LATENT EFFECT OF MICROBIAL INSECTICIDES AGAINST THE SECOND INSTAR LARVAE OF THE COTTON LEAFWORM , *Spodoptera littoralis* (Boisd.)

ELHAM F.M. ABDEL-RAHIM

Sides Agric. Res. Station, Plant Protection Res. Institute, ARC, Giza

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Abstract

The toxic effect of larval treatment of 2nd instar of *Spodoptera littoralis* with the biotic insecticides alone (1st test) or in mixtures (2nd test) was assay in the laboratory tests. The 2nd instar larvae were fed for 48h on leaves dipped for 5 seconds in the biotic insecticides alone or in mixtures of Bacillus (B) + Beauveria (Br), Bacillus (B) + Bioranza (Bz) and Beauveria (Br) + Bioranza (Bz) at the entire, half and quarter doses. Beauveria (Br) + Bioranza mixture was the most toxic one against the 2nd instar larvae, it induced 100,70 and 60% larval mortality at the entire, half and quarter doses, respectively . While, the larval treatment with Bacillus alone had the least effect, it induced10, 10 and5% at the three tested doses, respectively. Also, the larval treatment with the biotic-products induced pupal mortality%. Bacillus (B) + Bioranza mixture had the strongest effect, it induced 35.5,15 and13.3% at the three tested doses, respectively. While, Bioranza alone gave26.7, 13.3 and11.8% pupal mortality at the three tested doses. Therefore, Beauveria (Br) + Bioranza mixture had highest effect in the total accumulative mortality percent increase, it produced 100, 80 and65% at the three tested doses, respectively. Whereas, the bacillus alone treatment had the least effect. The biological parameters of the treated larval instars were obviously affected. The larval duration was prolonged, and Beauveria + Bioranza mixture had the most potent in this respect, it increased the larval period to29 and27.7d at the half and quarter doses, repectively, as compared to12.2d of control. But, the larval treatment with the biocides product alone recorded the longest period of pupal duration than that of biotic-products mixtures. The pupal weight was reduced, and the larval treatment with mixtures gave the highest effect in this respect, as compared with that of biotic-products alone. While the larval treatment with these compounds reduced the pupation%, and Beauveria + Bioranza mixture had the strongest effect, it reduced the pupation to20 and35% at the half and quarter doses, respectively, as compared to100% of control. Also, the larval treatment with these compounds decreased the adult emergence%, and Bacillus (B) + Bioranza mixture had the greatest effect, it decreased the moth emergence to24, 50 and53% at the three tested doses, respectively, as compared to100%of control. While ,the larval feeding on these compounds increased the pupal and adult malformations%, and Bacillus+ Bioranza mixture had the highest effect exceed 23.5, 11.1, 6.7 and 76, 47, 42% for malformed pupal and adults, respectively, as compared to0% of control. The larval treatment with biotic compounds inhibited the adult fecundity, and both Bacillus+ Bioranza and Beauveria + Bioranza mixture or

Bioranza alone retarded the fecundity to reach zero%, as compared to 501 and 510 eggs/female, respectively, of control. But only the treatment with Bacillus singly decreased the eggs hatching to reach 60.9%, as compared to100% of control. The larval treatment with Beauveria + Bioranza mixture had the strongest effect in adult longevity shorten to reach 5.6d, as compared to12d of control. Also, the sex ratio of the emerged adults was shifted in respect to control as result of the larval treatment with these products. The effect was pronounced with Beauveria +Bioranza mixture.

INTRODUCTION

The cotton leaf worm, *Spodoptera littoralis* (Boisd) is a key polyphagous pest in Egypt. Without a hibernation period the cotton leafworm (Clw) is active allover year. It was the most destructive insect pest of great variation of important vegetables and field crops, approximately 112 species belonging to 44 families are attacked by this pest. Among the wide range of hosts, cotton, soybean, maize, wheat and vegetable crops (e.g. tomato, potato and strawberry) are evidently favored by the worm and severe damage is annually caused to most crop growers. Opportunity using of microbial insecticides (Marie, 2007) is other trend will reduce the use of traditional chemical pesticides. One of the ecological advantages of microbial control agents is that they tend to be highly selective, infecting or killing a very narrow range of target pests Thus, it was safer substitute and the biodegradable biocontrol agents can provide important ecological benefits. Therefore, the biotic products use reduces the environmental contamination, pesticide risks. In spite of these advantages there are many constrains face the use of microorganisms. First, farmers used to get high mortality after short period when they apply the pesticides, but in the case of microbial agents the results need long time. So, farmers have to change their mind on biocides. Second, since the difference in efficacy of biocides products are depending on the time of application, mid-gut pH (e.g. bacteria and viruses), and host plant which insect feed on, insect instar and the environmental conditions. It is important to keep in mined all these parameters when we use biocides. Also, the farmers should be known all information about the time of application, efficacy and socioeconomic out put of using microorganisms (Hassan, 2005 and Temerak, 2006).

The principle aim of the present study was to evaluate the latent effect of some microbial insecticides against *S. littoralis* .

MATERIALS AND METHODS

1–Insect rearing.

The cotton leaf worm, *S. littoralis* was reared in the laboratory for several generations at room temp. ranged between 25 - 28 C° and 60 -65% R.H. Larvae were fed on castor bean leaves, *Ricinus communis* (L.) in a wide glass jars until pupation period and adults emergence. The newly emerged adults were mated inside glass jars supplied with a piece of cotton wetted 10% sugar solution as feeding source for the emerged moths and branches of Tafla (*Nerium oleander* L.) or castor bean leaves as an oviposition site (El- Defrawi *et al.*, 1964). Egg masses were kept in plastic jars until hatching.

2-Material used.

Protecto (*B.thuringensis*), Biovar (*B. bassiana*) and Bioranza(*M. anisoplae*) obtained from microbiology unit, Plant Protection Research Institute, Agriculture Res. Center. These compounds were evaluated in laboratory tests against the second instar larvae of *S. littoralis*, under laboratory conditions.

I-Protecto (WP) 10%, commercial product formulation contains 32 x 10^6 IU/mg of *Bacillus thuringiensis Subsp Kurstari*

300 gm. /100 liter water

п- Biovar (WP) 10%, commercial product formulation contains 32 x 10^3 viable spore/mg of *Beauveria bassiana*

200 gm. /100 liter water

ш- Bioranza (WP) 10%, commercial product formulation contains 32 x 10^3 viable spore/mg of *Metarhizium anisoplae*

200 gm. /100 liter water

3- Test procedures.

A weighted amount of powder of each of Protecto, Biovar and Bioranza was prepared in small doses according to the recommended rates starting of 3gm/litre of *Bacillus thuringiensis* and 2gm/litre of both *Beauveria bassiana and Metarhizium anisoplae* as stock solution (entire dose).Each bio-product was evaluated alone against the 2nd instar larvae at the entire, half and quarter doses. Mixtures of Bacillus and Biovar; Bacillus and Bioranza; and Biovar and Bioranza were prepared and tested against the same instar at the three doses (3:2, 3:2&2:2gm/litre, respectively). The castor leaves dipped in only water solution and used as control. Two drops of molleas honey were added in case of the bacterial solution for activation. The exposure of 2nd instar larvae to the biocides product depended upon the larval feeding for 48h on treated leaves with these products either alone or in mixtures feature in the first or second tests. After 48h., the treated leaves were replaced by another untreated one and the larvae fed on it until the pupation. Two replicates (consists of forty larvae for each dose for any of the three tested bio-products) were utilized in the two tests and control .Also, the observed malformations were recorded and photographed.

4-Statistical analysis:

The total percent of the larval mortality until pupation were recorded and corrected according to the check by using Abbott formula (Abbott, 1925). The different biological effects i.e. larval and pupal duration; pupal mortality, pupation and adults emergence percent, pupal weight were evaluated at the three tested doses. While Adult fecundity ,fertility ,longevity ,sex ratio were studied at the quarter dose, The obtained data of the biology were statistically calculated through Excel for windows computer program to determine the F-value, P-value and L.S.D (least significant difference) at 0.05 or 0.01 freedom degrees.

RESULTS AND DISCUSSION

1-Toxic effect:

Data illustrated in Table (1) and fig.1 showed the toxic effect of larval treatment of 2nd instar of *S. littoralis* with the biotic insecticides alone (1st test) or in mixture form (2nd test). Mixture of Beauveria (Br) + Bioranza (Bz) was the most toxic one against the second instar.It induced 100, 70 and 60% larval mortality at the entire ,half and quarter doses, respectively ,as compared to0% mortality of control. While

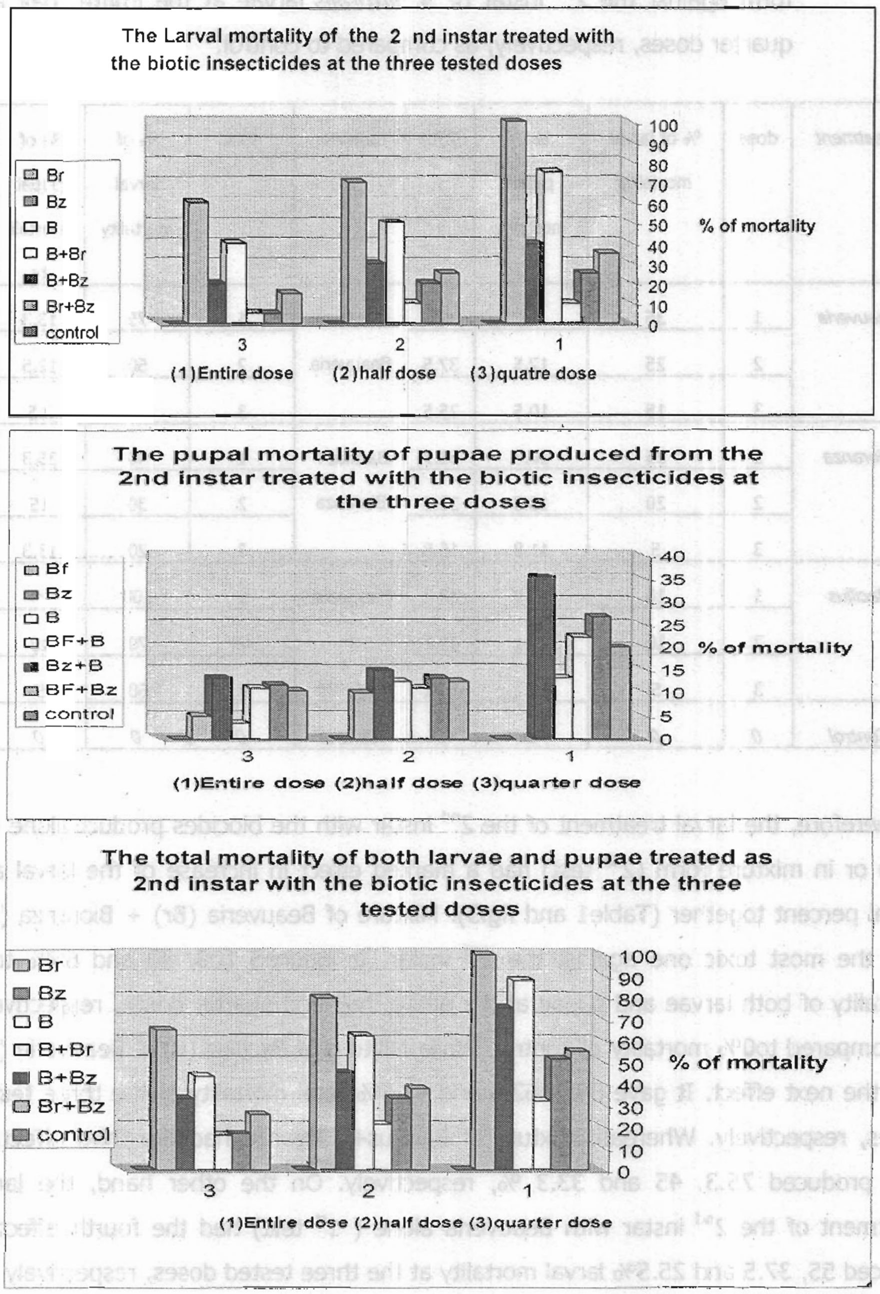
mixture of Bacillus (B) + Beauveria (Br) had the next effect. It gave 75, 50 and 40 % larval mortality at the three tested doses, respectively. Whereas, mixture of Bacillus+ Bioranza had the third effect. It was produced 40, 30 and 20 %, respectively. On the other hand, the larval treatment of the 2nd instar with only Beauveria (in the 2nd test) had the fourth effect, it induced 35,25 and 15%, larval mortality at the three tested doses, respectively .This is following by Bioranza treatment singly, it gave 25,20 and5% at the three tested doses, respectively. While the Bacillus alone had the least effect, it induced10, 10 and5% at the three tested doses, respectively.

On the other hand, the larval treatment of the 2nd instar with the biocides product alone (1st test) or in mixture form (2nd test) induced pupal mortality (Table1and fig.2). Mixture of Bacillus (B) + Bioranza (Bz) (2nd test) had the strongest effect on pupal mortality; it induced 35.3,15 and13.3% at the entire, half and quarter doses, respectively, as compared to0% of control. While the larval treatment with Bioranza alone (1st test) had the next effect on the pupal survive, it induced 26.7, 13.3 and11.8%pupal mortality at the three tested doses, respectively. While the Beauveria+ Bioranza treatment had the least effect, it induced 10 and 5% at the half and quarter doses, respectively.

Table. 1. The Latent effect of Beauveria, Bioranza and Bacillus alone or in mixtures form against the 2nd instar of *S. littoralis* larvae at the entire, half and quarter doses, respectively, as compared to control.

Treatment	dose	% of larval mortality	% of pupal mortality	Total	Treatment	dose	% of larval mortality	% of pupal mortality	Total
Beauveria	1	35	20	55	Bacillus+ Beauveria	1	75	13.3	88.3
	2	25	12.5	37.5		2	50	12.5	62.5
	3	15	10.5	25.5		3	40	3.5	43.5
Bioranza	1	25	26.7	51.7	Bacillus+ Bioranza	1	40	35.3	75.3
	2	20	13.3	33.3		2	30	15	45
	3	5	11.8	16.8		3	20	13.3	33.3
Bacillus	1	10	22.2	32.2	Beauveria + Bioranza	1	100	-	100
	2	10	11.1	21.1		2	70	10	80
	3	5	11.1	16.1		3	60	5	65
Control	*0*	*0*	*0*	*0*	*Control*	*0*	*0*	*0*	*0*

Therefore, the larval treatment of the 2nd instar with the biocides product alone (1st test) or in mixture form (2nd test) had a marked effect in increase of the larval and pupal percent together (Table1 and fig.3). Mixture of Beauveria (Br) + Bioranza (Bz) was the most toxic one against the 2nd instar. It induced 100, 80 and 65% total mortality of both larvae and pupae at the entire, half and quarter doses, respectively, as compared to0% mortality of control. While Mixture of Bacillus (B) + Beauveria (Br) had the next effect. It gave 88.3, 62.5 and 43.5% total mortality at the three tested doses, respectively. Whereas, mixture of Bacillus+ Bioranza had the third effect. It was produced 75.3, 45 and 33.3 %, respectively. On the other hand, the larval treatment of the 2nd instar with Beauveria alone (1st test) had the fourth effect, it induced 55, 37.5 and 25.5% larval mortality at the three tested doses, respectively following by Bioranza treatment alone, it gave 51.7, 33.3 and16.8% at the three tested doses, respectively. While the Bacillus alone treatment had the least effect on the total mortality, it induced 32.2, 21.1 and16.1% at the three tested doses, respectively, as compared to that of control.



Figs.(1- 3): illustrated the latent effect of Beauveria , Bioranza and Bacillus alone or in mixtures form against the 2nd instar of *S. littoralis* larvae at the entire, half and quarter doses, respectively , as compared to control.

These results agree with that obtained by Cipriano *et.al*(2010) who reported that Native strain BbPM (92.7%) and Bea-Sin™ (91.8%) of *Beauveria bassiana* (Bals.-

Criv.) killed significantly more larvae than did Meta-Sin™ (62.6%) of *Metarhizium anisopliae* during 2005 and 2006 year .They mentioned that the bioinsecticides were most effective early (on sampling days 7 and 14)and in general, viability of *B. bassiana* conidia ranged between 90 and 93% while that of Meta-Sin™ was 52% at three concentrations (1.2×10^{12}, 1.2×10^{9}, and 1.2×10^{6} conidia per hectare) of BbPM, Bea-Sin™, and Meta-Sin™ were applied to commercial cabbage, *Brassica oleracea* var. *capitata* They found that the native strain BbPM provided similar control as Bea-Sin™, and both killed significantly more cabbageworm larvae than did Meta-Sin™ .While, Arshad *et al.* (2009) indicated significant effects of Bt cotton on the percent cumulative mortalities of all instars compared with non-Bt cotton. They observed a significant higher mortality (100%) in neonates fed on Bt cotton leaves than those fed on Bt flower-bolls (93%).Whereas, Jolanta (2008) reported that mortality of *Otiorhynchus sulcatus* after the treatments with azadirachtin and *Beauveria brongniartii* ranged between 86–93%. He indicated that all treatments gave a very good control effect of the insect and the time to kill the insect with the fungus was longer than that with the neem treatments He found significant differences in the mean number of surviving stages of the insect between *Beauveria* and neem treatments, but the final total mortality was not statistically different. Also, Nguyen and Vo (2007) proved that *Metarhizium anisopliae* and *Beauveria bassiana* to exploit their potential for controlling the diamondback moth *Plutella xylostella* (DBM) in laboratory and greenhouse and they showed that all of four selected isolates of *M. anisopliae* and *B. bassiana* were found to be pathogenic to the tested DBM. They ensured that the *M. anisopliae.* (OM3-STO) isolate which was isolated from naturally infected DBM exhibited the highest infectivity to DBM. Ashfaq *et al.* (2001) reported that the mortality during the larval stage of *Pseudoplusia includens* increased linearly in response to an increase in the length of feeding time on Bt-cotton by first and third instars and the maximum mortality of about two out of three larvae occurred for first instars fed on Bt-cotton until pupation. Also, Zimmerman (1996) mentioned that all stages of *O. sulcatus* are susceptible to different entomopathogenic fungi used in field trials.

2. Latent effect:

2.1. Larval and pupal period

Data in Table (2) demonstrated that the treatment of 2nd instar larvae of *S. littoralis* with the biocides product alone (1st test) or in mixtures form(2nd test) at the entire, half and quarter doses, caused remarkable highly significant ($p<0.01$) increase in the larval periods. The effect was more pronounced with mixture of Beauveria (Br) + Bioranza (Bz) (2nd test), where the larval duration proved highly significant increase

($p< 0.01$) to average 29 and 27.7day for the treated 2nd instar at the half and quarter doses ,respectively, as compared to12.2d of control. Likewise, the larval treatment with mixture of Bacillus (B) + Beauveria induced highly significant ($p< 0.01$) increase of the larval duration to average 26.7, 26.2 and 26.1 at the entire, half and quarter doses. Whereas, the larval treatment with mixture of Bacillus+ Bioranza caused highly significant ($p< 0.01$) increase in the larval duration to average 20.3, 20.1 and 19. 1d at the three tested doses, respectively. Also, the larval treatment with Beauveria , Bioranza and Bacillus alone(1st test) induced highly significant ($p< 0.01$) increase in the larval duration averaged 21.8,20,19.8;20,19.8,19.7 and17.9,16.4 ,14.6d for three bio-products at the three tested doses, respectively, as compared to11.1 of control.

On the other hand, the second instar larvae feeding on the tested bio-products alone (1st test) or in mixture form (2nd test) at the three tested doses, highly significant ($p<0.01$) increased the pupal duration (Table.2) of the resulting pupae. The larval treatment with the bio-products alone had the highest effect on the pupal duration averaged18, 14, 13; 17, 15, 13 and 16.8, 14.4, 10.9d for pupae produced from the 2nd instar treated with Beauveria, Bioranza and Bacillus alone (1st test) at the three tested doses, respectively, as compared to10.3d of control. While The larval treatment with the biocides product mixture had the least effect in the pupal duration increase to average 14.8 ,13.9 ,12.9 ;13.2,12.5,11.8and -,14.5,14d for pupae resulting from larvae treated with mixtures of Bacillus+ Beauveria, Bacillus+ Bioranza and Beauveria+ Bioranza(2nd test) at the three tested doses, respectively, as compared to9.7d of control.

These results are agreement to those obtained by Arshad *et al.* (2009)who reported that there was a marked difference in larval development period of *Helicoverpa armigera* larvae fed on Bt cotton (27.75 days) and on non-Bt cotton (16.68 days) flower-bolls. Ashfaq *et al.* (2001) mentioned that the length of the larval developmental period of *Pseudoplusia includens* increased linearly with an increase in feeding time on Bt-cotton in first and third instars; while there was no significant response in the fifth instars. Also, Salama *et al.* (1981) recorded that the sublethal doses and length of exposure time to low endotoxin concentrations of three spore-δ-endotoxin preparations from *Bacillus thuringiensis, kurstaki* and *entomocidu* and Thuricide retard the larval development of lepidopterous cotton pests, *Spodoptera littoralis, Spodoptera exigua*, and *Heliothis armigera*.

Table. 2. The latent effect of Beauveria, Bioranza and Bacillus against the 2nd instar of *S. littoralis* larvae at the entire, half and quarter doses, respectively, as compared to control

Treatment	Dose gm/l	Larval duration Mean+S.D (days)	Pupal duration Mean+S.D (days)	Pupal weight Mean+S.D (mg)	Treatment	Dose gm/l	Larval duration Mean+S.D	Pupal duration Mean+S.D	Pupal weight Mean+S.D (mg)
Beauveria	1	21.8+2**	18+2.5**	288+36**	Bacillus+ Beauveria	1	26.7+1.8**	14.8+2.6**	241+24**
	2	20+1.3**	14+2.7**	302+22**		2	26.2+0.8**	13.9+1.8**	259+29**
	3	19.8+2.3**	13+1.2**	314+11**		3	26.1+2.5**	12.9+1.9**	286+55**
Bioranza	1	20+0**	17+1.4**	258+85**	Bacillus+ Bioranza	1	20.3+2.9**	13.2+0.4**	261+69**
	2	19.8+0.7**	15+4.8**	349+2.4**		2	20.1+1.6**	12.5+1.5**	341+41**
	3	19.7+1.5**	13.4+.8**	306+16**		3	19.1+1.8**	11.8+1.3**	355+13**
Bacillus	1	17.9+0.5**	16.8+0.7**	284+79**	Beauveria+ Bioranza	1	-	-	-
	2	16.4+1.7**	14.4+2.6**	297+3.8**		2	29+1.5**	14.5+4**	272+51**
	3	14.6+1.3**	10.9+1.2*	310+23**		3	27.7+1.7**	14+0.8**	323+47**
Control	0	11.1+2.1	10.3+1.0	382+11	Control	0	12.2+1	.9.7+1.5	390+42
F value		2499	791.8	661.2			821.2	61.247	26.70182
P value		0.0000001	0.00656	0.02			8.87E-11	0.000019	0.0112
L.S.D at 0.05		9	10.23	36.0			1.0278	1.134	43.1
0.01		*12.1*	*14.3*	*55.9*			*1.407*	*1.591*	*65.4*

** = Highly Significant (p<0.01)   * Significant (p<0.05)

S.D. =Standard deviation   Malfo. = Malformation%

L.S.D. = Least significant difference

2.2. The pupal weight:

The larval treatment of 2nd instar with the tested bio-products alone (1st test) or in mixture form (2nd test) at the three tested doses, highly significantly ($p<0.01$) reduced the pupal weight of the resulting pupae (Table,2).The effect was pronounced with the larval treatment with the three mixtures, Bacillus+ Beauveria, Bacillus+ Bioranza and Beauveria+ Bioranza(2nd test),it averaged 241,259,286;261,341,355 and - ,272 ,323 mg at the three tested doses ,respectively ,as compared to390mg of control. While, it averaged 288,302,314; 258,349,306and 284, 297, 310mg for pupae resulting from the larval treatment with Beauveria, Bioranza and Bacillus alone (1st test) at the three tested doses, respectively, as compared to382mg of the check.

These results are in agreement to those obtained by Arshad *et al.* (2009)Who mentioned that the pupae weight was significantly higher for larvae fed on non-Bt cotton as compared with Bt cotton plant parts (leaves & flowers-bolls). Ashfaq *et al.* (2001) reported that the pupal weight declined linearly in the first and fifth instars of *P. includens* in response to feeding time on Bt-cotton, although pupal weight also declined for third instars, the response was not linear. Also, Salama *et al.* (1981) found that the sublethal doses and length of exposure time to low endotoxin concentrations of three spore-δ-endotoxin preparations from *Bacillus thuringiensis, kurstaki* and *entomocidu* and Thuricide induced a significant reduction in the pupal weight.

2.3. Pupation and adult emergence:

Data presented in Table (3) demonstrated that the 2nd instar larvae of *S. littoralis* which feeding on leaves treated with Beauveria, Bioranza and Bacillus alone (1st test) or in mixtures form decreased the pupation percentage in respect to control. The effect was more pronounced with mixture of Beauveria (Br) + Bioranza (Bz) It decreased the pupation to 20 and 35% at the half and quarter doses, respectively, as compared to100% pupation of control. While mixture of Bacillus (B) + Beauveria (Br) had the next effect. It decreased the pupation to 12, 38 and 57 % at the three tested doses, respectively. Whereas, mixture of Bacillus+ Bioranza had the 3rd effect, where the pupation reached 25, 55 and 67 % at the three tested doses, respectively. On the other hand, the larval treatment of the 2nd instar with Beauveria alone (in the 1st test) had the

4th effect, it induced 45, 63 and 75 % at the three tested doses, respectively .This is following by Bioranza treatment alone, it gave 48 , 67 and 83 % at the three tested doses, respectively. While the Bacillus treatment singly had the least effect on the total mortality, it recorded 68, 79 and 84% at the three tested doses, respectively.

Table 3. The latent effect of Beauveria, Bioranza and Bacillus against the 2nd instar of *S. littoralis* larvae at the entire, half and quarter doses, respectively, as compared to control.

Treatment	Dose	%f Pupation		% of emergence		Treatment	Dose	%f Pupation		% of emergence	
	gm/l	Normal	Malf	Normal	Malf		gm/l	Normal	Malf	Normal	Malf
Beauveria	1	45+5**	11.1	60+10**	17	Bacillus+	1	12+2**	10.5	84+5.1**	12.5
	2	63+2**	6.7	75+5**	16	Beauveria	2	38+1.4**	6.3	94+4*	5
	3	75+5**	4.4	84+4*	15.4		3	57+1.3**	3.3	97+3n.s	0
Bioranza	1	48+3**	6.7	53+6**	31	Bacillus+	1	25+1.5**	23.5	24+4**	76
	2	67+2**	6.3	54+6.1**	13.3	Bioranza	2	55+5**	11.1	50+10**	47
	3	83+1**	0	65+5**	12		3	67+1.2**	6.7	53.3+7**	42
Bacillus	1	68+2**	22.2	78+2.1**	6.7	Beauveria+	1	-	-	-	-
	2	79+1**	11.1	83.3+3**	0	Bioranza	2	20+10**	10	67+3**	33.3
	3	84+2**	4.4	94.4*	0		3	35+5**	5	78+2**	22.2
Control	0	100	0	100	0	Control	0	100	0	100	0
F value		2736.9		112.814				9656.4		377.288	
P value		0.00395		0.01774				0.00101		0.05293	
L.S.D at 0.05		3.762		7.635				4.217		5.97	
0.01		6.89		*14.051*				*7.719*		*10.95*	

On the other hand, the larval treatment of the 2nd instar with the biocides product alone (1st test) or in mixture form (2nd test) affected the adult emergence percent (Table3). Mixture of Bacillus (B) + Bioranza (Bz) (2nd test) had the strongest in adult emergence percent decrease to reach 24, 50 and 53.3% at the entire, half and quarter doses, respectively, as compared to100% of control. While the larval treatment with Bioranza alone (1st test) had the next effect on the adult emergence, it gave 53, 54 and 65% of adult emergence at the three tested doses, respectively. While the Bacillus+ Beauveria mixture treatment had the least effect, it induced 84.2,94 and 97% at the half and quarter doses, respectively.

These results are agreement with Arshad *et al.* (2009) who recorded that only 7.0% larvae were survived fed on Bt cotton flower-bolls and pupated successfully. Also, Jolanta (2008) mentioned that some of the larvae *of Otiorhynchus sulcatus* treated with azadirachtin began pupation after the treatment than those infected by *Beauveria brongniartii.* Also, Salama *et al.* (1981) indicated that the sublethal doses and length of exposure time to low endotoxin concentrations of three spore-δ-endotoxin preparations from *Bacillus thuringiensis, kurstaki* and *entomocidu* and Thuricide the precentage of exposed larvae that survived and succeeded to pupate increased with the decrease in the toxin concentration and with the decrease in exposure time with decreasing in the adult emergence percent.

2.4. Morphogenetic effects:

Data presented in Table (3) found that the larval feeding of *S. littoralis* on the leaves of the biocides product alone (1st test) or in mixture form (2nd test) induced increase in the pupal malformations percent in relative to control. Mixture of Bacillus (B) + Bioranza (Bz) (2nd test) had the highest effect in increase of pupal malformation to reach 23.5, 11.1 and 6.7 % at the entire, half and quarter doses, respectively, as compared to0% of control. While the larval treatment with Bacillus alone (1st test) had the next effect in the malformed pupal increase to reach 22.2, 11.1 and 4.4% at the three tested doses .While, the larval treatment with the mixture of Bacillus+ Beauveria recorded 10.5, 6.3, and 3.3 at the three tested, respectively. Whereas, Beauveria+ Bioranza mixture gave 10 and 5% at the two tested doses, respectively. Hence, the larval treatment of Beauveria or Bioranza alone (1st test) gave11.1, 6.7, 4.4 and 6.7, 6.3, 0% at the three tested doses, respectively, as compared to that of the check (0%).

Likewise, the larval feeding of *S. littoralis* on the leaves of the biocides product alone (1st test) or in mixture form (2nd test) induced increase in the adult malformation percent, as compared to that of the control (0%).Also, mixture of Bacillus (B) + Bioranza (Bz) (2nd test) had the strongest effect in increase of the malformed adults to reach 76, 47 and 42%, as compared to0% of control. While the larval treatment with Beauveria+ Bioranza mixture gave 33.3 and 22.2% at the half and quarter doses, respectively. But Bioranza or Beauveria alone treatment had the next effect in increase of adult malformation percent to reach 31, 13.3, 12 and 17, 16, 15.4% at the three tested doses, respectively. Whereas, the larval treatment with Bacillus+ Beauveria mixture produced 12.5, 5 and 0% at the three tested doses, respectively. The treatment with Bacillus alone gave adult malformations percent but only in case at the entire dose reached 6.7%, as compared to that of control (0%).

Malformations of *S .littoralis* pupae resulting from the larval treatment of the 2nd instar with mixture of Bacillus and Bioranza in the present work mostly appeared as larval-pupal monstrosity with shifting in the body color, protrusions formation, and violent malformation in the general view of body with maintain with larval moulting skin(fig.1 and 2). Moreover, moth malformations showing body with poorly developed and twisted wings (fig.3) or moth failed to emerge from the pupal skin in the posterior half of body and had light malformed wings(fig.4)or moth failed to emerge from the pupal skin in the anterior half of the body(fig.5).Also, moth failed to emerge from the pupal skin in the anterior half of the body with onset of one wing and mouth parts(Fig.6).While, Malformations of pupae resulting from the larval treatment with Bioranza alone appears as monstrosity of pupae that bear numerous protrusions (fig.7).Whereas, the moth malformations showing body with violent malformation of one or two wings (fig.8 and 9). On the other hand, Malformations of pupae resulting from the larval treatment of the 2nd instar with mixture of Bacillus and Beauveria appears as larval-pupal intermediates with larval cuticle patches, head capsule and thoracic legs, posterior half of the body has the pupal properties (fig.10) or pupae showing body shrinkage (fig.11).Moreover, moth malformations showing variance degrees of deformed bodies and twisted wings (12, 13, 14, and15) or moth failed to emerge from the pupal skin in the posterior half of body with weakly wings (fig.16). Whereas, malformations of pupae resulting from the larval treatment of the 2nd instars with mixture of Beauveria and Bioranza showing undersized pupae(fig.17)or larval-pupal intermediates(fig.18).While ,malformed adults appears as adults with deformed wings(fig.19). Malformations of pupae resulting from the larval treatment with Beauveria alone appears as larval-pupal intermediates failed to remove the moulting larval skin(fig.20)or undersized pupae(fig.21).Hence the adult malformations appear as adults had complete mouth parts with deformed wings(fig22) or malformed adults with variance degrees of deformed bodies and twisted wings (23,24 and25). Malformations of pupae resulting from the larval treatment with Bacillus alone appears as larval-pupal monstrosity (fig.26) or abnormal pupae bear protrusions(fig.27).On the other hand, malformed adults having deformed twisting wings(fig.28,29),as compared to normal pupae and adult(fig.30 and31).

These results are agree with that obtained by Salama *et al.* (1981) who indicated that the sublethal doses and length of exposure time to low endotoxin concentrations of three spore-δ-endotoxin preparations from *Bacillus thuringiensis, kurstaki* and *entomocidu* and Thuricide appeared deformities in both pupae and moth populations.

2.5. Adult fecundity and fertility:

Data presented in Table (4) demonstrated that the treatment of 2nd instar of *S. littoralis* larvae with the biocides product alone (1st test) or in mixture form (2nd test) induced highly significant ($p<0.01$) reduction in the adult fecundity. Mixtures of both Bacillus+ Bioranza and Beauveria+ Bioranza (2nd test) and Bioranza alone (1st test) had the strongest effect in reducing the total number of eggs to reach 0%, as compared to 501 and 510eggs/female of control ,respectively. While the larval treatment with mixture Bacillus+ Beauveria decreased the total number of eggs to reach 44 eggs/f, Whereas, the larval treatment with both Beauveria and Bacillus alone (1st test) significant ($p<0.01$) reduced the total no. of eggs to 163 and 115 eggs/f, as compared to that of control (510 eggs/f).

On the other hand, the larval feeding on the biocides product alone (1st test) or in mixture form (2nd test) decreased the eggs fertility laid by adults female, as compared to that of control, but only in case of the larval treatment with Bacillus alone and mixture of Bacillus+ Beauveria, where the eggs fertility decreased to reach 60.9 and 84%, respectively, as compared to100 and 99.6%of control, respectively.

Table 4 . The latent effects of Beauveria, Bioranza and Bacillus against the 2nd instar of *S. littoralis* larvae at the quarter dose as compared to control

Treatment	Adult					Treatment	Adult				
	fecundity	fertility %	longevity	Sex ratio			fecundity	fertility %	longevity	Sex ratio	
	Mean +S.D		Mean +S.D	female	male		Mean +S.D		Mean +S.D	female	male
Beauveria	163+4**	97	8.1+2**	47	53	Bacillus + Beauveria	44**	84	7.6+1**	45	55.2
Bioranza	0**	0	7.2+1**	43.9	56	Bacillus + Bioranza	0**	0	7.3+0.8**	34.3	65.7
Bacillus	115**	60.9	12+3n.s	47.8	52	Beauveria+ Bioranza	0**	0	5.6+2**	60	40
Control	510+14	*100*	15+5	45	55	Control	501+250	99.6	12.3+1	45	55
F value	1589.9		22.403				909		44.182		
P value	0.01485		0.00174				0.006501		0.0002		
L.S.D at 0.05	82.3		3.35				110.1		2.9		
0.01	*144.3*		*4.75*				*181.2*		*4.1*		

Highly Significant (p<0.01)　　* Significant (p<0.05)

S.D. =Standard deviation　　Malfo. = Malformatiom%

L.S.D. = Least significant difference

These results are agree with those obtained by Fargues *et al.*(2004)who demonstrated that fecundity and egg hatching of *Leptinotarsa decemlineata* treated as newly moulted 4th instar larvae were monitored in surviving females mated with surviving males when reared at 22°C and the adult survivors showed a lessening of their reproductive potential during their whole life at the fungal infection and the reductions of the total number of eggs laid per female and the mean number of eggs per egg mass ranged from 20% to 56% and from 18% to 46%, respectively. They found that at 25°C, the fecundity of survivors was not affected by the fungal infection and the secondary effects of *B. bassiana* on the fecundity of the Colorado potato beetle are temperature-dependent Also, Sewify and Mabrouk (1991) reported that the fungus proved virulent against all stages of the mite and the treatment of the eggs resulted a reduced rate of hatchability. They mentioned that none of the fungal treated eggs hatched at 20'C and only 15% hatchability was obtained at 27'C and newly hatched larvae from treated eggs failed to develop and died soon after emergence. They indicated that the immature and mature stages were susceptible to fungal infection and newly deposited eggs appeared more susceptible to infection than those about to hatch (7 day old) and none of larvae, hatched from treated eggs, survived. Also, Salama *et al.* (1981) observed that the sublethal doses and length of exposure time to low endotoxin concentrations of three spore-δ-endotoxin preparations from *Bacillus thuringiensis, kurstaki* and *entomocidu* and Thuricide induced a reduction in eggs production of the moths and fertility of the egg together.

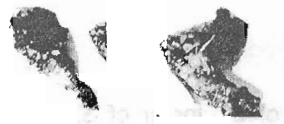
2.6. Adult longevity:

Table (4) showed that the larval treatment with the biotic insecticides alone (1st test) or in mixture form (2nd test) highly significant ($p<0.01$) decreased the adult longevity, as compared to that of control. Mixture of Beauveria+ Bioranza had the greatest effect in adult longevity decrease to reach 5.6d, as compared to12.3d of control. While the larval treatment with mixture of both Bacillus+ Beauveria and Bacillus+ Bioranza (2nd test) and Beauveria or Bioranza alone had a similar significant ($p<0.01$) effect in adult longevity decrease to reach 7.6, 7.3 and 8.1,7.2d, respectively, as compared to12.3 and15d that of control. While the larval treatment with Bacillus alone gave none significant decrease in the adult longevity reached to12d, as compared to15d of control.

These results are agree with that obtained by Salama *et al.* (1981) who recorded that the sublethal doses and length of exposure time to low endotoxin concentrations of three spore-δ-endotoxin preparations from *Bacillus thuringiensis, kurstaki* and *entomocidu* and Thuricide did not affect the longevity of the moths and the data showed no clear correlation with the concentration and time of larval exposure to the toxin.

2.7. Adult sex ratio:

Data obtained in Table (4) indicated that the larval treatment of 2nd instar of *S. littoralis* with the biotic insecticides alone (1st test) or in mixture form (2nd test) shifted the sex ratio in respect to control. The effect was more pronounced with Bioranza and Beauveria mixture, it increased the number of adult females to reach 60%, as compared to 45% of the check, and it decreased the adult males to reach 40%, as compared to 55% of control. Whereas, the larval treatment with both Beauveria and Bacillus alone had the next effect, it increased the adult females to reach 47and 47.8% ,respectively, as compared to 45% of control, and these decreased the adult males to reach53 and52%,respectively as compared 55% of control. While, the larval treatment with of Bacillus+ Beauveria mixture didn't affect the adult sex ratio. Whereas, the larval treatment with Bacillus+ Bioranza mixture and Bioranza alone had adversely effect on sex ratio. The effect was more pronounced with Bacillus+Bioranza mixture, it decreased the adult females to reach 34.3 %, as compared to 45%, and increased the adult males to reach 65.7%, as compared to55% of control. While the larval treatment with Bioranza alone decreased the adult females to 43.9%, and increased the adult males to reach 56%, as compared to45:55% of females and males of control, respectively.



(fig.3) moth malformations showing body with poorly developed and twisted wings

(Fig. 6) or moth failed to emerge from the pulpal skin in the anterior half of the body with onset of one wing and mouth parts

(fig.1 and 2) larval-pupal monstrosity with shifting in the body color, protrusions formation, and violent malformation in the general view of body with maintain the larval moulting skin.

Fig1-6: Malformations of *S .littoralis* pupae and adults resulting from the larval treatment of the 2nd instar with mixture of Bacillus and Bioranza

(Fig.4) or moth failed to emerge from the pupal skin in the posterior half of body and had light malformed wings

(Fig. 5) or moth failed to emerge from the pupal skin in the anterior half of the body

(Fig.7) pupae monstrosity that bear numerous protrusions.

(Fig.8and9), moth malformations showing body with violent malformation of one or two wings

Fig.7-9: Malformations of *S .littoralis* pupae and adults resulting from the larval treatment of the 2nd instar with Bioranza alone.

(Fig.16) or moth failed to emerge from the pupal skin in the posterior half of body with weakly wings.

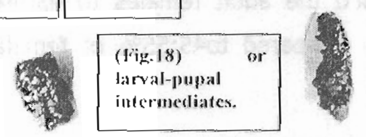
(Fig.10) larval-pupal intermediates with larval cuticle patches, head capsule and thoracic legs, posterior half of the body has the pupal properties.

(Fig.11) or pupae showing body shrinkage

(Fig.12, 13, 14 and15) moth malformations showing with variance degrees of deformed bodies and twisted wings

Fig10-16: Malformations of *S .littoralis* pupae and adults resulting from the larval treatment of the 2nd instar with mixture of Bacillus and Beauveria.

(Fig.17) undersized pupae

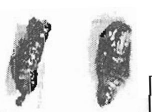


(Fig.18) or larval-pupal intermediates.

(Fig.19).malformed adults appears as adults with deformed twisting wings

Fig17-19: Malformations of *S .littoralis* pupae and adults resulting from the larval treatment of the 2nd instar with mixture of Beauveria and Bioranza

(Fig.20) larval-pupal intermediates failed to remove the moulting larval skin.



(Fig.21)or undersized pupae

(Fig.22) adults had complete mouth parts with deformed wings

(Fig.23, 24 and 25) malformed adults with variance degrees of deformed bodies and twisted wings.

Fig.20-25: Malformations of *S .littoralis* pupae and adults resulting from the larval treatment of the 2nd instar with Beauveria alone.

(Fig.26) as larval-pupal monstrosity.

(Fig.27) or abnormal pupae bear protrusions

Fig26-29: Malformations o Bacillus alone f *S .littoralis* pupae and adults resulting from the larval treatment of the 2nd instar with Bacillus alone.

(Fig. 28, and 29) malformed adults having deformed twisting wings

(Fig.30 and31) Normal pupae and adult of *S .littoralis*

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التأثيرات الجانبية لمركبات ميكروبية على العمراليرقى الثاني لدودة ورق القطن الكبرى

الهام فاروق محمود عبد الرحيم

محطة بحوث سدس. معهد بحوث وقاية النباتات. مركز البحوث الزراعية. الدقى . الجيزة

أجريت هذه الدراسة بغرض تقيم التأثير الجانبي لبعض المركبات الحيوية، البرتكتو(الباثيلس)، البيوفار والبيورنزا حيث اختبرت معمليا ضد يرقات العمر اليرقى الثاني لدودة ورق القطن.غذيت يرقات العمر الثاني لمدة٤٨ ساعة على ورق خروع مغطس في محلول احد المركبات المشار إليها والتي اختبرت اما بمفردها او فى مخاليط (الباثيلس مع البيوفار، الباثيلس مع البيورنزا، البيوفار مع البيورنزا) عند ثلاث جرعات ، كاملة ونصف الجرعة وربع الجرعة . وجد أن خليط البيوفار مع البيورنزا كان له التأثير الأقوى والغالب ضد يرقات العمر الثاني حيث انه أعطى نسب موت ١٠٠و٧٠و٦٠% عند التركيز الكلى والنصفي و الربعى بالتتالى . فى حين معاملة الباثيلس بمفردها اعطت التأثير الأخير .أيضا المعاملة اليرقيه أدت إلى موت عذري وكان لخليط الباثيلس مع البيورنزا له التأثير الأقوى حيث انه أعطى نسب موت عذري ٣٥,٥و١٥و١٣,٣ % عند التركيزات الثلاثة.كما ان المعامله بالبيورنزا مفردا سجل موت عذرى ٢٦,٧و١٣,٣و١١,٨ % عند التركيزات الثلاثة بالتتالي.وبناء على ذلك النسب الكلية لموت اليرقات والعذارى تأثرت نتيجة للمعاملة بهذه المركبات وكان لخليط البيوفار مع البيورنزا له التأثير الأقوى حيث انه أعطى نسب موت ١٠٠و٨٠و٦٥% عند التركيزات الثلاثة.في حين أن المعامله بالباثيلس بمفردها كان له التأثير الأخير. تأثرت المعايير البيولوجية لليرقات المعاملة بالمركبات الثلاثة بدرجة كبيره. وأدت المعاملة إلى زيادة العمر اليرقى وكان لخليط البيوفار مع البيورنزا له التأثير الأعلى حيث انه طول البقاء اليرقى إلى ٢٩و٢٧,٧ يوم عند التركيز النصفي والربع بالتتالي مقارنه بغير المعامل(١٢,٢ يوم).كما أن ألمعامله اليرقيه بالمركبات بمفردها كان له التأثير الأعلى فى زيادة البقاء العذرى عن المعاملة بمخاليط المركبات. فى حين ان المعاملة اليرقيه بمخاليط المركبات خفضت اكثر الوزن العذري. كما أن المعاملة اليرقيه بمخلوط البيوفار مع البيورنزا كان له التأثير الأقوى في خفض نسب التعذير إلى ٢٠و٣٥ %مع الجرعة ألنصفيه والربع مقارنه بالكنترول(١٠٠%). بينما المعاملة اليرقيه بهذه المركبات خفضت نسب خروج الفراش وكان لمخلوط الباثيلس والبيورنزا له التأثير الأكبر حيث انه سجل ٢٤و٥٠و٥٣ % عند الجرعات الثلاثه بالتتالى. فى حين ان التغذيه اليرقيه على هذه المركبات ذودت نسب التشوهات العذرية والحشرية وكان لخليط من الباثيلس مع البيورنزا له التأثير الأعلى في زيادة النسب التشوهات العذرية والحشرية إلى ٢٣,٥و١١,١و ٦,٧ : ٧٦و٤٦,٧و٥٠ % بالتتالي. كما أن ألمعامله اليرقيه بمخلوط الباثيلس مع البيورنزا او البيوفار مع

البيورنزا او معامله البيورنزا بمفردها كانوا لهم التأثير الأقوى في اضمحلال الخصوبة حيث انعدم وضع البيض بواسطة الإناث المعامل بالمقارنة ٥١٠ ، ٥٠١ بيضه لكل أنثى بالتتالي للكنترول .على الجانب الأخر المعاملة بالباثيلس بمفردها خفض نسب فقس البيض إلى ٦٠,٩ % مقارنه ١٠٠ % للكنترول. كما كان للمعاملة اليرقيه بخليط البيوفار مع البيورنزا له التأثير الاعلى في قصر عمر ألحشره الكاملة إلى ٥,٦ يوم مقارنه ١٢ يوم للكنترول. كما أن المعاملة اليرقيه بالمركبات مفردا اومخاليط لهم الأثر في تغير النسب الجنسية للذكور والإناث مقارنه بالكنترول وكان التأثيرأكثر ظهورا مع خليط البيوفار والبيورنزا .وبالتالي محاليل هذه المركبات بمفردها أو مخالطيها تكون فعالة لو استخدمت كرش ورقى ضد الآفة و تقلل من استخدام المبيدات المصنعة لتأثيرها الضار على البيئة.