

## SYNERGISTIC EFFECT OF SOME ADDITIVES WITH BIO-INSECTICIDES TO CONTROL THE PINK BOLLWORM, *Pectinophora gossypiella* (SAUND.)

Salem, M.S.

Plant Protection Research Institute, (A.R.C.) Dokki, Giza,

Egypt

msalem3030@gmail.com



### ABSTRACT

Some additive compounds of sorbic acid, glucose and glycine amino acid have been added to improve the potency of some bio-insecticides; (Bactericide) *Bacillus thuringiensis* var. *Kurstaki* and (Fungicide) *Beauveria bassiana* (Balsamo) formulations against newly hatched larvae of *Pectinophora gossypiella* (Saund.). Results obtained could be summarizing as follows:

*Bacillus thuringiensis* had the most bio-insecticides efficacy on *P.gossypiella* newly hatched larvae after 1,2&3 days from treatment.

Additives of glucose at concentrations of 0.1 and 0.05%, followed by sorbic acid at 0.1% were enhanced *B. thuringiensis* effect and caused the potentiation interaction on *P.gossypiella* larval mortality percentages. Other additive compounds caused additive interaction for *B. thuringiensis* except for additives of glycine (0.1& 0.025%) caused antagonism effect for *B. thuringiensis* and reduced from *P.gossypiella* larval mortality.

On the other hand, additives of glucose at concentration of 0.1% potentiate *B. bassiana* efficiency against *P.gossypiella* larvae. Other additive compounds of glucose (0.05 and 0.025%) and sorbic acid (0.1, 0.05 and 0.025%) caused additive interaction with *B. bassiana*. Meanwhile, additive of glycine decreased *B. bassiana* efficacy and caused antagonism effect on *P.gossypiella* larval mortality.

All additives compounds used had effect on some biological parameters of *P.gossypiella* when additives had interaction with *B. thuringiensis* or *B. bassiana* compared to use each of them alone. Additive of glucose had the best effect on the most biological parameters used, followed by sorbic acid, while; additive of glycine had the least interaction with the two bio-insecticides against the aforementioned pest.

**Keywords:** additives, sorbic acid, glycine, glucose, *Bacillus thuringiensis*, *Beauveria bassiana*, *Pectinophora gossypiella*.

### INTRODUCTION

The pink bollworm, *Pectinophora gossypiella* (Saunders) was described from larvae recovered from infested cotton bolls in India in 1843 (Noble, 1969). It was since become one of the most destructive pests of cotton in the major cotton growing regions of the world. In addition to infest many crops as okra, hibiscus, jute...exc.

Despite the growth of integrated and organic methods, the pesticides with serious health and environmental hazards are used and the number of sprays per season is high. The result is high input costs, health risks and the presence of pesticide residues in plants often exceeding the maximum residue levels. Pesticide use reduction targets along with requirement for farmers to follow crop specific integrated production guidelines are needed. Bio-insecticides of *B. thuringiensis* and *B. bassiana* that used in current study

were found among Integrated Pest Management Program because its efficacy and safety. Also, its efficacy can be enhance the formulation action and reduce the effective bio-pesticide dose required by additive compounds (Behle *et al.*, 1999).

Salama *et al.* (1990 a) demonstrated that the efficacy of Dipel (commercial formulation of *B. thuringiensis* var. *kurstaki*) was greatly enhanced in terms of larval population reduction and crop yield increase by adding potassium carbonate, zinc sulphate, or calcium carbonate to the bacterial spray or bait at a concentration of 0.075%. *Bacillus thuringiensis* var. *kurstaki* in the laboratory has been enhanced by the addition of toxic and non toxic compounds to the bacterial suspensions against the target larvae. Morris, *et al.* (1995) mentioned that the formulations include inorganic salts, tannin, organic acids, and oxidized carbohydrates causes an enhancement to the bacteria *Bt*.

Sabbour (2002) reported that metabolic acids (oxalic and citric acids) scored the highest enhancement in the efficiency of *M. anisopltae* against the first larval instars of *P. operculella*. In addition, Hsia, *et al.* (2014) evaluated the effect of additives (sulfonic acid, sodium salt, lignosulfonic acid, sodium salt, sodium acetate, sodium alginate and sodium glutamate) on conidial viability of *M. anisopliae* formulation as they were not harmful to the germination beyond 80%, when conidia were exposed to these additives. Amer, *et al.* (2014) tested some additive compounds of ascorbic acid, citric acid, glucose, L-alanine, potassium chloride and sodium carbonate that have been added to improve the potency of some entomopathogenics; *B. thuringiensis*; *M. anisopltae*; *H. bacteriophora* and *S. abbasi* formulations against 4<sup>th</sup> instars larvae of *S. littoralis*. *B. thuringiensis* had the most entomopathogenics efficacy on *S. littoralis* 4<sup>th</sup> instars larvae compared to other compounds used after 2 days from treatment. While, *M. anisopltae* was the most potent compound on *S. littoralis* 4<sup>th</sup> instars larvae after 4 and 7 days from treatments. Additives of ascorbic acid, potassium chloride and sodium carbonate were added at concentration of 0.025% enhance *B. thuringiensis* effect and caused the potentiation interaction. Other additive compounds caused additive interaction for *B. thuringiensis* except for additives of citric acid (0.1%) and glucose (0.1%) caused antagonism effect for *B. thuringiensis* and reduced from *S. littoralis* 4<sup>th</sup> instars larval mortality. On the other hand, additives of potassium chloride and sodium carbonate at concentration of 0.025% potentiate *M. anisopltae* efficiency against *S. littoralis* 4<sup>th</sup> instars larvae. Other additive compounds of ascorbic acid (0.05 and 0.025%), citric acid (0.1, 0.05 and 0.025%), glucose (0.05%), potassium chloride (0.1%) and sodium carbonate (0.05%) caused additive interaction with *M. anisopltae*. Meanwhile, the other tested additives decreased *M. anisopltae* efficacy and caused antagonism effect on *S. littoralis* larval mortality.

The aim of the present study to evaluate some compounds of glucose, glycine and sorbic acid represent in different groups were used as additives to determine which of them can potentiate the efficiency of *Bacillus thuringiensis* var. *kurstaki*, and *Beauveria bassiana* (Balsamo) formulations

against newly hatched larvae of *Pectinophora gossypiella* (Saund.) efficacy and biological parameters.

## MATERIALS AND METHODS

### A- Bio-insecticides.

**1- Bactericide:** Dipel-2x, a commercial formulation of *Bacillus thuringiensis* var. Kurstaki, a product of Vailent Bioscience Corporation, USA was obtained from May trade company, Giza, Egypt. About 3200 International units potency per mg. spores and protein crystals, of the active ingredient constituted 6.4% of the formulation and the dose rate was 200 gm/feddan.

**2- Fungicide:** Biosect, a local commercial formulation of *Beauveria bassiana* (Balsamo) and it is a product of Organic Company for Biotechnology, Egypt. The international unit was  $32 \times 10^6$  viable spores per mg. The active ingredient was 10% and the recommended application rate was 200gm per 100 liter water/Faddan.

### B- Additives.

The additive compounds used were grouped as follow:

1- **Organic acid:** Sorbic acid; getting from Chemicals limited Company.

2- **Amino acid:** Glycine; packed and distributed by El-Nasr Adweic Company, Egypt, according to the method of PROLABO.

3- **Sugar:** Glucose; getting from Lamia Company for chemical.

All additives compounds used at concentrates of 0.1, 0.05 and 0.025% were tested singly and combined with bio-insecticide compounds.

### C- Experimented insect.

A laboratory strain of the newly hatched larvae of the pink bollworm was reared in the Bollworms Department, Plant Protection Research Institute, Agriculture Research Center on semi artificial diet as described by Rashad and Ammar (1985). Rearing conditions were controlled at  $27 \pm 1^\circ\text{C}$  and 65-75% RH with complete darkness all day time.

### D- Bioassay of *Bacillus thuringiensis* and *Beauveria bassiana*:

To deduce the  $\text{LC}_{50}$  of *B. thuringiensis* and *B. bassiana* against newly hatched larvae of *P. gossypiella*. Four serial dilutions of *B. thuringiensis* or *B. bassiana* (40, 10, 2.5 and 0.625 g/L) or ( $128 \times 10^6$ ,  $32 \times 10^6$ ,  $8 \times 10^6$  and  $2 \times 10^6$  IU/L for *B. thuringiensis*) and ( $128 \times 10^{10}$ ,  $32 \times 10^{10}$ ,  $8 \times 10^{10}$  and  $2 \times 10^{10}$  CFU'S/L for *B. bassiana*) were prepared and five replicates/ dilution were done. Two gm of semi artificial diet/Petri-dish (9 cm diameter) were mixed with 1 cm of each concentration from the tested bio-insecticides. The Petri-dish used as control was treated with 1 cm distilled water mixed with 2 gm artificial diet. Twenty five newly hatched larvae of *P. gossypiella*/replicate exposed for about 6 hours for each replicates/ concentrate/ tested bio-insecticide and kept at  $27 \pm 1^\circ\text{C}$  and 65-75% R.H. Then the alive larvae transferred to untreated artificial diet to investigate the alive and dead larvae after 24, 48 & 72 hours after treatment.

#### E. Biological parameters:

Newly hatched larvae of *P. gossypiella* treated by LC<sub>50</sub>'s of bio-insecticides with additive compounds (at concentrations of 0.1, 0.05 and 0.025%) and each of them singly. The following biological parameters were investigated as follows:

**1- Larval and pupal durations** (in days).

**2- Pupation and moths emergency percentages.**

% Pupation= No. produced pupae/Total tested larvae X100

% Moths emergency = No. emerged moth/total tested larvae X100

**3- Larval mortality percentage:** Larval mortalities were corrected according to Abbott's formula (1925).

All biological parameters of *P. gossypiella* were analyzed using Costat statistical program software, 1990 and Duncan's multiple range test (Duncan, 1955) at 5% probability level to compare the differences among time means.

#### F- Statistical analysis.

a- LC<sub>50</sub> & LC<sub>90</sub> and slope values were measured by according to Finny (1971) by using Ldp line software (www.Ehabbakr software/Ldp line).

b- The combined (joint) action of the different mixtures was expressed as Co-toxicity factor (Sun and Johnson, 1960) to observe the differentiation between the potentiation, antagonism and additive effects using the following equation:

$$\text{Co-toxicity Factor} = (O - E) \times 100/E$$

Where O is observed mortality (%) and E is expected mortality (%).

This factor differentiates the results into three categories. A positive factor of  $\geq 20$  indicates potentiation, a negative factor of  $\leq -20$  indicates antagonism, and the intermediate values of  $> -20$  to  $< 20$  indicates an additive effect.

## RESULTS AND DISCUSSION

#### A- Efficacy of bio-insecticides against *Pectinophora gossypiella* larvae.

Cotton bollworm, *P. gossypiella* newly hatched larvae was treated by tested bio-insecticides; Bactericide (*B. thuringiensis* var. *kurstaki*) and Fungicide (*B. bassiana*, Balsamo.) to evaluate the efficacy of them against *P. gossypiella* larvae.

Table 1 showed that *B. thuringiensis* was more efficacy than *B. bassiana* on *P. gossypiella* newly hatched larvae after 1, 2& 3- day from treatment especially, at 3-day (LC<sub>50</sub>: 0.012 and 0.035 gm/L for *B. thuringiensis* and *B. bassiana*, respectively). Also, the same table observed that susceptibility of *P. gossypiella* to *B. thuringiensis* was higher than to *B. bassiana* as recorded in the slope values in Table 1.

**Table (1): Efficacy of *Bacillus thuringiensis* and *Beauveria bassiana* against newly hatched larvae of *Pectinophora gossypiella*.**

Bio-insecticides	LC <sub>50</sub> (gm/L) ± 95% Confidence limits	LC <sub>90</sub> (gm/L) ± 95% Confidence limits	Slope
After 1 day from treatment			
<i>Bacillus thuringiensis</i> (IU/L)	1.45 (0.08±2.54) 46.4x10 <sup>5</sup>	51.51(41.54±62.03) 1648.3 x10 <sup>5</sup>	0.151± 0.1721
<i>Beauveria bassiana</i> (CFU S /L)	4138 (3255±5219) 132416 x10 <sup>9</sup>	51417 (25588±66523) 1645344 x10 <sup>9</sup>	0.612± 0.3579
After 2 days from treatment			
<i>Bacillus thuringiensis</i> (IU/L)	0.07 (0.01±0.1) 2.24 x10 <sup>5</sup>	33.64 (29.32±37.37) 1076.5 x10 <sup>5</sup>	0.348± 0.1807
<i>Beauveria bassiana</i> (CFU S /L)	467 (353.2±689.3) 14944 x10 <sup>9</sup>	6554 (5339.1±7654.5) 209728 x10 <sup>9</sup>	0.179± 0.1453
After 3 days from treatment			
<i>Bacillus thuringiensis</i> (IU/L)	0.012 (0.001±0.041) 0.384 x10 <sup>5</sup>	11.30 (7.40±15.13) 361.6 x10 <sup>5</sup>	0.430± 0.1858
<i>Beauveria bassiana</i> (CFU S /L)	0.035 (0.014±0.084) 1.12 x10 <sup>9</sup>	69.96 (55.15±78.81) 2238.7 x10 <sup>9</sup>	0.245± 0.1604

Akici, *et al.* (2014) stated that *Bacillus thuringiensis* subsp. *kurstaki* to be the most effective causing 100% mortality within 10 days after treatment on *S. littoralis* 3<sup>rd</sup> instars larvae. While, fungi can spread fast among hosts horizontally via aerially produced conidia and infects its host by penetration of the cuticle by its germ hyphae. The fungi grow within the internal fluids of insect integument, sponging degraded proteins & fat bodies, and produces toxins which kill the host. The active phase of fungi are spores attacking insect cuticle and/or as a stomach poison and must be eaten by the lepidopterous larvae, therefore; it could be active feeding on treated exposed plant parts. Also, Al-Kherb (2014) carried out the capabilities of two entomopathogenic fungi, *B. bassiana* and *M. anisopliae*, in controlling eggs and the 1<sup>st</sup> instars larvae of *S. exigua* Hub. in the laboratory. Eggs and 1<sup>st</sup> instars larvae of *S. exigua* were treated with different concentrations of conidia of two entomopathogenic fungi. Three weeks after treatment, spore concentration 10x10<sup>5</sup> spores/ml resulted in the highest mortality (50 and 37.6%) with eggs and (100 and 88%) with 1<sup>st</sup> instars larvae when treated by *B. bassiana* and *M. anisopliae*, respectively.

**B- Effect of bio-insecticide and additive combinations on *Pectinophora gossypiella* larvae.**

Some additive compounds of glucose, glycine and sorbic acid, at concentrations of 0.1, 0.05 and 0.025% were added to LC<sub>50</sub> of bio-insecticides compounds, *B. thuringiensis* and *B. bassiana* for activation purpose against *P. gossypiella*.

### 1- *Bacillus thuringiensis*.

Some chemical additives have been adopted to increase the potency of *B. thuringiensis* against the cotton bollworm, *P. gossypiella*. Procedures were based on the incorporation of some selected essentially non toxic and low cost compounds with different modes of action with the endotoxin to increase its activity. Among the compounds tested were some organic acid salt (sorbic acid), sugar (glucose) and amino acid (glycine).

As illustrated in Table (2), all the additives caused 4% mortality percentage of *P. gossypiella* larvae. Additives of glucose (0.1 and 0.05%) and sorbic acid (0.1%) caused potentiation interaction with LC<sub>50</sub> value of *B. thuringiensis* against larvae. Opposite, the additive of glycine (0.1 and 0.025%) caused contributed effect with *B. thuringiensis* activity and appeared antagonism interaction. Other additives caused additive effect with *B. thuringiensis* activity against *P. gossypiella* larvae.

Salama, *et al.* (1990 a) subsisted that the low pH of the gut juice of *S. littoralis* larvae is a main factor contributing to the weak susceptibility of this species to many *B. thuringiensis* preparations and reported that, the addition of such alkaline compounds will change the pH of the gut, thus enhancing the endotoxin breakdown and release of toxic fragments. So, potassium carbonate enhanced the effectiveness of *B. thuringiensis* against *S. littoralis* and *A. ipsilon* on soybean and various crops; also, increased the toxicity of *B. thuringiensis* var. *kurstaki* HD-1 at (0.5%) against *S. littoralis* as compared to the control. Also, mentioned that potassium carbonate tested as an inorganic salt to enhance the activity of *B. thuringiensis* against *Plutella xylostella* and there was a highly significance after the combination of potassium carbonate at the tested concentrations with *B. thuringiensis* (HD-234) at LC<sub>50</sub> tested against 1<sup>st</sup> instars larvae of *P. operculella* and *H. armigera* and it showed effective enhancer for *B. thuringiensis*.

**Table 2: Effect of some additives on *Bacillus thuringiensis* activity against newly hatched larvae of *Pectinophora gossypiella* after 3-day from treatment.**

Treatments	Corrected mortality %		Expected Mortality %	Co-toxicity factor	Type of interaction
	Additive alone	Additive+ <i>B. thuringiensis</i>			
<i>B. thuringiensis</i>	51	-	-	-	-
Glucose 0.1%	4	78	55	41.8	Potentiation
Glucose 0.05%	4	74	55	34.5	Potentiation
Glucose 0.025%	4	64	55	16.4	Additive
Glycine 0.1%	4	25	55	-54.5	Antagonism
Glycine 0.05%	4	54	55	-1.82	Additive
Glycine 0.025%	4	38	55	-30.9	Antagonism
Sorbic acid 0.1%	4	72	55	30.9	Potentiation
Sorbic acid 0.05%	4	58	55	5.45	Additive
Sorbic acid 0.025%	4	46	55	-16.4	Additive

In addition, Salama *et al.* (1990a) used amino acids for *B. thuringiensis* activation and mentioned that potential effect of such *B. thuringiensis* protein solubilizing agents was due to the reduction of disulfide bonds in the

endotoxin molecule to sulfahydryl groups, increasing endotoxin solubility in the insect gut and thus high larval mortality. Most of the nitrogen in the insect haemolymph is found in the form of amino acids, the alteration of the haemolymph composition may interfere with the normal physiological processes. Among the tested amino acids, L-arginine and L-serine and they showed to be effective enhancer for *B. thuringiensis* against *P. operculella* larval mortality. Morris *et al.* (1995) reported that amino acids may lead to leakage to the haemocoel in an unorganized manner. It is expected that, the regulatory mechanism of the insect would be challenged to reset the natural composition of haemolymph possibly through specific reaction. Also, the same authors mentioned that sorbic acid was tested as one of this group, and it showed to have an antagonistic effect against *B. thuringiensis*. When *B. thuringiensis* (HD-234) tested alone at LC<sub>50</sub>, the larval mortality of *P. operculella* and *H. armigera* was 47 and 51%, respectively; it was markedly decreased to reach 39 and 39%, respectively, after the incorporation of sorbic acid at 0.1% concentration. Where sorbic acid was ineffective as enhancer for the activity of *B. thuringiensis* against *M. Configurata*. Amer, *et al.* (2014) stated that *B. thuringiensis* had the most efficacy on *S. littoralis* 4<sup>th</sup> instars larvae after 2 days from treatment. Additives of ascorbic acid, potassium chloride and sodium carbonate were added at concentration of 0.025% enhance *B. thuringiensis* effect and caused the potentiation interaction. Additives of citric acid (0.1%) and glucose (0.1%) caused antagonism effect for *B. thuringiensis* and reduced from *S. littoralis* 4<sup>th</sup> instars larval mortality.

**a- *Beauveria bassiana*.**

Fungicide, *B. bassiana* had corrected mortality percentage 50% for *P. gossypiella* newly hatched larvae, this mortality percentage increased to 68% when glucose (0.1%) added to LC<sub>50</sub> *B. bassiana* and the potentiation interaction appeared as illustrated in Table (3). Additive compounds of sorbic acid (0.1, 0.05 and 0.025%) and glucose (0.05 and 0.025%) caused additive interaction to *B. bassiana* against *P. gossypiella* larvae. Contrary, additive of glycine (0.1, 0.05 and 0.025%) had antagonism interaction when added to *B. bassiana* and decreased from the efficacy of *B. bassiana* against *P. gossypiella* larvae.

**Table(3):Effect of some additives on *Beauveria bassiana* activity against newly hatched larvae of *Pectinophora gossypiella* after 3-day from treatment.**

Treatments	Corrected mortality %		Expected Mortality %	Co-toxicity factor	Type of interaction
	Additive alone	Additive+ <i>B. bassiana</i>			
<i>B. bassiana</i>	50	-			
Glucose 0.1%	4	68	54	25.9	Potentialion
Glucose 0.05%	4	48	54	-11.1	Additive
Glucose 0.025%	4	48	54	-11.1	Additive
Glycine 0.1%	4	30	54	-44.4	Antagonism
Glycine 0.05%	4	28	54	-48.1	Antagonism
Glycine 0.025%	4	38	54	-29.6	Antagonism
Sorbic acid 0.1%	4	62	54	14.8	Additive
Sorbic acid 0.05%	4	60	54	11.1	Additive
Sorbic acid 0.025%	4	50	54	-7.41	Additive

Similar results were obtained by Sabbour (2002) who found that calcium carbonate at concentration of 0.05% combined with the fungi caused 79% reduction in LC<sub>50</sub> of *B. bassiana* against *P. operculella*. Where Potassium carbonate at 0.05% concentration caused 21 and 41% reduction in LC<sub>50</sub> of *B. bassiana* and *M. anisopliae* against *P. operculella*, respectively. Amer, *et al.* (2014) stated that *M. anisopliae* was the most potent compound on *S. littoralis* 4<sup>th</sup> instars larvae after 4 and 7 days from treatments. Additives of potassium chloride and sodium carbonate at concentration of 0.025% potentiate *M. anisopliae* efficiency against *S. littoralis* 4<sup>th</sup> instars larvae. Other additive compounds of ascorbic acid (0.05 and 0.025%), citric acid (0.1, 0.05 and 0.025%), glucose (0.05%), potassium chloride (0.1%) and sodium carbonate (0.05%) caused additive interaction with *M. anisopliae*.

The obtained results in Table (3) of using amino acid of glycine caused antagonism interaction with LC<sub>50</sub> of *B. bassiana* against *P. gossypiella* larvae. The results were matched with Sabbour (2002) who mentioned that L-arginine and L-serine at a concentration of 0.05% caused 47 and 50% reduction in LC<sub>50</sub> of *B. bassiana* and *M. anisopliae* against *P. operculella*, respectively and caused 57 and 73% reduction in LC<sub>50</sub> of *B. bassiana* and *M. anisopliae*, respectively.

#### **B- Biological parameters of *Pectinophora gossypiella* affected by bio-insecticide and additive treatments.**

##### **1-Larval and pupal durations.**

Tables (4&5) showed that larval duration (from newly hatched until pupation) increased when the cotton bollworm, *Pectinophora gossypiella* treated as newly hatched larvae in the most treatments of additives only as well as with those treated by LC<sub>50</sub>'s of *Bacillus thuringiensis* with glycine (0.1, 0.05 & 0.025%) compared to the control (16 days). Opposite was happened in bio-insecticide with additives of glucose and sorbic; in addition, treatment



of glucose only (0.025%), the larval durations were decreased than control. Another trend was found in Table 5, each of bio-insecticide alone and *Beauveria bassiana* with additive of glycine (0.1, 0.05 and 0.025%) treatments had the same value of control.

Alive larvae of *P. gossypiella* and complete to pupal stage had pupal duration increased about one day than control value (5 days) in all the treatments of bio-insecticides with additives or each of them if used singly.

### 2- Larval mortality percentage.

The normal larval mortality percent of *P. gossypiella* was 4%, that percent increased and ranged from 14 to 54 % when the newly hatched larvae treated by additive compounds Table (4), When the newly hatched larvae treated by LC<sub>50</sub> of *B. thuringiensis* or *B. bassiana*, the larval mortality were 88 and 90%, respectively. Meanwhile, larval mortality was the highest that reached to 100% when newly hatched larvae of *P. gossypiella* treated by additives of glucose and sorbic when added at concentrations of 0.1, 0.05 and 0.025% to *B. thuringiensis* or *B. bassiana* as showed in Table (5).

**Table 4: Effect of tested additives on some biological parameters of *Pectinophora gossypiella* treated as newly hatched larvae.**

Treatments	Larval duration (days)	% Larval Mortality	Pupal duration (days)	% Pupation	% Moths emerged
Control	16 <sup>a</sup>	4 <sup>g</sup>	5 <sup>a</sup>	96 <sup>a</sup>	96 <sup>a</sup>
Glucose 0.1%	19 <sup>a</sup>	28 <sup>c</sup>	6 <sup>a</sup>	72 <sup>e</sup>	72 <sup>e</sup>
Glucose 0.05%	18 <sup>a</sup>	20 <sup>e</sup>	6 <sup>a</sup>	80 <sup>cd</sup>	80 <sup>cd</sup>
Glucose 0.025%	7 <sup>b</sup>	14 <sup>f</sup>	6 <sup>a</sup>	86 <sup>b</sup>	86 <sup>b</sup>
Glycine 0.1%	18 <sup>a</sup>	40 <sup>d</sup>	6 <sup>a</sup>	60 <sup>f</sup>	60 <sup>f</sup>
Glycine 0.05%	18 <sup>a</sup>	24 <sup>d</sup>	6 <sup>a</sup>	76 <sup>de</sup>	76 <sup>de</sup>
Glycine 0.025%	18 <sup>a</sup>	16 <sup>f</sup>	6 <sup>a</sup>	84 <sup>bc</sup>	84 <sup>bc</sup>
Sorbic acid 0.1%	19 <sup>a</sup>	54 <sup>a</sup>	7 <sup>a</sup>	46 <sup>g</sup>	46 <sup>g</sup>
Sorbic acid 0.05%	18 <sup>a</sup>	40 <sup>b</sup>	6 <sup>a</sup>	60 <sup>f</sup>	60 <sup>f</sup>
Sorbic acid 0.025%	17 <sup>a</sup>	20 <sup>e</sup>	6 <sup>a</sup>	83 <sup>bc</sup>	83 <sup>bc</sup>
LSD <sub>0.05</sub>	2.849	2.998	2.583	4.137	4.137

### 3- Pupation and moth emergency percentages.

Pupation percentages were affected by the different treatments as in Tables (4&5). The pupation percentage had highly decreased especially in treatments of *B. thuringiensis* (12%) or *B. bassiana* (10%) singly or combined with glycine (0.1, 0.05 & 0.025%) treatments, it ranged between 20 and 24%.

Moth emergency percentage was affected as the same trend that obtained previously in pupation parameter.

**Table (5): Effect of tested additives and bio-insecticides on some biological parameters of *Pectinophora gossypiella* treated as newly hatched larvae.**

Treatments	Larval duration (days)	% Larval Mortality	Pupal duration (days)	% Pupation	% Moths emerged
<i>Bacillus thuringiensis</i>	16 <sup>ab</sup>	88 <sup>b</sup>	6 <sup>a</sup>	12 <sup>b</sup>	12 <sup>b</sup>
Glucose 0.1% + <i>Bacillus thuringiensis</i>	8 <sup>ef</sup>	100 <sup>a</sup>	- <sup>b</sup>	- <sup>c</sup>	- <sup>c</sup>
Glucose 0.05%+ <i>Bacillus thuringiensis</i>	9 <sup>def</sup>	100 <sup>a</sup>	- <sup>b</sup>	- <sup>c</sup>	- <sup>c</sup>
Glucose 0.025%+ <i>Bacillus thuringiensis</i>	11 <sup>bcdef</sup>	100 <sup>a</sup>	- <sup>b</sup>	- <sup>c</sup>	- <sup>c</sup>
Glycine 0.1%+ <i>Bacillus thuringiensis</i>	17 <sup>a</sup>	76 <sup>cd</sup>	6 <sup>a</sup>	24 <sup>a</sup>	24 <sup>a</sup>
Glycine 0.05%+ <i>Bacillus thuringiensis</i>	17 <sup>a</sup>	77 <sup>cd</sup>	6 <sup>a</sup>	23 <sup>a</sup>	23 <sup>a</sup>
Glycine 0.025%+ <i>Bacillus thuringiensis</i>	17 <sup>a</sup>	78 <sup>cd</sup>	6 <sup>a</sup>	22 <sup>a</sup>	22 <sup>a</sup>
Sorbic acid 0.1%+ <i>Bacillus thuringiensis</i>	11 <sup>bcdef</sup>	100 <sup>a</sup>	- <sup>b</sup>	- <sup>c</sup>	- <sup>c</sup>
Sorbic acid 0.05%+ <i>Bacillus thuringiensis</i>	13 <sup>abcde</sup>	100 <sup>a</sup>	- <sup>b</sup>	- <sup>c</sup>	- <sup>c</sup>
Sorbic acid 0.025%+ <i>Bacillus thuringiensis</i>	14 <sup>abcd</sup>	100 <sup>a</sup>	- <sup>b</sup>	- <sup>c</sup>	- <sup>c</sup>
<i>Beauveria bassiana</i>	16 <sup>ab</sup>	90 <sup>b</sup>	6 <sup>a</sup>	10 <sup>b</sup>	10 <sup>b</sup>
Glucose 0.1% + <i>Beauveria bassiana</i>	6 <sup>f</sup>	100 <sup>a</sup>	- <sup>b</sup>	- <sup>c</sup>	- <sup>c</sup>
Glucose 0.05%+ <i>Beauveria bassiana</i>	13 <sup>abcde</sup>	100 <sup>a</sup>	- <sup>b</sup>	- <sup>c</sup>	- <sup>c</sup>
Glucose 0.025%+ <i>Beauveria bassiana</i>	15 <sup>abc</sup>	100 <sup>a</sup>	- <sup>b</sup>	- <sup>c</sup>	- <sup>c</sup>
Glycine 0.1%+ <i>Beauveria bassiana</i>	16 <sup>ab</sup>	74 <sup>d</sup>	6 <sup>a</sup>	26 <sup>a</sup>	26 <sup>a</sup>
Glycine 0.05%+ <i>Beauveria bassiana</i>	16 <sup>ab</sup>	74 <sup>d</sup>	6 <sup>a</sup>	26 <sup>a</sup>	26 <sup>a</sup>
Glycine 0.025%+ <i>Beauveria bassiana</i>	16 <sup>ab</sup>	80 <sup>c</sup>	6 <sup>a</sup>	20 <sup>a</sup>	20 <sup>a</sup>
Sorbic acid 0.1%+ <i>Beauveria bassiana</i>	10 <sup>cdef</sup>	100 <sup>a</sup>	- <sup>b</sup>	- <sup>c</sup>	- <sup>c</sup>
Sorbic acid 0.05%+ <i>Beauveria bassiana</i>	12 <sup>abcde</sup>	100 <sup>a</sup>	- <sup>b</sup>	- <sup>c</sup>	- <sup>c</sup>
Sorbic acid 0.025%+ <i>Beauveria bassiana</i>	16 <sup>ab</sup>	100 <sup>a</sup>	- <sup>b</sup>	- <sup>c</sup>	- <sup>c</sup>
LSD <sub>0.05</sub>	4.519	4.443	2.183	4.076	4.076

Generally, additives of glucose, followed by sorbic acid had increased from *B. thuringiensis* and *B. bassiana* efficacy against *P. gossypiella* larvae compared to each of bio-insecticide or additive efficiency when used alone. While, additive of glycine had the least interaction with bio-insecticides

against *P. gossypiella* larvae. Larvae of *P. gossypiella* affected by the aforementioned treatments and clearly appeared in its biological parameters especially larval mortality percent that reached to 100% in the most treatments of bio-insecticides that added to tested additives.

## REFERENCES

- Abbott, W.W. (1925). A method of computing the effectiveness of an insecticide. *Journal of Economic Entomology*, 18: 265-267.
- Akici, F.Z.; Sevim, A.; Demirbag, Z. and Demir, A. (2014). Investigating internal bacteria of *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae) larvae and some *Bacillus* strains as biocontrol agents. *Turk J Agric.* 38: 99-110.
- Al-Kherb, W.A. (2014). Virulence bio-Assay efficiency of *Beauveria bassiana* and *Metarhizium anisopliae* for the biological control of *Spodoptera exigua* Hübner (Lepidoptera: Noctuidae) eggs and the 1<sup>st</sup> Instars Larvae. *Australian Journal of Basic and Applied Sciences*, 8(3): 313-323.
- Amer, R.A.; Nouh, G.M.; Yacoub, Sh.S. and Elhosary, R.A. (2014). Role of some additives for enhancing entomopathogenic biocontrol agents against larvae of the cotton leaf worm, *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae). *Egypt. Jour. Of Biol. Pest. Cont.* 24(2): 399-405.
- Behle, R.W.; Tamez-Guerra, P.; Shasha, B. and McGuire, M. (1999). Formulating bioinsecticides to improve residual activity. *Formulations Forum'99. Orlando, FL, USA*; March 3–5.
- Duncan, D.B. (1955). Multiple ranges and multiple F.test. *Biometrics*. 11:1-42.
- Finney, D.J. (1971). Probit Analysis, Cambridge: *Cambridge University Press*.
- Hsia, I.C.; Islam, M.T.; Ibrahim, Y.; How, T.Y. and Omar, D. (2014). Evaluation of Conidial Viability of Entomopathogenic Fungi as Influenced by Temperature and Additive. *Int. J. Agric. Biol.*, 16: 146–152
- Morris, O.N.; Converse, V. and Kanagaratnam, P. (1995). Chemical additive effects on the efficacy of *Bacillus thuringiensis* Berliner subsp. *kurstaki* against *Mamestra configurata* (Lepidoptera: Noctuidae). *Journal of Economic Entomology*, 88: 815-824.
- Noble, L.W. (1969). Fifty years research on the pink bollworm in the united states agriculture. *Handbook No. 357, Washington, D.C.* 20402.
- Rashad, A.M. and E.D. Ammar (1985): Mass rearing of the spiny bollworm, *Earias insulana* (Boisd.) on semi artificial diet. *Bull.Soc.Ent.Egypte*, 65: 239-244.
- Sabbour, M.M. (2002). The role of chemical additives in enhancing the efficacy of *Beauveria bassiana* and *Metarhizium anisopliae* against the

- potato tuber moth *Phthorimaea operculella* (Zeller) (Lepidoptera: Gelechiidae). *Pakista Journal of Biological Science*, 5: 1155-1159.
- Salama, H.S.; Saleh, M.R.; Moawad, S. and Shams-El-Din, A. (1990 a). Spray and dust applications of *Bacillus thuringiensis* Berliner and Lannate against *Spodoptera littoralis* (Boisd) (Lepidoptera, Noctuidae) on soybean plants in Egypt. *J. Appl. Entomol.*, 109: 194-199.
- Sun, Y.P. and Johnson, E.R. (1960). Analysis of joint action of insecticides against house flies. *J. Econ. Entomol.*, 53, 887-892.

### التأثير التنشيطي لبعض الإضافات مع المبيدات الحيوية لمكافحة دودة اللوز القرنفلية

محمد سالم محمد سالم

معهد بحوث وقاية النباتات- مركز البحوث الزراعية- الدقى- الجيزة- مصر

تم إضافة بعض المركبات: حامض السوربيك- الجلوكوز- الحامض الأميني جليسين وذلك بغرض تحسين فاعلية مستحضرات المبيدات الحيوية الحشرية لبكتيريا الـ *Bacillus thuringiensis* var. *Kurstaki* - فطر الـ *Beauveria bassiana* (Balsamo) على يرقات الفقس الحديث لدودة اللوز القرنفلية (*Pectinophora gossypiella* (Saund.) . ويمكن تلخيص النتائج كما يلي :

أظهرت بكتيريا الـ *B. thuringiensis* كفاءة أعلى على يرقات الفقس الحديث لدودة اللوز القرنفلية بالمقارنة بفطر الـ *B. bassiana* بعد مرور 1, 2, 3 أيام من بداية المعاملة . أدت إضافة الجلوكوز بتركيزات 0.1 - 0.05 % بليه السوربيك (0.1%) إلى تأثير تقوية لبكتيريا *B. thuringiensis* وزيادة نسبة موت اليرقات. بينما أظهرت المركبات الأخرى تأثير إضافة (0.1 - 0.025%) ما عدا مركب الجليسين الذى أظهر تأثيرا تضاديا لبكتيريا *B. thuringiensis* وخفض النسبة المئوية للموت اليرقى لدودة اللوز القرنفلية . إضافة الجلوكوز بتركيز 0.1% أدى إلى تأثير تقوية لفطر *B. bassiana* لمكافحة يرقات دودة اللوز القرنفلية. كما سببت المركبات المضافة الأخرى (الجلوكوز بتركيزات 0.05 - 0.025% وحمض السوربيك بتركيزات 0.1 - 0.05 - 0.025%) تأثيرا إضافيا لفطر *B. bassiana* بينما سبب مركب الجليسين تأثيرا تضاديا للفطر على النسبة المئوية لموت يرقات دودة اللوز القرنفلية . أثرت معاملات المبيدات الحيوية والإضافات معا على القياسات البيولوجية لدودة اللوز القرنفلية وتأثيرا واضحا مقارنة باستخدام أى منهم بمفرده. وكان مركب الجلوكوز أفضل المركبات المضافة تأثيرا على القياسات البيولوجية بليه فى ذلك حمض السوربيك. بينما أعطى الجليسين أقل تأثيرا على كفاءة المبيدين الحيويين ضد يرقات دودة اللوز القرنفلية .