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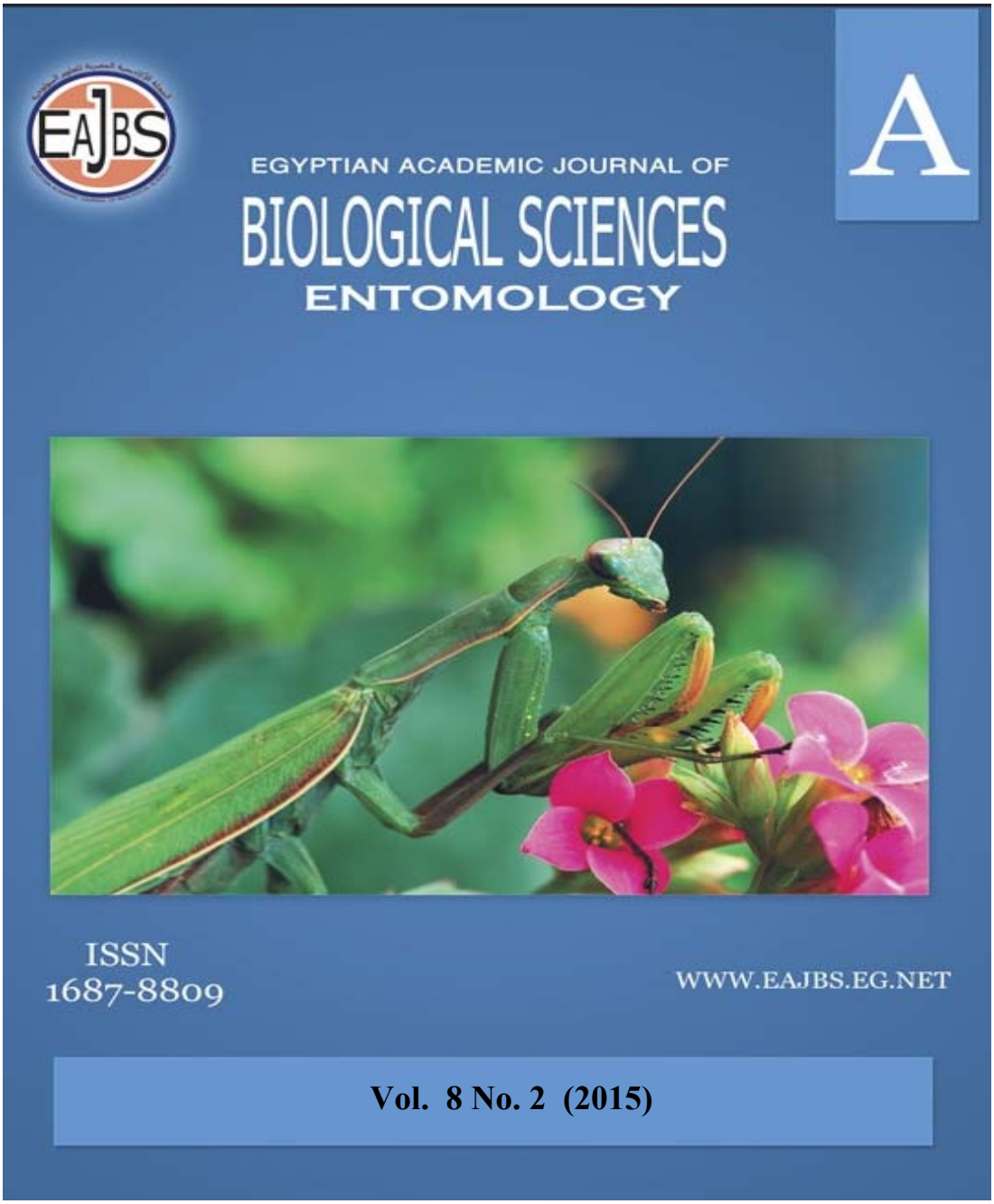

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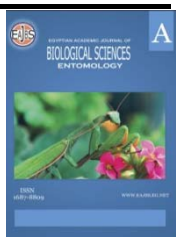

Strategies to Enhance the Toxicity of *Bacillus thuringiensis* Against *Spodoptera littoralis* (Boisd.)

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ABSTRACT

Bacillus thuringiensis (*Bt)* is an aerobic, spore forming bacterium that produces crystal inclusions that contain Insecticidal Crystal Proteins (ICPs) used to control many major pests. ICPs, or Cry proteins, are in the form of protoxins in the crystal. ICPs are solubilized and processed to toxic peptides by gut proteases in insects. Because proteases are important to toxicity, research into interactions of proteases with *Bt* proteins may lead to improved toxin efficacy. Toxicity of two commercial formulations of *Bt* was evaluated against 2nd instar larvae of *Spodoptera littoralis*. Dipel DF was more effective than Dipel 2X (LC_{50} were 0.13 and 0.17, respectively). In addition, rice bran is an agricultural waste which contains protein, fiber and carbohydrate content. It was tested in the laboratory to determine its stimulatory effects on *S. littoralis* protease. Adding rice bran extract to commercial formulation of *Bacillus thuringiensis var. kurstaki*, (Dipel DF) and / or (Dipel 2X) revealed a potential action with Co-toxicity factor +34.33 and + 21.38, respectively indicating good potential for rice bran extract to enhance *B. thuringiensis*-based formulations. Sodium bicarbonate ($NaHCO_3$), calcium carbonate ($CaCO_3$) and sodium dodecyl sulphate (SDS) were also added to both Dipel DF and Dipel DX. Data showed that all tested bio agents and their mixture with different additives induced significant reduction in both pupation percentage and pupal weight (except in case of $NaHCO_3$). The reduction effect was significantly high in Dipel DF with rice bran extract compared with the control. The pupal malformation ranged from 2.82 to 13.33%. Adult malformation resulted from treated 2nd instar larvae showed also varying degrees of deformities as a side effect of bioinsecticide alone and its combination with additives. The biochemical analysis revealed a significant increase in the proteolytic activity in all treated samples and a significant decrease in the activity of trehalase, invertase and amylase, as compared to control group.

INTRODUCTION

Bacillus thuringiensis (*Bt*) is a bacterium that forms crystals, an inactive protoxin complex, containing insecticidal proteins (ICPs) which are used to control lepidopteran, dipteran, and coleopteran insects (Schnepf *et al.,* 1998). In Lepidopera, gut proteases process ICPs from 130-140 KDa protoxins to toxic proteins of approx. 60-70 KDa.(Oppert, 1999). Protoxins are solubilized at the high alkaline environments and cleaved by proteinases in the midgut of these insects to form active toxins which then bind to specific sites on midgut brush border membranes, leading to pore formation and cell lysis. *B. thuringiensis* produce other pathogenic factors, which might contribute to virulence. These factors include phospholipases, β-

exotoxins, proteases, chitinases and vegetative insecticidal proteins (VIPs) (Estruch, 1996 and Schnepf *et al*., 1998). Also, it produces antibiotic compounds having antifungal activity (Stabb *et al*., 1994). However, protein toxins are more effective than these factors and allow the development of the bacteria in dead or weakened insect larvae. Granule and spray able starch-based formulations of *Bacillus thuringiensis* were developed to entice feeding in order to attract insects to the specific whorl locations and to compensate for *Bt* being distasteful to insects (McGuire *et al.,* 2000). Additives to the cornstarch-based granular formulation allowed a 75% reduction in the amount of *Bt* applied without loss of efficacy (McGuire and Shasha, 1995). So, Additives are compounds that can enhance the formulation action (Rodham *et al.,* 1999) and reduce the effective biopesticide dose required (Behle *et al.,* 1999). Rice bran is an agricultural waste which contains protein, fibre and carbohydrate content. (Poopathi, 2010) utilized the abundant supply of these bioorganic wastes as fermentation media for *B. thuringiensis* serovar *israelensis* (*Bti*) strains to synthesize mosquitocidal toxins. Metal ions such as Ca2+, Mg2+, Mn2+, Zn2+, Cu2+ and Fe2+ are essential for production of the highest sporulation and δ-endotoxin formation by *Bt* (Faloci *et al*., 1986, Sadek, 2000 and Icgen *et al*., 2002). SDS breaks up the two- and three-dimensional structure of the proteins by adding negative charge to the amino acids. Since like charges repel, the proteins are more-or-less straightened out. The aim of the work to evaluate some additives like rice bran extract, sodium bicarbonate ($NaHCO_3$), calcium carbonate ($CaCO_3$) and sodium dodecyl sulphate (SDS) to determine which of these additive compounds can potentiate the efficiency of *B. thuringiensis* var. *kurstaki* from two commercial formulations against *Spodoptera littoralis* 2nd instar larvae with reference to their effects on some enzyme activities that related to their mode of action like protease and carbohydrates hydrolyzing enzymes.

MATERIALS AND METHODS

Insects:

The culture of the cotton leafworm, *Spodoptera littoralis* (Boisd.) was obtained from a laboratory strain, in the Cotton Leafworm Research Dept, Plant Protection Research Institute, Agricultural Research Centre, Dokki, Giza. The insect was reared on castor leaves, *Ricinus communis*, under laboratory conditions at 27 ± 2 °C and 60 ± 5 % R.H. 2nd instar larvae were used in the current work.

Biopesticide:

Bacillus thuringiensis var. kurstaki as two commercial formulations were obtained from Plant Protection Research Institute, Agricultural Research Centre, Cairo, Egypt. Dipel 2X 6.4 % and Dipel DF 54 %.

Additives:

The additives used were rice bran extract, sodium bicarbonate ($NaHCO_3$), calcium carbonate ($CaCO_3$) and sodium dodecyl sulphate (SDS). Rice bran extracted according to (Poopathi, 2010).

Bioassay:

Preliminary test were carried out using series of concentrations (in water) ranged from 0.25 to 0.03 % for each of the bio-agent, Dipel DF and Dipel 2X using leaf-dipping technique. Castor bean leaves, *Ricinus communis,* were dipped in each concentration then left to dry at room temperature and these were offered to 2nd instar larvae. Larvae were allowed to feed for 48 hrs. then, they were provided with fresh and untreated castor bean leaves. Larvae that fed on untreated castor bean leaves

were used as control. In all treatments, four replicates were carried out for each concentration; each replicate consisted of 25 larvae. The larval mortality percentages were determined after two, three and five days from treatment. The data were then subjected to probit analysis (Finney, 1971) to obtain the LC_{50} values of both bio-agents after 120 hrs. from treatment.

Combined action

Preliminary experiment: using additives alone, without combination with bio-agents, were tested firstly at 0.01% concentrations to evaluate the effect of them against the target insect. The additives were tested in combination with bio-agents at LC_{50S}. Mortality were recorded after 5 days and corrected by Abbott's formula (Abbott, 1925).

Combined action was expressed as Co-toxicity factor and was estimated by the equation of (Mansour *et al.*, 1966) as follows.

$$\text{Co-toxicity factor} = \frac{[\text{Observed mortality \%} - \text{Expected mortality\%}]}{\text{Expected mortality \%}} \times 100$$

Where: Observed mortality % is the mortality of individuals treated by the combination, Expected mortality % is the sum of mortalities of each material when used singly.The interaction result was assessed as, 'potentiation' when Co-toxicity factor was greater than or equal to 20; 'addition' when Co-toxicity factor was between −20 and +20; and 'antagonism' when Co-toxicity factor was less than or equal to −20.

Biochemical Analysis

The samples of larvae used in enzyme assays were obtained from those subjected and fed on the median lethal concentration LC_{50} of Dipel DF, Dipel 2X and the promising mixtures resulting from combine action experiment compared with untreated larvae.

Preparation of samples for biochemical analysis:

Fifteen starved larvae from each treatment and control were homogenized in distilled water using a Teflon homogenizer surrounded with a jacket of crushed ice for 3 minutes. Homogenates were centrifuged at 6000 r.p.m. for 10 minutes at 5°C. The supernatant was stored at –20 °C until analysis. Three replicates were used for each biochemical determination.

Determination of Protease activity:

The proteolytic activity was determined by the casein digestion method described by (Ishaaya *et al*., 1971).

Determination of Carbohydrates hydrolyzing enzymes:

The principle based on the digestion of trehalose, sucrose and starch by trehalase, invertase and amylase, respectively according to the method described by (Ishaaya and Swirisk, 1976). The free aldehydic group of glucose formed after trehalose, sucrose and starch digestion were determined using 3.5 dinitrosalicylic acid reagent. Trehalase reaction mixture consisted of 0.2 ml of 3% trehalose (substrate), 0.18 ml acetate buffer (pH 5.40 and 20 µl of larval homogenate). Invertase reaction mixture consisted of 0.2 ml of 4% sucrose (substrate) and 20 µl of larval homogenate. Amylase reaction mixture consisted of 0.2 ml of 2% starch (substrate), 0.16 ml of phosphate buffer (pH 6), and 40 µl larval homogenate.

Statistical analysis:

Statistical analysis was carried out using Analysis of Variance (one way ANOVA) test through "SPSS-Computer Program". Means were compared using Duncan's Multiple Range test.

RESULTS AND DISCUSSION

The median lethal concentrations LC_{50} of Dipel DF and Dipel 2X.

Toxicity of two commercial formulations of *Bacillus thuringiensis* was evaluated against 2nd instar larvae of *S. littoralis* by leaf dip bioassay (Figs. 1 and 2).

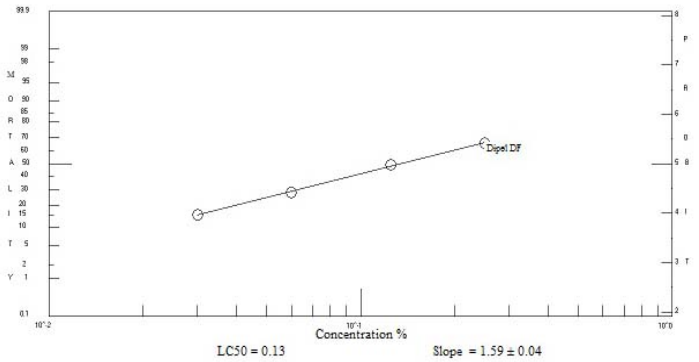

Fig.1: Log concentration probit line showing response of 2nd instar larvae of *S. littoralis* to Dipel DF.

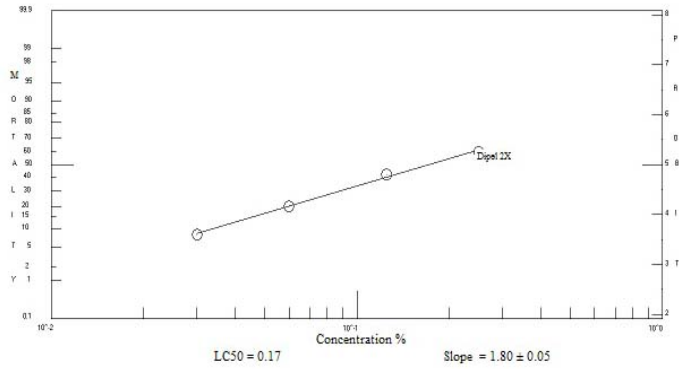

Fig.2: Log concentration probit line showing respons of 2nd instar larvae of *S. littoralis* to Dipel 2X.

The median lethal concentrations LC_{50}s of Dipel DF and Dipel 2X were 0.13 and 0.17% after five days from treatment. Based on LC_{50} values, data revealed that Dipel DF was more effective than Dipel 2X. These results are in agreement with those recorded by (Haggag, 2013) who revealed that the tested Bt-formulations had insecticidal activity against 2nd and 4th instars of *S. littorals* larvae, where Dipel DF highly killed the insect larvae, followed by Dipel 2X. Indeed, Dipel DF contains *Bt* sub. *Kurstaki*, fermentation solides, spores and insecticidal toxins. While Dipel 2X, contains only *Bt* sub *kurstaki*.

Susceptibility of the 2nd instar larvae of *S. littoralis* toward Dipel DF, Dipel 2X and some additives.

Preliminary experiment using additives alone [rice bran extract, sodium bicarbonate ($NaHCO_3$), calcium carbonate ($CaCO_3$), sodium dodecyl sulphate (SDS)] at 0.01% concentrations and bio-agents [Dipel DF and Dipel 2X] at LC_{50} to evaluate the effect of them against *S. littoralis* 2nd instar larvae Table (1).

Table 1 : Susceptibility of the 2nd instar larvae of *S. littoralis* toward Dipel DF, Dipel 2X and some additives at different time intervals.

Treatments (Conc.)	Corrected larval mortality % at different time		
	48 hrs.	72 hrs.	120 hrs.
Dipel DF (0.13%)	6.0	32.3	48.5
Dipel 2X (0.17%)	0.0	21.2	50.1
Rice bran extract(0.01%)	0.0	1.0	5.1
$NaHCO_3$ (0.01%)	0.0	2.0	3.0
$CaCO_3$ (0.01%)	0.0	0.0	2.0
SDS (0.01%)	4.0	6.1	8.1

The mortality of second instar larvae by Dipel DF started at 48 hrs. While the mortality by Dipel 2X start after this time. This result may be due to the difference between their formulations. The larval mortality caused by additives alone ranged from 2 to 8.1 after 120 hrs. from treatments. Similar results were obtained by (Morris *et al*., 1995) that larval mortality caused by different concentrations from calcium carbonate or SDS alone ranged from 2.0 to 3.0 or 2.0 to 12.6%, respectively.

The combine action.

The data of evaluated bio- agents / additives combinations against *S. littoralis* 2nd instar larvae are cited in Table (2).

Table 2: The combine action of some additives mixed with bio-agents against *S. littoralis* 2nd instar larvae.

Bio-agents / Additives	Dipel DF			Dipel 2X		
	Observed mortality %	Expected mortality %	Co-Toxicity factor	Observed mortality %	Expected mortality %	Co-Toxicity factor
Rice bran extract (R.B)	72	53.6	+ 34.33(p)	67	55.2	+ 21.38(p)
$NaHCO_3$ 0.01%	34	51.5	- 33.98(a)	44	53.0	- 16.98(d)
$CaCO_3$ 0.01%	64	50.5	+ 26.73(p)	56	52.0	+ 7.69(d)
SDS 0.01%	67	56.6	+ 18.37(d)	58	58.2	- 0.34 (d)
(R.B)+ $NaHCO_3$ 0.01%	23	56.6	- 59.36(a)	42	58.2	- 27.84(a)
(R.B)+ $CaCO_3$ 0.01%	78	55.6	+ 40.28(p)	60	57.2	+ 4.90(d)
(R.B)+ SDS 0.01%	66	61.7	+ 6.97(d)	64	63.3	+ 1.11(d)

(a) = antagonism (p) = potentiation (d) = additive effect

Results showed that the combinations of Dipel DF or Dipel 2X with rice bran extract revealed a potential action**.** (Poopathi, 2010) developed a culture media based on rice bran for *B. thuringiensis* serovar *israelensis* (*Bti*) and recorded their efficacy of the produced protein toxin against three mosquito species (*Culex quinquefasciatus*, *Anopheles stephensi* and *Ae.aegypti*). In addition, rice bran is an agricultural waste which contains protein, fibre and carbohydrate content. This may stimulate protease production in target insect gut which considered important to transfer protoxins to form active toxins. Also, combinations of Dipel DF with calcium carbonate ($CaCO_3$) showed a potential action**.** Similar results were obtained by (Morris *et al*., 1995) who state that $CaCO_3$ increased the efficiency of the endotoxin of *B. thuringiensis* var. *kurstaki* (Dipel) against *M. configurata* by 1.6 fold at 0.05% concentration. Some ions may act as cofactors in the proteolytic process of the ingested delta-endotoxin, facilitating the cleavage of the protoxin in the insect gut (Fast, 1981). Our results agree with (Couch and Ross, 1980) who reported that calcium salts increased the proteolytic process of the intact crystal of *B.*

thuringiensis, thus yielding either increased levels to the active toxin fractions responsible for exerting the mode of action of the toxin on the target insect. In addition, (Narayanan *et al*., 1976) suggested that the low pH of the gut juice of *S. littoralis* is a main factor contributing to the weak susceptibility of this species to many *B. thuringiensis* preparations. (Ragaei, 1990) and (Nickerson, 1980) reported that, the addition of such alkaline compounds will change the pH of the gut, being more alkaline and thus enhancing the endotoxin breakdown and release of toxic fragments. On the other hand, sodium bicarbonate ($NaHCO_3$) recorded antagonism action with both bio-agents. The combinations of Dipel DF with SDS revealed an additive action + 18.37. Similar results were obtained with (Morris *et al*., 1995) who declare that SDS increased the efficiency of the endotoxin of *B. thuringiensis* var. *kurstaki* (Dipel) against *M. configurata* by 1.8-fold at 0.01% concentration. The protein solubilizing agents (SDS) might have increased the endotoxin solubility by reducing the disulphide in *Bt* protein molecules, thereby effecting the midgut epithelial cells to increase the permeability to *Bt* (Salama *et aI.,* 1989). (Mohamed *et al*., 2010) recorded that calcium carbonate increase the pH value of *Bt*.

The latent effect of the tested bio-agents and their mixture with some additives.

The latent effects of the tested bioinsecticides and additives combinations on the 2nd instar larvae of *S. littoralis* are shown in Tables (3). Pupation percentage, pupal weight, adults emergence percentage and morphological malformations of pupae and emerged adults were investigated and recorded.

Table 3: The latent effect of the tested bio-agents and their mixture with some additives on some biological aspects of *S. littoralis*.

Treatments (Conc.)	Pupation %	Pupal weight (g.± SE)	Malformed pupae %	Adult emergence %	Malformed adults %
Dipel DF (0.13%) +	46	0.296±0.002b	8.69	39	7.69
Rice bran extract(0.01%)	28	0.272±0.001c	10.71	21	9.52
$NaHCO_3$ (0.01%)	59	0.318±0.001a	3.39	51	3.92
$CaCO_3$ (0.01%)	30	0.289±0.004bc	6.67	22	13.64
SDS (0.01%)	32	0.292±0.001b	9.38	28	3.57
(R.B)+ $NaHCO_3$(0.01%)	71	0.332±0.003a	2.82	66	3.03
(R.B)+ $CaCO_3$ (0.01%)	18	0.288±0.002bc	11.11	12	16.67
(R.B)+ SDS (0.01%)	31	0.281±0.003bc	6.45	28	3.57
Dipel 2X (0.17%) +	45	0.291±0.003b	13.33	36	13.89
Rice bran extract(0.01%)	34	0.283±0.001bc	11.76	27	14.81
$NaHCO_3$(0.01%)	49	0.298±0.001b	6.12	42	4.76
$CaCO_3$ (0.01%)	41	0.301±0.002b	7.32	35	11.42
SDS (0.01%)	42	0.297±0.001b	7.14	33	12.12
(R.B)+ $NaHCO_3$(0.01%)	47	0.324±0.008a	4.26	41	4.88
(R.B)+ $CaCO_3$ (0.01%)	39	0.294±0.001b	7.69	31	6.45
(R.B)+ SDS (0.01%)	33	0.278±0.002c	3.03	29	6.89
Control	98	0.321±0.004a	0.0	96	0.0

Within each column, means with the same letter are not significantly different ($p < 0.05$).

Data clearly indicated that all tested bio-agents and their mixture with different additives induced significant reduction in both pupation percentages and pupal weight except in the case of $NaHCO_3$ showed no significant differences. The reduction effect was significantly high in Dipel DF + rice bran extract (0.272 g.) compared with (0.321 g.) in control. Weight reduction of pupae could be attributed to the effect of larval feeding inhibition caused by *Btk* resulting in lower food intake,

digestion and assimilation (Ignoffo and Gregory, 1972). The descending order of reduction in adult emergence in different treatments is Dipel DF + R.B + $CaCO_3$ (12%), Dipel DF + R.B (21%), Dipel DF + $CaCO_3$ (22%), Dipel 2X + R.B (27%) and so on. Adult mortality may be due to the latent toxic effects of the tested material, and this was agreed with that obtained by (Mohamed *et al.,* 2005; Koja *et al.,* 2006 and Younes *et al.,* 2008).

The pupal malformation ranged from 2.82 to 13.33%. Malformations were high in Dipel 2X (13.33%), Dipel DF + R.B + $CaCO_3$ (11.11%), Dipel 2X + R.B (11.76%) and Dipel DF + R.B (10.71%). The abnormalities of the resulted pupae showed in Figure (3). They were characterized by the following; Larval pupal intermediate with larval head and thoracic legs, but the abdomen is pupal stage (Fig. 3-B & C), undifferentiated body segments (Fig. 3-D) and small size directed downward head capsule (Fig. 3- E & F). Normal pupa (Fig. 3- A).

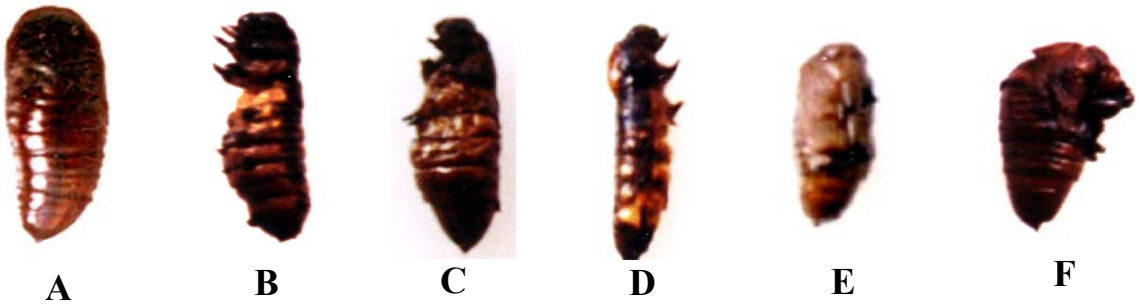

Fig. (3): Showing the normal and malformed structure of *S. littoralis* pupae produced after treated 2nd instar larvae with the tested bio-agents and their mixture with some additives.

S. littoralis resulted from treated 2nd instar larvae showed also varying degrees of deformities as a side effect of bioinsecticide alone and its combination with additives (Fig. 4). The malformations include the attachment of pupal cuticle and the adult failed to emerge (Fig. 4- B & C), adult attached to old cuticle of pupa by the legs (Fig. 4- D), enlargement in the abdominal region and severe shrinkage of appendages especially wings (Fig. 4- E) and wings were folded and extremely reduced in size (Fig. 4- F). Normal adult (Fig. 4- A).

Fig. (4): Showing the normal and malformed structure of *S. littoralis* adults produced after treated 2nd instar larvae with the tested bio-agents and their mixture with some additives.

Bio- agents alone and its combination with additives showed an increase in the percentage of malformed pupae and adults. The reason of malformations may be due to the reduction in proteins, transaminase enzymes, carbohydrate hydrolyzing enzymes and lipids and these results and observations are in agreement with those of

(Attalla *et al.,* 2003). Further, these effects might also be due to inhibition of nucleic acid and protein synthesis by *Btk* exotoxin (Kim *el al.,* 1972).

Biochemical Analysis.

Because proteases play an important role in the solubilization and activation of *Bt* protoxins, in which believes they are necessary in early step in Bt mode of action. In addition, proteases act as membrane receptors for *Bt* toxins. (Valaitis *et al.,* 1995 and Lee *et al.,* 1996) documented Aminopeptidase N, in the brush border membrane of the gypsy moth, *Lymantria dispar* as a Cry1Ac binding protein. Similar results showed by (Gill *et al.,* 1995) on the lepidopteran insect *Heliothis virescens*. So, in the present study we recorded the changes in total proteolytic activity of larvae fed on the median lethal concentration LC_{50} of Dipel DF, Dipel 2X and the promising mixtures resulting from combine action experiment compared with untreated larvae (Table 4). Results showed a significant increase in the proteolytic activity in all treated samples as compared to control groups.

Table 4: Effect of the tested bio-agents and their mixture with some additives on some digestive enzymes.

Treatments (Conc.)	Activity Ux100/gm. (Mean ± SE)		Mean carbohydrate hydrolyzing enzymes activity µg glucose/min /gm. ± S. E.					
	protease		Trehalase		Invertase		Amylase	
	72hrs.	% change	72hrs.	% change	72hrs.	% change	72hrs.	% change
Dipel DF (0.13%) +	61.2±1.5 c	+ 32.47	223.3±2.7 c	- 28.84	289.3±5.6 b	- 28.20	204.8±3.4 b	- 15.69
Rice bran extract (0.01%)	73.0±1.6 b	+ 58.00	235.3±3.6 c	- 25.02	224.4±2.6 d	- 44.35	152.8±2.6 d	- 37.09
$CaCO_3$ (0.01%)	61.8±1.1 c	+ 33.77	223.8±5.8 c	- 28.68	275.3±3.5 c	- 31.67	204.5±2.1 b	- 15.81
(R.B)+ $CaCO_3$(0.01%)	82.2±1.2 a	+ 77.92	205.8±3.9 d	- 34.42	175.8±4.4 e	- 56.37	185.1±1.8 c	- 23.80
Dipel 2X (0.17%) +	48.2±2.4 d	+ 4.33	263.8±3.9 b	- 15.93	308.2±6.8 b	- 23.50	233.0±2.7 a	- 4.08
Rice bran extract (0.01%)	72.3±1.1 b	+ 56.49	203.2±4.7 d	- 35.25	232.7±2.7 d	- 42.24	185.5±2.6 c	- 23.63
Control	46.2±2.1 d	0.0	313.8±6.5 a	0.0	402.9±1.3 a	0.0	242.9±7.8 a	0.0

Within each column, means with the same letter are not significantly different ($p < 0.05$).

In addition, all treatments caused significant decrease in the activities of trehalase, invertase and amylase as compared to control group. Carbohydrates are reserves as glycogen and trehalose which can be converted into glucose for the support of all life processes. During metamorphic changes glycogen and treholase supply glucose which provides an energy source and a substrate for the synthesis of pupal and adult tissues, especially the cuticle. So, the inhibition of trehalase observed in the present work might affect chitin build-up. (Meisner *et al.,* 1978) state that, trehalase has the important function for liberating glucose for energy, and is activated during moulting to generate glucose for chitin build up. In conclusion, Dipel DF was more effective against 2nd larval instar of *S. littoralis* than Dipel 2X, may be due to its formulation. Adding rice bran revealed a potential action with both formulations. Adding rice bran and calcium carbonate to Dipel DF caused the most significant increase in protease activity compared with the control group.

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ARABIC SUMMERY

استراتيجيات لتعزيز سمية بكتيريا *Bacillus thuringiensis* ضد دودة ورق القطن

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بكتيريا الباسيللس ثورينجينسيس تنتج بلورات تحتوي على البروتينات السامه المستخدمة لمكافحة العديد من الآفات. هذه البلوات تتواجد بحالة غير نشطة الى ان تنكسرلتعطى البيتيدات السامة بواسطة كل من الوسط القلوى وانزيم البروتياز داخل المعى المتوسط للحشرة. ولاهمية كل من قلوية الوسط المتواجد به البلورات وانزيم البروتياز لانطلاق البيبتيدات السامة فأن تنشيط هذان العاملان من الممكن ان يؤدي إلى تحسين فعالية سمية البكتيريا. ولذلك فقد تم اختبار نوعين من المستحضرات البكتيريه (Dipel DF) و (Dipel 2X) ضد يرقات الطور الثاني لدودة ورقة القطن سبودوبترا ليتوراليس و كان Dipel DF هو الأكثر كفاءة حيث كان LC_{50} 0.13 و 0.17 ل Dipel DF و Dipel 2X لهما على التوالي. وحيث ان نخالة الارزتعتبر من المخلفات الزراعية التى تحتوى على البروتينات و الالياف و الكربوهيدرات فمن الممكن الاستفادة منها فى الدراسة الحاليه باختبارها في المختبر لتحديد قدرتها على زيادة كفاءة اثنين من المستحضرات التجارية من بكتيريا الباسيلاس ثيرينجنسيس (Dipel DF) و (Dipel 2X) و ذلك باضافة مستخلص نخالة الأرز للمستحضرات المختبرة. كما تم أيضا اختبار بعض الأملاح مثل بيكربونات الصوديووم و كربونات الكالسيوم و صوديوم دودوسيل سلفات لزيادة كفاءة كلا المستحضرين Dipel DF, Dipel 2X منفردين او مجتمعين مع مستخلص نخالة الارز و كانت النتيجة أن كل هذه الإضافات (ما عدا بيكربونات الصوديوم) قد أحدثت انخفاض معنوي في نسبة العذارى و أوزانها. نسبة العذارى المشوهة تراوحت ما بين 2.82-13.33%. كما كانت هناك تشوهات في الحشرات البالغة. أوضحت الدراسة البيوكيميائية أيضا ارتفاع معنوي في نشاط انزيم البروتياز و انخفاض معنوي في انزيم التريهاليز، و الأميليز و الإنفيرتيز مقارنة بالمجموعة الضابطة. كما اوضحت النتائج قدرة مستخلص نخالة الارز على زيادة نشاط انزيم البروتياز المختبر. وهذا يشير إلى إمكانية جيدة لاستخدام مستخلص نخالة الأرز لتعزيز تركيبات الباسيللس ثورينجينسيس.