmid and subsequently lost their ability to produce crystal proteins (Cry^-), were small and smooth. While, colonies phenotype of *B. thuringiensis* strains that have their plasmid and subsequently has the ability to produce crystal proteins (Cry^+) were big and rough (Fig.3).

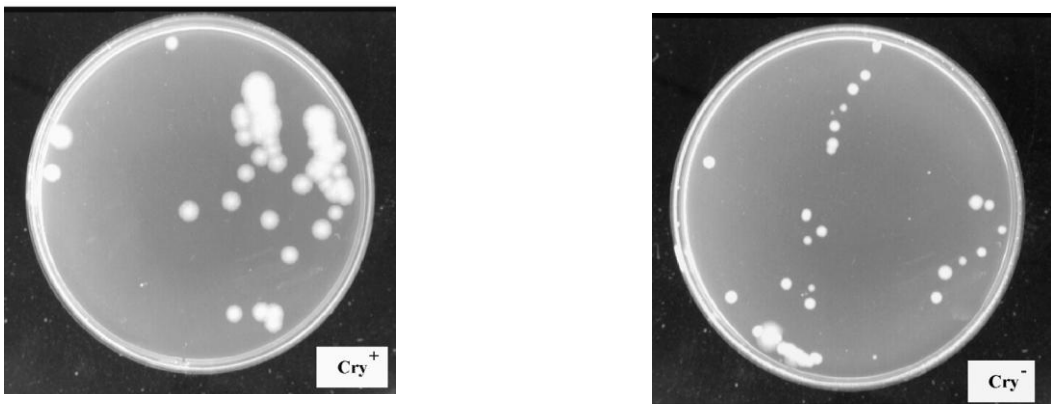

Fig.(3). Colonies with spores and crystal protein of mutants were examined after 72 hrs by phase contrast microscope.

Table (2). Data of UV irradiation against spores of *B.thuringiensis* wild type (M_5) for different periods (1-10 hrs) at 10^{-10} dilution factor.

Treatment period (hrs)	Dilution factor	Colonies No.			Average No. colonies
		Rep.1	Rep.2	Rep.3	
0 (control)	10^{-10}	285	223	222	243
1	10^{-10}	250	300	350	300
2		36	56	45	45.6
3		37	26	42	35
4		30	25	33	29.3
5		16	13	14	14.3
6		9	14	11	11.3
7		12	6	10	9.3
8		7	8	11	8.6
9		10	6	8	8
10		7	10	5	7.3

One hundred forty four mutants were selected according to colony morphology, where colonies with rough with big size, and bipyramidal shape crystal protein. So, they were selected and tested for their ability to produce dark brown pigment.

Screening of melanin in UV mutants:

After UV experiments, selected mutants were grown on NCM broth medium with L-tyrosine, incubated at 30^{o}C for 24 hrs, then incubated at 42^{o}C [18] for 7 days. Growth and pigment was observed. Results were registered after 3and 7 days (Table3). Firstly, L-tyrosine inhibited some mutants' growth in the first 3 days of the experiment (like mutants No. 14, 26, 33, 52, 58, 69, 74, 79, 82, 99, 103, 109, 116, 124 and 142) and after 7 days, growth and pigment were appeared in a slow way in these mutants. Secondly, the mutants' No. 11, 12, 14, 26 and 28 gave low pigment, while other mutants (1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 13, 15, 16, 17, 18, 19, 20, 21, 22, 24, 25, 27, 29) were grown but didn't give any pigments. Finally, some other mutants, like 106, 119 and 130, did not grow at all for the 7 days with the presence of L-tyrosine.

Table (4) showed that variability between mutants with melanin productivity when compared with the wild type isolate (M_5) when measured by spectrophotometer at different wavelength, 400, 500 and 600 nm, according to [19].

Figure (4) showed the highest 10 UV mutants with melanin production and their numbers were 65, 62, 64, 32, 118, 131, 123, 132, 103 and 85 where the absorption at 400nm by spectrophotometer were 2.322, 1.505, 1.303, 1.245, 1.242, 1.222, 1.212, 1.18 and 1.172, respectively.

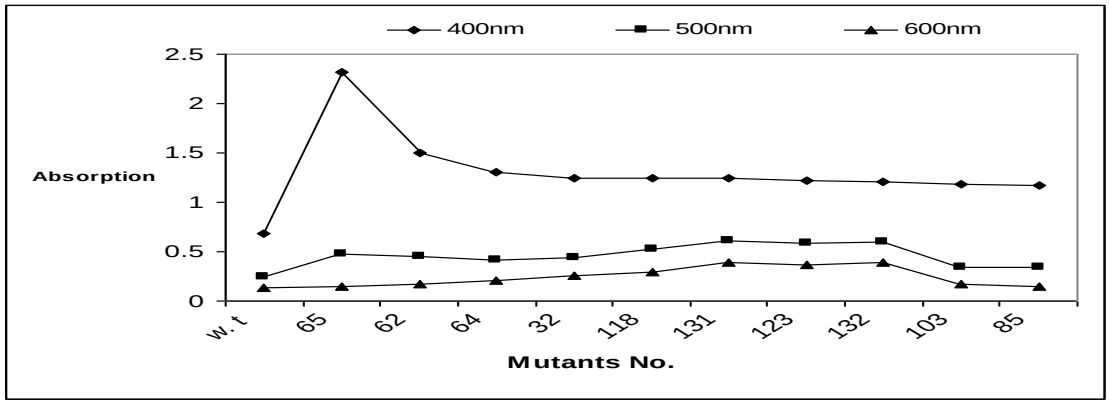

Fig. (4). The highest ten UV mutants with dark brown pigment productivity compared with the wild type isolate at 400-500-600 nm. wavelength.

Relation between UV irradiation periods and pigment productivity:

Data of UV experiments, with different exposure periods, and melanin productivity revealed that there is no relation between periods of UV irradiation and melanin productivity (Fig.5). Whereas, mutants No. 65, 62 and 64 which were irradiated for 7 hours, were the highest melanin productivity (2.322, 1.505 and 1.303 at 400 nm). While, mutants No. 118, 131, 123 and 132 which were irradiated for 9 hours, were less in melanin productivity (1.242, 1.242, 1.222 and 1.212 at 400 nm).

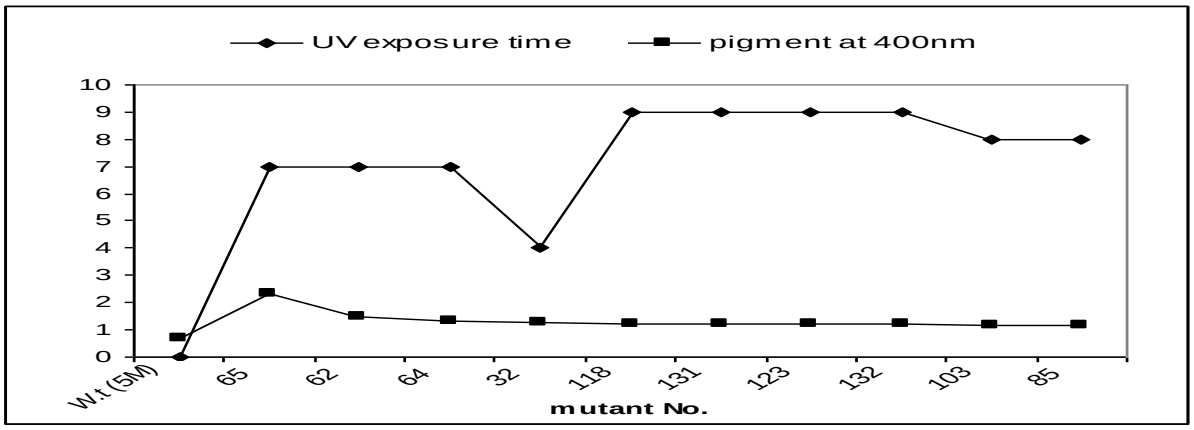

Fig. (5): Relationship between UV irradiation periods and pigment productivity of UV mutants.

Mortality and LC_{50} values calculations of *B. thuringiensis* mutants:

Assessment of mutants' toxicity was expressed as mortality percentage of larvae after 4 days following treatment, as well as in the control. A constant concentration (1500 µg/ml) from all the mutants was used for screening experiments, to detect the most toxic mutants. Table (5) and Figure (6)showed that from 10 mutants, used for screening their activities against 3rd instar larvae of the cotton leaf worm, *S. littoralis* ،only 3 mutants gave

mortality ranged between 90% to 63% these were mutants No. 62, 65 and 85 gave 90, 77 and 63% mortalities; respectively and were the most toxic mutants.

On the other hand, mutants No. 123, 118, 32, 103, 65, 131 and 132 gave 53, 46, 40, 29, 28, 25 and 22% mortalities, respectively compared with 7% mortality in the control.

Tables (6 and 7) showed Results of bioassay experiments using the highest three active mutants (62, 65 and 85) at five different concentrations (1500, 1000, 500, 250 and 125 µg/ml) against 3rd instar larvae of *S. littoralis* with the mortalities calculated and corrected according to Abbott's formula [20]. The bioassay results of these mutants were compared with the results of the wild type isolate (M_5). The LC_{50} values were, 52, 94 and 330 µg/ml for the 62, 65and 85 mutants respectively. The results of the variance and Chi^2 for the three mutants (62, 64 and 85), compared with the wild type isolate (M_5), showed that the percentage mortality at the diagnostic LC_{50} values in the susceptible population of *S. littoralis* were considered significantly.

Examination of mutants and wild type isolate by transmission electron microscope:

After screening and bioassay experiments, the highly toxic 3 mutants, were selected for examination with transmission electron microscope and were compared with the wild type isolate (M_5). Crystal proteins, with bipyramidial shape and active against Lepidopteran insects were detected in all the selected mutants 62, 65 and 85 (Fig.7).

Table (3): Capacity of *B. thuringiensis* mutants producing melanin following incubation at 42°C

Mutant No.	Growth		Pigment	Mutant No.	Growth		Pigment	Mutant No.	Growth		Pigment	Mutant No.	Growth		Pigment	Mutant No.	Growth		Pigment
	After 3 days	After 7 days			After 3 days	After 7 days			After 3 days	After 7 days			After 3 days	After 7 days			After 3 days	After 7 days	
11	✓		+	49	✓		++	75	✓		+	98	✓		+++	123	✓		+++
12	✓		++	50	✓		++	76	✓		+++	99	-	✓	+	124	-	✓	++
14	-	✓	+	51	✓		+	77	✓		+++	100	✓		+	125	✓		+++
23	✓		+	52	-	✓	++++	78	✓		++	101	✓		+++	126	✓		++
26	-	✓	+	53	✓		+	79	-	✓	+	102	✓		+	127	✓		++
28	✓		+	54	✓		+	80	✓		++	103	-	✓	++	129	✓		++
30	✓		+	55	✓		++	81	✓		+	104	✓		+	131	✓		+++
31	✓		++	56	✓		+	82	-	✓	+	105	✓		+	132	✓		+++
32	✓		+	57	✓		+	83	✓		+	107	✓		++	133	✓		++
33	-	✓	+++	58	-	✓	++	84	✓		+	108	✓		++	134	✓		++
34	✓		++	59	✓		+	85	✓		++++	109	-	✓	++	135	✓		+++
	✓				✓				✓				✓				✓		
35			+	61			++	86			+	110			++	136			++
36	✓		++	62	✓		+++++	87	✓		+	111	✓		++	137	✓		++
37	✓		+	63	✓		+	88	✓		+	112	✓		++	138	✓		++
38	✓		+	64	✓		+++	89	✓		++	113	✓		+	139	✓		+++
39	✓		+	65	✓		++++++	90	✓		+	114	✓		+	140	✓		++
42	✓		++	66	✓		++	91	✓		+	115	✓		+	141	✓		+++
43	✓		++	67	✓		+	92	✓		+	116	-	✓	++	142	-	✓	++
44	✓		++	69	-	✓	+	93	✓		+	117	✓		++	143	✓		++
45	✓		++	71	✓		++	94	✓		+++	118	✓		+++	144	✓		+++
46	✓		++	72	✓		++	95	✓		++	120	✓		++				
47	✓		++	73	✓		++	96	✓		+	121	✓		+				
48	✓		+	74	-	✓	+	97	✓		+	122	✓		++				

Note: Number of plus sign (+) represents the capacity of *B. thuringiensis* mutants to produce melanin.

Activity of delta- endotoxin crystals in *Bacillus thuringiensis* mutants as influenced by UV Treatments

Table (4): Light absorption in 400-500-600 nm wavelength of pigment produced by UV mutants of *B. thuringiensis*

Mutant No.	400 nm	500 nm	600 nm	Mutant No.	400 nm	500 nm	600 nm	Mutant No.	400 nm	500 nm	600 nm	Mutant No.	400 nm	500 nm	600 nm
W.T.	0.678	0.249	0.131	109	0.96	0.247	0.111	56	0.861	0.284	0.15	105	0.692	0.2	0.09
65	2.322	0.473	0.146	81	0.958	0.256	0.12	66	0.851	0.325	0.177	84	0.681	0.248	0.141
62	1.505	0.446	0.175	44	0.953	0.277	0.04	54	0.85	0.288	0.158	72	0.679	0.23	0.127
64	1.303	0.413	0.212	59	0.952	0.316	0.167	110	0.842	0.364	0.225	39	0.639	0.058	0.19
32	1.245	0.443	0.254	53	0.951	0.377	0.222	116	0.842	0.336	0.179	82	0.593	0.197	0.099
118	1.242	0.526	0.297	124	0.95	0.405	0.236	138	0.832	0.301	0.164	36	0.576	0.165	0.076
131	1.242	0.61	0.387	107	0.942	0.372	0.2	74	0.83	0.346	0.203	102	0.574	0.209	0.123
123	1.222	0.587	0.36	133	0.942	0.398	0.226	11	0.827	0.389	0.219	38	0.548	0.038	0.165
132	1.212	0.598	0.388	62	0.932	0.44	0.272	37	0.825	0.26	0.135	96	0.544	0.224	0.133
103	1.18	0.338	0.165	127	0.932	0.368	0.204	43	0.805	0.252	0.131	90	0.531	0.212	0.134
85	1.172	0.34	0.146	137	0.93	0.389	0.234	50	0.804	0.244	0.125	14	0.527	0.137	0.059
125	1.162	0.466	0.27	48	0.926	0.288	0.145	104	0.782	0.287	0.158	80	0.518	0.146	0.063
135	1.142	0.472	0.265	51	0.922	0.346	0.201	142	0.782	0.251	0.13	94	0.489	0.195	0.114
139	1.142	0.433	0.226	35	0.915	0.319	0.178	114	0.781	0.28	0.158	99	0.482	0.117	0.065
101	1.126	0.376	0.192	23	0.911	0.298	0.161	52	0.772	0.272	0.153	88	0.48	0.194	0.127
122	1.112	0.456	0.265	34	0.902	0.313	0.173	55	0.77	0.172	0.064	58	0.478	0.111	0.045
61	1.095	0.308	0.147	47	0.893	0.305	0.169	121	0.762	0.278	0.154	98	0.469	0.173	0.092
141	1.092	0.51	0.311	117	0.892	0.319	0.173	28	0.757	0.329	0.177	100	0.435	0.146	0.087
77	1.072	0.253	0.103	75	0.889	0.369	0.226	49	0.753	0.236	0.125	86	0.42	0.177	0.12
31	1.037	0.259	0.101	111	0.886	0.364	0.22	57	0.753	0.236	0.122	87	0.402	0.139	0.09
126	1.032	0.449	0.276	33	0.883	0.222	0.105	113	0.752	0.281	0.162	79	0.38	0.114	0.033
67	1.013	0.251	0.106	71	0.88	0.231	0.101	52	0.747	0.138	0.037	93	0.36	0.098	0.054
144	1.012	0.401	0.243	42	0.879	0.295	0.156	45	0.746	0.199	0.092	26	0.357	0.083	0.031
129	1.002	0.425	0.243	120	0.878	0.35	0.198	143	0.722	0.287	0.17	69	0.345	0.093	0.031
136	1.002	0.379	0.211	112	0.872	0.316	0.163	115	0.721	0.246	0.134	91	0.342	0.073	0.036
12	0.977	0.465	0.231	30	0.869	0.282	0.153	78	0.714	0.244	0.126	92	0.324	0.101	0.062
134	0.972	0.366	0.201	46	0.869	0.291	0.158	63	0.712	0.208	0.103	89	0.296	0.063	0.03
108	0.962	0.379	0.218	140	0.862	0.32	0.176	83	0.697	0.249	0.139	97	0.279	0.037	0.011

Table (5). The mortality percentages of cotton leafworm, *S. littoralis*, resulted after the treatment with *B. thuringiensis* mutants.

Mutants No.	Mean of dead larvae	% Mortality
Control	0.7	7%
62	9	90%
64	7.7	77%
85	6.3	63%
123	5.3	53%
118	4.6	46%
32	4	40%
103	2.9	29%
65	2.8	28%
131	2.5	25%
132	2.2	22%

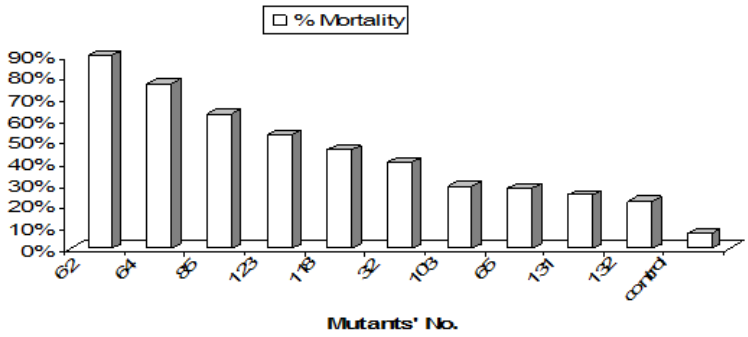

Fig. (6) Mortality of the highest ten *B. thuringiensis* mutants with dark brown pigment against *S. littoralis*.

Table (6) the bioassay data of mortality of *S. littoralis* larvae treated with different concentrations from the best three *B. thuringiensis* mutants.

Mutants	Conc. (µg/ml)	% Mortality	% Corrected Mortality
w.t (M_5)	1500 1000 500 250 125	95 87 80 60 50	94 83 75 50 38
62	1500 1000 500 250 125	90 87 80 70 69	89 85 78 67 65
65	1500 1000 500 250 125	75 76 65 63 58	72 69 61 58 53
85	1500 1000 500 250 125	70 68 60 57 40	67 64 56 50 33

Table (7): Statistical analysis of the bioassay data of *B. thuringiensis* UV mutants performed using State program.

Mutant No.	LC_{50}	Slope	Confidence limits		Variance	Chi^2
			Low	High		
w.t (M_5)	213	1.611	57.65	368.98	2.249343 E-02	5.43944 E-05
62	52	0.7989	-399.2987	502.99	0.4015654	1.50237 E-05
64	94	0.4678682	-1519.49	1707.671	0.6138609	3.933907 E-06
85	330	0.7549516	-141.116	801.7019	6.535133 E-02	6.294251 E-05

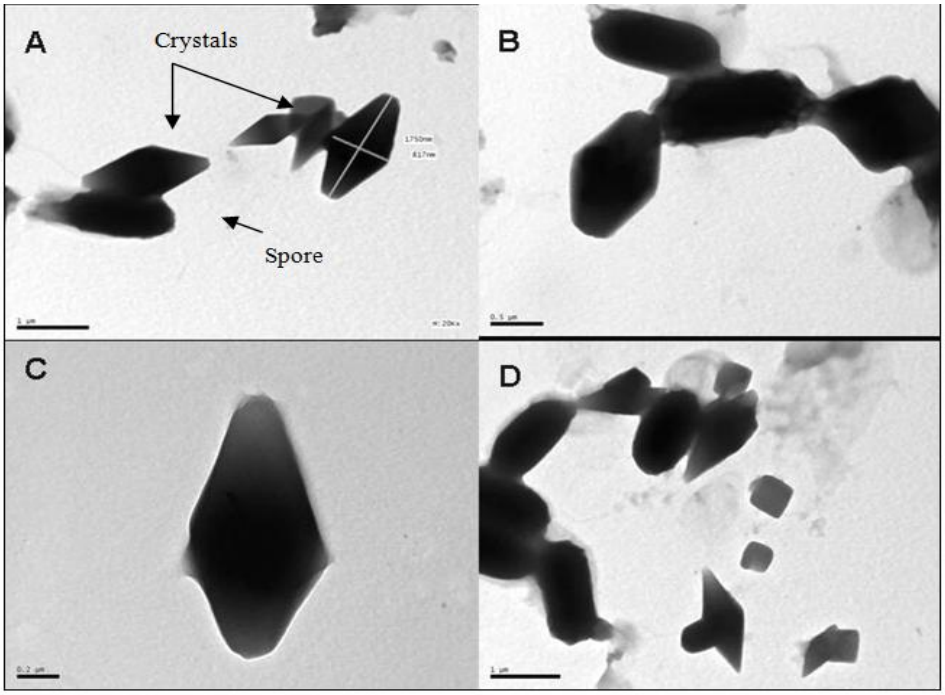

Fig. (7): A photo with transmission electron microscope to the wild type isolate of *B. thuringiensis* (A), 62 (B), 65(C) and 85 (D) mutants showing the diameter of a crystal protein, with bipyramidial shape.

DISCUSSION

The aim of this study was to improve the ability of the *B. thuringiensis* promising isolates, to produce melanin pigment which led to resistance against UV radiation damage and subsequently to protect *B. thuringiensis* toxicity against the cotton leafworm under field conditions.

The melanin pigment is well known as a protective agent from the damaging effects of UV radiation. This means that, the highly dark brown pigment produced by the *B. thuringiensis* isolate the highly the ability to UV damage resistance._ So, with the results illustrated in Table (1), the isolate (J) gave the highest production of dark pigment and it was, subsequently, a UV resistant isolate. While, the isolates M_5 and M_1 were low with their

pigment productivity and, subsequently, they had a low resistance to UV. While other isolates, (M_6, M_8, C, ATCF, M_{12} and M_{13}) had no pigment at all. So, they considered to be sensitive to UV radiation [19,14,15,18,&16]

The characteristics of UV inactivation of sporeforming and other bacteria phenotypes or shape were reported and discussed by [21] and [12]. Also, [3] described the inactivation of *B. thuringiensis* spores and their treatment with two protectants at wavelengths of the near-ultraviolet and visible spectra 254 nm gave more than 50% protection against UV irradiation.

All the 144 mutants were identical with colony morphology with rough big size bipyramidal shape crystal protein, so, they were selected to be tested for their ability to produce dark brown pigment, according to[17]. With the mutants that were survived after exposure to UV radiation for 10 hours, were few, smooth colonies and most of them lost the ability to produce crystal protein when they examined with phase contrast microscope. Loss of plasmids occurs both spontaneously and during exposure to curing condition in *B. thuringiensis* subsp. *thuringiensis* [22].

Results in Table (6) of the bioassay experiments revealed that the insecticidal activity of the *B. thuringiensis* UV resistance mutants, which had the ability to produce melanin pigment, were lower than the insecticidal activity of the wild type that was sensitive to UV radiation. These results were agreed with some reports, whereas [16] had a *Bt* L-7601 strain that was isolated as a wild type with a high UV resistance, and it was shown to produce melanin, which is an excellent UV protective agent for *Bt* formulations. But they found that the formulations of *Bt* L-7601 strain had low toxicity to insects and suggested that, in the future, they can introduce the *mel* gene into high toxic *B. thuringiensis* strains to solve the problem of *B. thuringiensis*'s susceptibility to UV, by constructing a melanin-producing transgenic bacterium.

The results of bioassay studies for the three mutants, compared with the wild type isolate, revealed that the LC_{50} value for the mutant 62 was lower than LC_{50} value of the wild type isolate and the other mutants (65 and 85) giving 52 µg/ml compared to 213, 94 and 330 µg/ml for the wild type isolate and the mutants 64 and 85, respectively. These results are in a great agreement with some previous reports. Whereas, [14, 15 &18] have obtained brown pigment producing mutants of Bt-m-8, Bt-m and *B. thuringiensis* strain 94001, respectively by artificial-mutation of *B. thuringiensis* subsp. *kurstaki* after UV radiation. They assayed toxicity against *Plutella xylostella*, *S. littoralis* and *Heiothis armigera*, respectively. They suggested that the insecticidal activity of *B. thuringiensis* that produced melanin was significantly higher after UV irradiation than when melanin was not produced. Electron microscope was used to state the regular and irregular shapes of *B. thuringiensis* isolates at the sporangium stage [23]. Also, [24] described a comprehensive ultra structural analysis of sporulation and parasporal crystal development for *B. thuringiensis* using electron microscope.

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فاعلية الأشعة فوق البنفسجية علي بعض طفرات من عـزلات بكتيـريا الباسيلس ثورينجيانسس ضد الحشرات حرشفية الأجنحة

عاطف عبد الرازق[1]، فاطمة طلخان[2]و مروة عزام[2] ،
المركز القومى للبحوث – قسم آفات ووقاية النبات[1] - قسم الوراثة والسيتولوجى
جمهورية مصر العربية

المستخلص

تم قياس قدرة بعض العزلات من بكتيريا الباسيلس ثورينجيانسس في إنتاج صبغات بنية قاتمة اللون وذلك كمقياس لمناعتها ضد الأشعة فوق البنفسجية . من هذه العزلات تم تحديد عزلة محلية وإستخدامها كسلالة برية وذلك لتحسين قدرتها علي مقاومة الظروف البيئية الصعبة في الحقل والمتمثلة في تأثيرات الأشعة فوق البنفسجية للشمس والتي تخفض من فاعلية البكتيريا في الحقل. قمنا بتعريض العزلة المذكورة لفترات متفاوتة للأشعة فوق البنفسجية الصناعية وبإستخدام فترات تراوحت بين 1- 10 ساعات. الطفرات التي نتجت من هذه التأثيرات تم إختبارها من الناحية المورفولوجية بإستخدام الميكروسكوب الضوئي. ولقد تم تحديد حوالي 144 طفرة من هذا التعريض للأشعة ولكنا وجدنا فقط عشرة منها ذات فاعلية علي دودة ورق القطن. من بين هذه العزلات كانت الطفرات 62، 65 و58 من اشد واكثر الطفرات فاعلية. تم إختبار وجود الجرثومة البلورية دلتا اندوتوكسين بعد تعرضها للأشعة الفوق البنفسجية بإستخدام الميكروسكوب الإليكتروني ووجدت من النوع الهرمي المتميز بكفائته ضد العديد من الحشرات حرشفية الأجنحة. هذه الدراسة اثبتت اهمية العمل المعملي والتكنولوجيا الحيوية في خدمة المجال الزراعي بالحصول علي طفرات وعزل سلالات ذات كفائة عالية بمكن ان تساهم الي حد كبير في برامج المكافحة الميكروبية للآفات الي جانب ان إنتاجها ايضا يمكن ان يسهم في القضاء تدريجيا من الإعتماد علي المبيدات الكيميائية وبالتالي الحصول علي منتج زراعي عالي الجودة خالي من الملوثات وبالتبعية الحفاظ علي الصحة العامة للمزارعين والمستهلكين للمنتجات الزراعية وبالتالي زيادة الإنتاج ورفع الاقتصاد القومي.