Research Journal of Agriculture and Biological Sciences, 2(6): 243-248, 2006
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Comparative Biological Activity of the Residual *Bacillus Thuringiensis* Strains Against a Susceptible Laboratory Strain of Pink Bollworm, *Pectinophora Gossypiella* Saunders, (Lepidoptera: Gelechiidae)

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Abstract: We evaluated effects of *Bacillus thuringiensis* subspp. *aizawai* and *kurstaki,* in the same concentration as those isolated from sprayed plants at 0, 1, 3 and7 days post spraying, on the biological and reproductive potential factors of *Pectinophora gossypiella.* Our investigation showed that mortality percentages for larvae and pupae were reduced at the tested concentrations isolated from the one to seven days after treatments. Also, a prolongation of larval durations as males and females were significant to control ones. The percentage emergence and malformation of adults seemed not clearly affected due to the tested concentrations. Inconsistent significance for the adult longevity, pre-oviposition period and oviposition period between some concentrations of both *B.thuringiensis* strains were recorded. Generally, egg production showed a significant increase as concentrations decreased for both *B. thuringiensis* strains. Regarding percentage of hatching inconsistent relation between the different concentrations of both *B. thuringiensis* subspp. *aizawai* and *kurstaki*, were recorded and this may be due to malformation of adults. However, a significant reduction in some of these parameters at different concentrations of both *B. thuringiensis* strains was recorded compared to control ones. This inconsistent relation may be due to that the tested concentrations used were closer to each other.

Key words: *Pectinophora gossypiella*, *Bacillus thuringiensis* subsp. *kurstaki* and subsp *aizawai,* residual activity, biological development

INTRODUCTION

The pink bollworm, *Pectinophora gossypiella* (Saunders), continues to be the most serious insect pest of cotton world wide. Damage is caused in the late season, as developing larvae tunnel through the boll wall and then lint fiber as they move from seed to seed. The burrowing activity stains lint, destroy fiber and reduce seed weight, vitality and oil content. The pink bollworm cut holes in boll walls as they leave bolls for pupation. Holes may become infected with boll rotting organisms[4]. The evolution of insecticide resistance, which has been documented in more than 500 insect species including pink bollworm[9], threatens agriculture and human health world wide. The myriad examples of insecticide resistance offer rich opportunities for examining hypotheses about how response to selection is affected by various factors[19,25,6]. Problems with insect resistance to insecticides, resurgence of secondary pests and regulatory actions limiting availability of some widely used cotton insecticides[18].

Continuing concern about the need for new approaches to pink bollworm control has resulted in research exploring the potential of mating disruption with the sex pheromones[24]. Attracticides and release of sterile insects[11]. All these approaches focus on manipulating of the reproductive biology of the insects to achieve population suppression. On the other hand, mating disruption result in prevention, reduction or delay of mating. However, all these control measures are not lethal to the insect and still there is a need for use of chemical insecticides besides these control strategies.

Bacillus thuringiensis can reduce reliance on sprayed insecticides[23]. This pathogen is less harmful to non target organisms, including beneficial insects, wildlife and people[7]. Foliar applied formulations of *B. thuringiensis* had also been used for control of pink bollworm in cotton production[3,8], though its use has declined since the introduction of *B.t.* cotton varieties.

The purpose of the present study is to explore the effects of residual bacterial strains on the biological and reproductive potential factors of pink bollworm including larval and pupal development, egg production, hatching, adult longevity and mortality measures of larvae and pupae.

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MATERIALS AND METHODS

The microbial biopesticides used in this study were:

- *B. thuringiensis* subsp. *aizawai* used at rate of (500g/400 L water)
- *B. thuringiensis* subsp. *kurstaki*, used at the rate of (300 g/400 L water).

The bacterial strains were suspended in 1/2 L water and sprayed by a pressure sprayer (1.1liter) on cotton plants (5 months old) previously planted in pots (30 cm diameter), fifteen pots were used for each treatment. Zero time, one day, 3 days and seven days after application of the bacterial pathogens, the residual spore deposits on plants were determined as recorded by Karima and Abdel-Razek[14]. These residual spore counts after each period were used as the concentrations tested in this experiment. These counts were (15×10^5, 11×10^5, 10×10^5, 45×10^4) and (23×10^5, 15×10^5, 12×10^5, 34×10^4) for *B. thuringiensis* subsp. *aizawai* and *B. thuringiensis* subsp. *kurstaki* at 0, 1, 3 and 7 days ,respectively .The biological activity of these residues against larval instars, pupae and adults of pink bollworm (PBW) were evaluated as follows: a volume of 1 ml suspension of each aforementioned bacterial concentration was homogeneity mixed with 1 gm of artificial diet[17] but with out the antimicrobial agents, then placed in Petri-dish (9 cm diameter) . Fifty neonate larvae of a susceptible laboratory strain of pink bollworm were added to each Petri-dish. A control experiment was done, but Petri-dishes were prepared with diet mixed with water only. Larvae of all treatments were allowed to feed on the treated diet for one and half hour then transferred individually to glass tubes (2×7 cm) containing untreated artificial diet. Tubes were plugged with cotton plugs and incubated at 26±1°C and 70±5 % RH. Mortalities of larval instars, pupal stage, larval period, larval weight, pupal period, pupal weight, % emergency of adults, malformation of adults were recorded. After emergence three pairs of adults were kept in glass tubes and the pre-oviposion, oviposition periods, the longevity of both sexes, egg production and percent hatching were recorded for the control and treatments.

Statistical analysis: Mortality records were corrected by Abbott's formula Abbott's[1]. All biological data were subjected to analysis of variance (ANOVA). In addition means were separated by Duncan's multiple range tests.

RESULTS AND DISCUSSIONS

Effects on larval and pupal mortalities: Data in Table 1 show that percentage mortalities in 1st and 2nd larval instars, total larval instars, pupae from total number of larvae used didn't show any relation between different concentrations isolated from different periods after spraying with *B. thuringiensis* subsp. *aizawai*.

On the other hand, a gradual decrease in percentage mortalities of 1st and 2nd larval instars, total larval instars, and pupae from total number of larvae used were recorded as compared with the different periods of the isolated *B. thuringiensis* subsp. *kurstaki* (Table 1). The female larvae of pink bollworm as feeding on residual spore counts of *B. thuringiensis* subsp. *aizawai* showed a significant elongation of larval period as compared with the control. Also, all the treatments were significantly different from each other and from the control treatment except the first treatment with (24.0±3.3), (21.7±2.4) and (19.4±2.7) days as compared by 15.8±1.6 days for the control. The male larvae showed the same trend of retardation of larval period except that no significance at the level of 0.05% was recorded between the concentrations from the 3 and 7 days period of isolation (Table 2). Data from treatments with *B. thuringiensis* subsp. *kurstaki* at period of isolation

Table 1: Effects of the residual *B. thuringiensis* strains on pink bollworm (PBW) larval and pupal mortalities.

Bacterial strain	Period of isolation	Concentration (spores/ml)	% Mortality in 1st and 2nd instars only	% Mortality in Total larval instars	% Mortality in Pupae from total No. of larvae used
B. thuringiensis	0 time	15×10^5	14.38	5.3	7.37
subsp. *aizawai*	1 day	11×10^5	20.40	23.36	28.3
	3 days	10×10^5	31.10	26.8	35.68
	7 days	45×10^4	16.28	18.92	26.06
B. thuringiensis	0 time	23×10^5	31.04	32.78	41.6
subsp. *kurstaki*	1 day	15×10^5	16.86	18.24	44.1
	3 days	12×10^5	10.02	5.68	15.3
	7 days	34×10^4	10.46	3.38	18.6

Table 2: Effects of the residual *B. thuringiensis* strains on pink bollworm (PBW) larval and pupal durations and larval weights separated as females and males.

Bacterial strain	Period of isolation	Concentration (spores/ml)	Larval duration (days) Mean±SD. ♀	Larval duration (days) Mean±SD. ♂	Larval weight (gm) Mean±SD. ♀	Larval weight (gm) Mean±SD. ♂	Pupal duration (days) Mean±SD. ♀	Pupal duration (days) Mean±SD. ♂
B. thuringiensis	0 time	5×10^5	17.3±2.9^{d}	17.1±2.2^{d}	0.0327±0.006^{ab}	0.0271±0.005^{a}	13.5±1.5	13.6±2.1
subsp. *aizawai*	1 day	11×10^5	24.0±3.3^{a}	23.7±2.6^{a}	0.0285±0.005^{bcd}	0.0208±0.005^{a}	13.0±2.5	14.6±2.2
	3 days	10×10^5	21.7±2.4^{b}	21.3±4.7^{bc}	0.0266±0.007^{cd}	0.0226±0.004^{a}	13.3±0.6	13.0±1.9
	7 days	45×10^4	19.4±2.7^{c}	18.4±2.8^{c}	0.0302±0.006^{bc}	0.0255±0.004^{a}	14.5±1.2	15.3±1.5
B. thuringiensis	0 time	23×10^5	23.4±4.3^{ab}	23.8±2.6^{a}	0.0284±0.007^{bcd}	0.0241±0.005^{a}	13.9±2.4	14.3±1.0
subsp. *kurstaki*`	1 day	15×10^5	23.2±3.5^{ab}	20.6±2.5^{c}	0.0291±0.009^{bc}	0.0341±0.004^{a}	13.1±2.6	12.9±2.9
	3 days	12×10^5	22.9±2.9^{ab}	22.4±2.9^{ab}	0.0264±0.004^{cd}	0.0215±0.0036^{ca}	14.9±1.0	14.9±1.2
	7 days	34×10^4	21.8±2.3^{b}	20.7±2.8^{c}	0.0241±0.006^{d}	0.0236±0.0039^{a}	14.7±2.6	13.8±1.9
Control			15.8±1.6^{d}	15.4±1.3^{e}	0.0346±0.005^{a}	0.0263±0.0035^{d}	13.00±2.1	13.0±1.2
LSD			1.614	1.523	0.00412	0.00136	n.s.	n.s.

n.s. no significance. Means in the same column followed by the same letter are not significantly different at the level of 5 %.

Table 3: Effects of the residual *B. thuringiensis* strains on pink bollworm (PBW) moth emergence , malformation and longevity separated as females and males.

Bacterial strain	Period of isolation	Concentration (spores/ml)	% Emergence ♀	% Emergence ♂	% adult emergence from the total treated larvae	% Malformation ♀	% Malformation ♂	Longevity (days) Mean±SD. ♀	Longevity (days) Mean±SD. ♂
B. thuringiensis	0 time	5×10^5	100	93.8	67.4	6.3	0.0	34.6±6.9^{a}	27.0±7.0^{b}
subsp. *aizawai*	1 day	11×10^5	84.6	80.0	41.3	18.2	0.0	25.1±9.2^{c}	24.1±7.3^{bc}
	3 days	10×10^5	66.6	93.8	41.2	0.0	6.6	28.1±7.4^{bc}	27.1±8.7^{b}
	7 days	45×10^4	90.5	100	73.2	16.6	0.0	34.3±3.7^{a}	33.5±4.3^{a}
B. thuringiensis	0 time	23×10^5	83.3	90.9	43.5	0.0	0.0	24.6±11.3^{c}	20.0±7.6^{cd}
subsp. *kurstaki*`	1 day	15×10^5	61.5	80.0	54.5	12.5	0.0	30.8±6.3^{b}	27.1±7.6^{b}
	3 days	12×10^5	85.5	94.1	69.6	0.0	25.0	25.6±8.6^{c}	19.4±6.3^{d}
	7 days	34×10^4	77.3	85.7	48.9	5.9	0.0	23.6±4.7^{c}	21.0±5.5^{cd}
Control			83.3	85.0	72.2	0.0	5.9	35.4±7.3^{a}	33.4±7.6^{a}
LSD								4.362	4.282

Means in the same column followed by the same letter are not significantly different at the level of 5 %.

0 time, 1 day , 3 and 7 days did not show any significant difference for female larval duration between treatments but a significant difference recorded between them and the control experiment. The male larvae showed a significant retardation in duration as compared with the control once (Table 2).

The larval weight for males at both treatments with *B .thuringiensis* subsp. *kurstaki* and *aizawai* At the different concentrations were not significant except with the control treatments. The female weights of control treatment show clear significance with treatments except with first treatment.

Pupal duration did not have any significance for female and male pupae between treatment and control with a mean duration of 13 days at the control of both male and female pupae (Table 2).

Percentage of female and male emergence showed gradual decrease from 100% to 66.6 % from 0 to 3 days of isolation and 90.5 % for females treated with *B. thuringiensis* subsp. *aizawai* at the concentration isolated from period of 7 days. For males a gradual increase from 80% at 1day period to 100% at 7 days period for the same treatments compared to 85% for the control experiment. For treatments with *B. thuringiensis* subsp. *kurstaki*, the

Table 4: Effects of the residual *B. thuringiensis* strains on pink bollworm (PBW) pre-oviposition period , oviposition period, number of eggs per female and percentage of hatching.

Bacterial Strain	Period of isolation	Concentration (spores/ml)	Pre-oviposition period (days) Mean±SD.	Oviposition period (days) Mean±SD	Egg No. / female Mean±SD	% Hatching
B. thuringiensis	0 time	5×10^5	3.0 ± 1.4^{f}	24.0 ± 3.4^{a}	67.1 ± 12.9^{abc}	27.6
subsp. *aizawai*	1 day	11×10^5	13.3 ± 1.1^{a}	5.3 ± 2.1^{e}	10.2 ± 8.0^{e}	18.6
	3 days	10×10^5	7.25 ± 1.7^{c}	12.0 ± 1.4^{d}	33.5 ± 3.5^{de}	41.7
	7 days	45×10^4	5.0 ± 1.4^{de}	18.0 ± 2.7^{bc}	82.1 ± 13.5^{ab}	14.6
B. thuringiensis	0 time	23×10^5	7.0 ± 1.0^{c}	14.3 ± 4.9^{cd}	10.2 ± 3.9^{e}	10.0
subsp. *kurstaki`*	1 day	15×10^5	9.5 ± 0.07^{b}	10.7 ± 2.5^{de}	58.5 ± 40.0^{bcd}	31.0
	3 days	12×10^5	4.16 ± 1.4^{ef}	24.1 ± 8.6^{a}	46.6 ± 32.8^{cd}	32.6
	7 days	34×10^4	4.25 ± 1.2^{ef}	14.7 ± 3.8^{c}	53.4 ± 37.8^{bcd}	15.8
Control			5.87 ± 1.2^{cd}	21.1 ± 7.2^{ab}	91.3 ± 28.5^{a}	71.0
LSD			1.374	5.515	27.00	

Means in the same column followed by the same letter are not significantly different at the level of 5 %.

different periods of isolation showed relationship concerning emergence (Table 3). In a trial of testing percent adult emergence from the total larvae used at the beginning of the experiment, data showed a gradual increase in % emergence from 41.3-73.2 as concentrations isolated from 1 to 7 days decreased. Also, an increase from 43.5-69.6% as concentrations isolated from 0-3days decreased. The control adult emergence was 72.2 % .

Malformation of control adults was zero for females and 5.9% for males. This increased to 6.3, 18.2 and 16.6% for females reared as larvae on *B. thuringiensis.* subsp. *aizawai* at periods of 0 times, 1day and 7days, respectively, and to 6.6% for males reared as larvae on the same treatment at the period of 3 days (Table 3). Also, female larvae reared on *B. thuringiensis* subsp. *kurstaki* showed a malformation as adults of 12.5 and 5.9 % at the isolation period of 1 day and 7 days compared with zero malformation at control treatment (Table 3).

The females' longevity showed a significant decrease from 25.1 and 28.1days for the treatments of *B. thuringiensis* subsp. *aizawai* isolated at periods of 1day and 3days compared with control. The males on the other hand did not show significance between treatments 0, 1, 3 days except with treatment of 7 days period which was not significantly different compared with control experiment (Table 3).

The treated larvae with *B. thuringiensis* subsp. *kurstaki* generally did not show a clear significant in female and male adults longevity at the different concentrations tested, while a significant reduction in these treatment compared with control was recorded (Table 3).

Table 4 showed that the pre-oviposition period was significantly affected at the most tested concentrations for both treatments with *B. thuringiensis* subsp. *aizawai* and *kurstaki* as compared with control and with a gradual decrease in this period as concentrations decreased for the periods from 1-7 days. inconsistent. The other treatment (*B. thuringiensis* subsp. *kurstaki*) showed significance with control except for the first concentration isolated after 0 times. The same trend also, occurred for the oviposition period with maximum oviposition period 24.0 days for treatment at zero time with no significance with control 21.1 days, for the treatment with *B. thuringiensis* subsp.*aizawai*. While with *B.thuringiensis* subsp. *kurstaki,* the oviposition period seemed to be significant with the control except the treatment at 3 days of isolation, with no clear relation between concentrations (Table 4).

With respect to egg production in the treatment with *B. thuringiensis.* subsp. *aizawai* the isolated concentration at 1day and 3 days were significantly reduced compared with the control while, those at zero time and 7 days were not significant. On the other hand, treatments with *B. thuringiensis.* subsp. *kurstaki* were significantly reduced the egg production of pink bollworm. The percentage of egg hatching at both treatments was reduced at the tested concentrations compared with the control with inconsistent relation between the tested concentrations (Table 4).

Cotton (*Gossypium barbadense* L.) in Egypt is attacked by various insect pests and diseases during the different stages of its development. The pink bollworm, *P. gossypiella* (Saunders) is among the primary pests of cotton causing most damage.

The system of cotton peat management adopted in Egypt is described in relation to the economic thresholds of infestation of various species. The management system includes cultural, mechanical, chemical and microbial control methods [20]. Hosny[12] accomplished a season-long control of pink bollworm with 3-4 applications of pheromone, gossyplure. The application of it at the pin square stage of the cotton plant is

achieving control by mating disruption comparable to 3-4 application of broad spectrum chemical insecticides and this has resulted in reduction of up to three pesticide application in areas where the pink bollworm infestation is exceptionally high.

The foregoing results indicate that toxins produced by different strains of *B. thuringiensis* can have different spectra of activity against pink bollworm. Burgerjon and Dulmage[5,21] and Salama *et al*. reported similar results judged by the variation in the units/mg of several formulations of different varieties of *B. thuringiensis.* Our results showed that several life-history characteristics of pink bollworm were influenced to varying degrees by different strains of *B. thuringiensis* and also within the strains by the different concentrations used. Although the different strains of *B. thuringiensis* did not show any correlated percentage larval and pupal mortalities, it seemed to be reduced starting from the period of 1 day to 7 days of isolation of concentrations tested.

The results obtained revealed that *B. thuringiensis* subsp. *kurstaki* gave promising results on prolongation of larval duration as compared with the other strain *B. thuringiensis* subsp. *aizawai* and with the control. These results are inconsistent with those reported by Salama *et al.* and Abdel-Razek[22,2] for *Plodia interpunctella*, *Sitotroga cerealella* and *Cadra cautella*, *Tribolium confusum,* respectively. The larval weights for males and females were significantly reduced compared to control but with no differences between both sexes.

Ignoffo and Graham[13] recorded the susceptibility of *P. gossypiella* to spores of *B. thuringiensis* and the pupation rate of survivors exposed to spores as newly hatched larvae was inhibited and pupal weight decreased with increase in concentration.

The percentage of emergence and malformed adults seemed to be not clearly affected; this may be due that the concentrations tested were nearly lower.

The longevity of either sex was not affected by treatment with *B. thuringiensis* subsp. *aizawai* as compared to control, while those treated as larvae with *B. thuringiensis* subsp. *kurstaki* showed significance with control. Due to the fact that the isolated bacterial strains from plants represented the different concentrations used and while they seemed to be closer to each other, the resulting parameters of pre-oviposition period, oviposition period, egg production and percent hatching in general showed insignificant between the different concentrations of both *B. thuringiensis* subspp. *aizawai* and *kurstaki*, although a significant reduction was recorded in some of these parameters compared to the control.

The aforementioned observations with our laboratory susceptible pink bollworm strain could be explained due to the binding affinity of *B. thuringiensis* toxins to target sites in the brush border membrane vesicles (BBMVs) of larval pink bollworm midgut is high, this is why in resistant insects this binding affinity was reduced or the toxins did not bind to any additional binding sites as described by Karim *et al.*[15,10] and Gonzalez-Cabrera *et al.* Also, because the larval midgets of pink bollworm are alkaline and contain proteases that help in the solubilization and activation of *B. thuiringiensis* toxins. The same finding was reported by Meyer *et al.*[16]. From the mortality records for pink bollworm in this study we conclude that the concentration used as isolated from the cotton plants at the aforementioned periods, could be under the sublethal doses that's why the results for some biological parameters dose not have any correlated significance although in most cases there were a significance compared with the control experiment. However, the biological effects recorded so far may reduce the ability of pink bollworm to cause damage to cotton plants. These effects could be increased by increasing the concentrations.

Accordingly, continuous or intermittent exposure of newly hatched larvae of pink bollworm to lower concentrations as that used may be as significant as death itself for effective control of the insect pest from one side to prevent the introduction of resistance among these susceptible strain of pink bollworm to *B. thuringiensis* biopesticides.

ACKNOWLEDGEMENTS

Authors are grateful to board of directors, entomologists and technicians of both the National Research Centre and Plant Protection Research Institute, whose generous sharing of information and financial support made this work possible.

REFERENCES

1. Abbott, W.S., 1925. A method of computing the effectiveness of an insecticide. J. Econ. Entomol., 18: 265-267 .
2. Abdel-Razek, A.S., 1998. Biological efficacy of some commercial and isolated varieties of *Bacillus thuringiensis* on the development of *Cadra cautella* (Walker) and *Tribolium confusum* Jacqueline du vall on stored crushed corn .IOBC bulletin vol. 21(3): 67-74 .
3. Baun, J.A., T.B. Johnson, and B.C. Carlton, 1999. *Bacillus thuringiensis* natural and recombinant bioinsecticides products. In: Methods in Biotechnology volume 5 (Hall, F.R. and J.J. Mean) Totowa, NJ: Humana Press, pp: 189-209.

4. Bohmfalk, G.T., 1982. Identification ,Biology and management of cotton insects. Texas Agricultural Extension Service, B-933.
5. Burgerjon, A. and H. Dulmage, 1977. Industrial and International Standardization of microbial pesticides, *Bacillus thuringiensis*. Entomophaga, 22: 121-129 .
6. Carriere, M.A., 2003. Haplodiploidy, sex and the evolution of pesticide resistance. J. Econ. Entomol., 6: 1626-1640.
7. Charles, J.A., A. Delecluse, and Nielson-LeRoux, 2000. Entomopathogenic bacteria from laboratory to field application, Kluwer Academic Publications, Donlrecht, The Netherlands.
8. Dulmage, H.T., 1981. Insecticidal activity of isolates of *Bacillus thuringiensis* and their potential for pest control in Microbial control of pests and plant diseases 1970-1980 (Burges, H.D. Ed.) New York, N.Y. Academic Press, pp: 193-222.
9. Georghiou, G.P. and A. Lagunes-Tejeda, 1991. The occurrence of resistance to pesticides in arthropods. Food and Agricultural Organization of the United Nations, Rome.
10. Gonzalez-Caberera, J., B. Escniche, B.E. Tabashnik and J. Ferre, 2003. Binding of *Bacillus thuringiensis* toxins in resistant and susceptible strains of pink bollworm, *Pectinophora gossypiella*. Insect Biochemistry and Molecular Biology 33 (9): 929-935.
11. Graham, H.M., 1978. Sterile pink bollworm: field release for population suppression. J. Econ. Entomol., 71: 233-235.
12. Hosny, M.M., 1988. The role of pheromones in the management of pink bollworm infestation in Egyptian cotton fields. Agriculture Ecosystems and Environment, vol: 21(1-3): 67-83 .
13. Ignoffo, C.M. and H.M. Graham, 1967. Laboratory and field cage test with *Bacillus thuringiensis* against pink bollworm larvae. J. Invertebr. Pathol., 9 (3): 390-394.
14. Karima, A. El-Lebody and A.S. Abdel-Razek, 2005. Evaluation of residual effects of the microbial insecticide *Bacillus thuringiensis* subsp. alone or mixed with *Phothorimea operculella* virus (POGV) against *Pectinophora gossypiella* (Lepidoptera: Gelechiidae). Bull. Ent. Soc. Egypt, Econ. Ser., 31: 215-228.
15. Karim, S., S. Riazulddin, F. Gould and D.H. Dean, 2000. Determination of receptor binding properties of *Bacillus thuringiensis* σ-endotoxin to cotton bollworm (*Helicoverpa zea*) and pink bollworm *(Pectinophora gossypiella*) midgut brush border membrane vesicles. Pesticide Biochem. Physiol., 67(3): 198-216.
16. Meyer, S.K., B.E. Tabashnik, Y.B. Liu, M.C. Wright, and B. Federici, 2001. Cyt1A from *Bacillus thuringiensis* lacks toxicity to susceptible and resistant larvae of diamondback moth (*Plutella xylostella*) and pink bollworm *(Pectinophora gossypiella*) Appl. Environ. Microbiol., 67(1): 462-463.
17. Rashad, A.M. and E.D. Ammar, 1984. Mass rearing of the spiny bollworm, *Eareas insulana* (Biosd.) on semiartificial diet. Bull. Ent. Soc. Egypt, 65: 239-244.
18. Ridgway, R.L., 1983. Evolution of cotton insect management with special reference to the boll weevil USDA Agricultural Handbook 589.
19. Roush, R.T. and J.A. Mckinzie, 1987. Ecological genetics of insecticide and acaricide resistance. Ann. Rev. Entomol., 32: 361-380.
20. Salama, H.S., 1983. Cotton-pest management in Egypt. Crop Protection, 2(2): 183-191.
21. Salama, H.S., M.S. Foda, and A. El-Sharaby, 1981. Potency of spore σ-endotoxin complex of *Bacillus thuringiensis* against some cotton pests. Z. angew. Entomol., 91: 388-398.
22. Salama, H.S., R. Aboul-Ela, A. El-Moursy, and A.S. Abdel-Razek, 1991. Biology and development of some stored grain pests as affected by σ-endotoxin and β-exotoxin of *Bacillus thuringiensis*. Biocontrol Science and Technology (1): 281-287.
23. Shelton, A.M., T.Z. Zhan, and R.T. Roush, 2002. Economic ecological food safety and social cocequences of the deployment of *Bt* transgenic plants. Ann. Rev. Entomol., 47: 845-881.
24. Shory, H.H. 1975. New advances in pink bollworm control. In: Proceedings Beltwide Cotton Production-Mechanization conference. New Orleans, pp: 24-25.
25. Tabashnik, B.E., 2002. Pesticide resistance. In: Encyclopedia of Evolution (M. Pagel, Ed.). Oxford University Press, New York, USA, pp: 880-883.