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Compatibility and interaction between the entomopathogenic bacteria *Bacillus thuringiensis* (Berliner) and some synthetic insecticides used to control cotton leafworm, *Spodoptera littoralis* (Boisd.)

Hassan Mohamed El-Saadany[1], Aziza Hassan Mohamady[2*] and Ahmed Adly Ibrahim[1]

[1] Bio- insecticides production Unit, Plant Protection Research Institute, Agriculture Research Center, Giza, **Egypt**
[2] Bioassay Department, Central Agriculture Pesticides Laboratory, Agriculture Research Center, Giza, **Egypt.**

***Correspondence:** aziza_1972@yahoo.com **Accepted:** 30 Jul. 2019 **Published online:** 20 Aug. 2019

As a selective biological agent, *Bacillus thuringiensis* var. *kurstaki* (Berliner) (*Bt*) has been widely used to control *Spodoptera littoralis* (Boisd.). The objective of this study was to evaluate the compatibility between a local isolate *B. thuringiensis* and some recommended insecticides at the recommended field rate/concentration (RC), half and 1/4 RC in culture medium. Results showed a significant difference in the number of *Bt* colonies formed following direct exposure to the tested insecticides (chloropyrofos-methyl, lambda-cyhalothrin, methomyl and flufenoxuron). RC, half and 1/4 RC of Chloropyrofos-methyl as well as RC of lambda cyhalothrin inhibited the growth of *Bt* by 100%. In contrast, emamectin benzoate at all concentrations used and methomyl at (half & 1/4 RC) had no effect on the number of *Bt* colonies when compared to control. The comparative efficacy of tested compounds against 2nd larval instar of *S. littoralis* revealed that emamectin benzoate was the most effective compound (with LC_{50} = 0.0503 ppm) compared to the other compounds, while methomyl was the least toxic compound (with LC_{50} = 7.42 ppm) after 48h from treatment. Interaction bioassay showed potentiation effect of emamectin benzoate at zero and 24 h, while lambda-cyhalothrin and flufenoxuron exhibited additive effect. In contrast, antagonistic effect was observed with chloropyrofos-methyl and methomyl treatments at zero time, while 24h after treating the larvae with *Bt*, both exhibited potentiation and additive effect, respectively. The findings of the present study suggested that, application of the tested insecticides after 24h from larval exposure to *Bt* were more effective than when combined with *Bt* at zero time or individual application.

Keywords: Compatibility, *Bacillus thuringiensis*, Synthetic insecticides, *Spodoptera littoralis*

INTRODUCTION

The cotton leafworm, *Spodoptera littoralis* (Boisd.), is one of the most destructive agricultural lepidopterous pests. It can attack numerous economically important crops all the year round such as cotton, *Gossypium hirsutum* L., peanut, *Arachis hypogaea* L., soybean, *Glycine max* L. and vegetables in Africa, Asia and Europe (Bayoumi et al., 1998 and Pineda et al., 2007). This pest causes considerable damage for many field and vegetable crops in Egypt. To combat this pest, many chemical insecticides belonging to different groups have been registered and recommended to use for its control according to the approved agricultural pest control recommendations (Anonymous, 2012). Repeating and intensive use of conventional

insecticides such as organophosphate, carbamate and pyrethroids against *S. littoralis* have led to the development of insect resistance, and subsequently affected the implementation of pest control programs and increased environmental contamination (Smagghe et al., 1999 and Aydin & Gürkan, 2006). To overcome these problems, new insecticide groups that produced from natural agents or formulations that disrupt the physiological processes of the target pest have been introduced and registered as alternatives for use in integrated pest management programs (Dhadialla et al., 1998; Thompson et al., 2000; Smagghe et al*.,* 2003 and Nedal and Hassan, 2009). So, the application of these products such as chitin synthesis inhibitors (CSIs) and bio-pesticides that showed high selectivity and low toxicity to human and environment is highly appreciated (Teran-Vargas et al., 1997; Furlong et al., 1994; Grafton-Cardwell et al., 2005 and Defago et al., 2006).

Chitin synthesis inhibitors act by interfering with chitin biosynthesis during moulting period in insects, which confers a remarkable action specificity with low harm to beneficial arthropods (Consoli et al., 2001 and Wakgari & Giliomee, 2003) and humans (Grafton-Cardwell et al., 2005).

Emamectin benzoate is a novel generation of avermectin that generated from the modified fermentation of the soil microorganism, *Streptomyces avermitilis* (Crouch et al., 1997). It acts as a chloride channel activator by binding gamma amino butyric acid (GABA) receptor and affecting the glutamate-gated chloride channels causing a flow of chloride ion into neuronal cells which disrupting nerve impulses. This resulting in, irreversible paralysis, cessation of feeding and death within 3-4 days (Dunbar et al., 1998; Ishaaya et al., 2002 and Grafton-Cardwell et al., 2005). Emamectin benzoate has high efficacy against Lepidoptera insects including *Spodoptera exigua, Helicoverpa zea*, and *S. littoralis* (Trumble et al., 1987; Lopez et al., 2010; El-Sheikh, 2015) and has low activity against most beneficial arthropods (Jansson et al., 1997)

B. thuringiensis (*Bt*) endotoxins are the most important microbial insecticides used in the world (BenFarhat-Touzri et al., 2013) as an alternative or supplement to chemical insecticides. (*Bt*) endotoxins are effective in controlling different cotton pests including *S. littoralis* but not their natural enemies (Torres et al., 2006; Armengol et al., 2007; Brookes & Barfoot, 2008 and Mhalla et al., 2018). However, some shortcomings limit its usage, such as its narrow spectrum of activity and short persistence in the field (Satinder et al., 2006 and Sleem et al., 2012). Thus, the combination of bio-agent with chemical insecticides was tested as attempt to increase the efficiency of the bio-agent, minimize the use of chemical insecticides and reduce the environmental pollution. Previous studies showed potentiation of *Bt* by addition of toxic and non-toxic compounds (Khalique and Ahmed 2005; Wang & Huang 1999 and Morris et al., 1995).

Despite importance of mixtures, the interaction between *Bt* and chemical insecticides has rarely been investigated (Salama et al., 1984; Morales-Rodriguez & Peck, 2009 and Amizadeh et al., 2015).

Therefore, the current study was conducted to evaluate the compatibility between *B. thuringiensis* spores with certain recommended synthetic insecticides under laboratory conditions, and the comparative efficacy of these insecticides against the 2nd larval instar of *S. littoralis*. The optimal time to apply the synthetic insecticides with or after larval exposure into *B. thuringiensis* spores to achieve the effective control of *S. littoralis* was also determined.

MATERIALS AND METHODS

The experiments were carried out under laboratory conditions (25 ± 2°C, 65 ± 5% R.H.) at the Bio-insecticides Production Unit, Plant Protection Research Institute, Agriculture Research Center, Ministry of Agriculture, Dokki, Giza, Egypt.

Tested insect:

A laboratory strain of cotton leafworm*, S. littoralis* was provided by Central Agriculture Pesticides Laboratory (CAPL), Dokki, Giza. This strain was reared on castor bean leaves as described by (El–Defrawi et al., 1964) in laboratory under constant conditions of 25 ± 2ºC and 65 ± 5% R.H. without any exposure to insecticides.

Tested compounds:

Entomopathogenic bacteria:

Bacillus thuringiensis, subspecies kurstaki was kindly provided by insect Pathogen Production Unit, Plant Protection Research Institute, ARC, Ministry of Agriculture, Egypt. Culture of *Bt* was carried out according to Attathom et al. (1995) as follows: T3 medium was prepared which composed of tryptone 3.0g, tryptose 2.0g, yeast extract 1.5g, $MnCl_2$ 0.005g and NaH_2PO_4. H_2O 8.9g, adjusted pH to 6.8 and the final volume was made up to 1

liter with distilled water. The sterilized medium was inoculated and incubated on a shaker (142 rpm) at 37ºC for 72 h. The number of CFU/ml of the suspension, which resulted from the previously technique of production, was determined by plate count method (Atlas, 2004).

Synthetic insecticides:

In this study we used five synthetic insecticides that locally recommended in control *S. littoralis* (Table 1).

Bioassay experiments

Effect of some synthetic insecticides on *B. thuringiensis* growth:

Efficacy of some synthetic insecticides on the growth of *B. thuringiensis* was investigated using the method described by Ibrahim et al. (2009). Each 100 ml portion of the medium was dispensed into a 250 Erlenmeyer conical flask and autoclaved at 121ºC for 20 minutes and then cooled to about 45ºC. Stock solutions of the insecticides were prepared in sterilized distilled water and incorporated into each flask to provide RC, half and 1/4 RC. Each plate was inoculated with 1ml from 72h old culture of *B. thuringiensis*. Each flask was shaken well and poured into 3 sterilized Petri-plates (9 cm in diameter). A medium without insecticides served as a control. The inoculated plates were incubated at 37±1ºC. After 24h of incubation, the growth of *B. thuringiensis* colony in the Petri-plates treated with different insecticides at different concentrates was recorded.

Efficacy of some Synthetic insecticides and *B. thuringiensis* spores against the 2nd larval instar of S. *littoralis:*

Bioassay tests were carried out under laboratory conditions to evaluate the efficacy of the tested compounds against newly ecdysed 2nd larval instars of *S. littoralis* using leaf-dipping technique as described by Shepard (1958). A Serial of concentrations for each compound were prepared in distilled water, then fresh castor bean leaves were dipped in each concentration for 20 seconds and left to dry at room temperature before being offered to larvae. Three replicates with ten larvae per replicate were tested for each concentration, and each bioassay was repeated three times. Control larvae were fed on water-treated leaves. The larvae were exposed and fed on treated leaves for 48h, and mortality percentages were recorded after 24 and 48h for chloropyrofos-methyl, lampda-cyhaothrin, and methomyl. While in case of flufenoxuron, emamectin benzoate and *Bt* spores suspension, the survival larvae were transferred to feed on untreated leaves for another 24 h, and the mortality were recorded after 48 and 72h for flufenoxuron and emamectin benzoate and after 48 and 72h, 96 and 168h for *Bt* spores suspension. Mortality percentages were corrected as compared to control larvae according to Abbott's formula (Abbott, 1925). To estimate LC_{25}, LC_{50} and slope values, the corrected mortality percentages were subjected to Probit analysis using Ldp-line software according to Finney (1971).

Interaction between *B. thuringiensis* spores suspensions and some synthetic insecticides:

This experiment has been done in order to define the optimal time for applying synthetic insecticides and *Bt*. The aim behind this was to determine the best time of exposure that might show high efficacy when both synthetic insecticide and *Bt* were applied together at the same time or in different times. Joint toxic action between the *Bt* spores suspension and the tested insecticides were evaluated against the 2nd larval instar of *S. littoralis* at two different time intervals according to the following. 1) The larvae were treated with only LC_{50} of *B. thuringiensis* spores suspension or LC_{25} of each synthetic insecticide. 2) The larvae were treated with mixture of LC_{50} of *B. thuringiensis* spores suspension and LC_{25} of each synthetic insecticide (zero time). 3) The larvae were exposed into LC_{50} of *B. thuringiensis* spores suspension only for 24h then, the same larvae were treated with LC_{25} of each synthetic insecticide (24h). Three replicates with ten 2nd instar larvae per each replicate were used for each treatment and bioassays were repeated 3 times. Also, three replicates were used as control which fed on water-treated leaves. The observed mortality percentage was recorded after four days of each treatment. The expected mortality for the mixture was calculated by sum of the observed mortalities of each concentration used in the mixture.

Table (1): Synthetic insecticides used in bioassay tests.

Chemical group	Common name	Trade name	Manufacturer	Rate of application

Organophosphate	**Chloropyrofos-methyl**	**Ictan 50% EC**	**Icta**	**1L /Feddan**
Synthetic pyrethroids	**Lambada-cyhalothrin**	**Lambada super 10% WP**	**Eid**	**50g /100L water**
Avermectin	**Emamectin benzoate**	**Proclaim 5% SG**	**Syngenta**	**60g /Feddan**
Carbamates	**Methomyl**	**Neomyl 90% SP**	**KZ**	**300g /Feddan**
Chitin synthesis inhibitors	**Flufenoxuron**	**Novo 10% DC**	**Soltair**	**200 cm / Fedden**

The co-toxicity factors were determined according to (Mansour et al., 1966) as follows

$$\text{Co-toxicity factors (CTFs)} = \frac{\text{Observed \% mortality} - \text{Expected \% mortality}}{\text{Expected \% mortality}} \times 100$$

This factor was used to categorize the results into three categories as follow: Co-toxicity factors ≥ +20 meant potentiation; co-toxicity factors < - 20 meant antagonism; and co-toxicity factors between -20 and +20 meant additive effect.

Statistical analysis:

The mortality data were corrected using Abbott's formula (Abbott, 1925). Probit analysis was performed for calculating LC_{25}, LC_{50}, and slope values according to Finney (1971) using Ldp-line Sofware. The interactions between the tested insecticides and *Bt* were determined by comparing expected and observed mortalities based on the equation described by Mansour et al. (1966).

RESULTS AND DISCUSSION

Effect of some synthetic insecticides on *B. thuringiensis* growth:

The effect of synthetic insecticides used in controlling *S. littoralis* on *B. thuringiensis* growth was investigated.

Data presented in Table (2) showed that, there was no reduction in the growth of *Bt* when exposed to emamectin benzoate, at the three concentrations tested and methomyl at half and 1/4 RC. On the other hand, chloropyrofos-methyl showed 100% inhibition of *Bt* growth at the three tested concentrations and lambada- cyhalothrin at RC. Whereas flufenoxuron with all concentrations, methomyl at RC and lambada- cyhalothrin at half and 1/4 RC gave a remarkable reduction in the growth of *Bt* when compared with control.

Results revealed that, there was a significant difference in the number of *Bt* colonies formed following direct exposure to the different tested insecticides. Also, it can be notice that, some of these insecticides not affect on the number of *Bt* colonies when compared with control. This would indicate that *Bt* might use these insecticides as supplementary nutrient sources by degrading them. This ability of *Bt* has also been shown by other researchers (Jaques and Morris, 1981; Mandal et al., 2013; Amizadeh et al., 2015 and Narkhede et al., 2017) who reported that most insecticides are compatible with *Bt* having little or no effect on spore germination and cell multiplication. Also they further reported that compatibility of *Bt* and chemical insecticides at low concentrations of Carbamates and Organophosphates, did not affect bacterial growth but improved it, while others specially Chlorinated hydrocarbons inhibited growth. In contrary, our results revealed that, in chloropyrofos-methyl treatments no colonies were formed, because of chloropyrofos-methyl may have antibiotic or toxic activity against *Bt*. The current results are in agreement with Batista Filho et al. (2001) who stated that endosulfan and monocrotophos used at maximum concentrations (2.5L / ha and 2250 ml/ ha, respectively) reduced the production conidia and vegetative growth, whereas at minimum concentrations (0.5 L/ha and 300ml/ ha respectively) they had no effect on the fungal growth. Also Amizadeh et al., 2015 reported that, in metaflumizone treatments, no *Bt* colonies were formed.

Table (2): In vitro compatibility of *Bacillus thuringiensis* with some synthetic insecticides.

Synthetic insecticides Treatments	Emamectin benzoate	Flufenoxuron	Methomyl	Lambada-cyhalothrin	Chloropyrofos-methyl

RC	**4.138×10^{12} ± 2.9038×10^{11} *** (f)**	**4.13×10^{9} ± 8.7×10^{7} *** (c)**	**4.022×10^{11} ± 7×10^{9} *** (e)**	**0 ± 0 *** (a)**	**Ve ± 0 *** (a)**
1 / 2 RC	**3.22×10^{13} ± 6.557×10^{10} *** (g)**	**4.13×10^{11} ± 6.95×10^{9} *** (e)**	**4.092×10^{13} ± 3.81×10^{11} (i)**	**4.1×10^{8} ± 1×10^{7} *** (b)**	**Ve ± 0 *** (a)**
1 / 4 RC	**3.49×10^{13} ± 5.43×10^{11} *** (h)**	**4.1×10^{11} ± 5.2×10^{9} *** (e)**	**4.16×10^{13} ± 8.72×10^{11} (i)**	**4.07×10^{10} ± 1.074×10^{9} *** (d)**	**Ve ± 0 *** (a)**
Control	**$4.028 \times 10^{13} \pm 2.90379 \times 10^{11}$ (i)**				

RC: Recommended concentrate, 1/2RC: Half of the Recommended concentrate 1/4RC: Fourth of the Recommended concentrate, *****: Without chemical insecticide, -ve: No growth, Ve: Values represent means ± SE M (n = 15), Significance level: * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$ compared with control, The same letter in the same column represent no significant differences.

Table (3): Efficacy of some synthetic insecticides and *B. thuringiensis* on 2nd larval instar of *S. littoralis*.

Tested compounds	Intervals	2nd larval instar		
		LC_{25} values in ppm (CL)	LC_{50} values in ppm (CL)	Slope ± SE
Chloropyrofos - methyl	24	4.11 (1.92-5.99)	10.42 (7.41-15.60)	1.67 ± 0.3749
	48	2.0866 (0.7456- 3.32)	4.82 (2.89-6.59)	1.86 ± 0.4018
Lambda-cyhalothrin	24	0.3055 (0.1044-0.4824)	0.8516 (0.5622-1.2673)	1.51 ± 0.3676
	48	0.1226 (0.0465-0.2017)	0.3508 (0.2174 – 0.4943)	1.48 ± 0.2796
Methomyl	24	5.24 (2.46 – 7.99)	14.91 (10.27- 21.07)	1.49 ± 0.2725
	48	3.22 (1.28- 4.98)	7.42 (4.74- 10.06)	1.86 ± 0.3945
Emamectin benzoate	48	0.0201 (0.0099- 0.03011)	0.0503 (0.0346- 0.0681)	1.69 ± 0.2885
	72	0.0112 (0.0031- 0.019)	0.0279 (0.0149- 0.0393)	1.70 ± 0.3954
Flufenoxuron	48	0.1829 (0.0757- 0.287)	0.5035 (0.3332- 0.6953)	1.53 ± 0.2977
	72	0.0391 (0.0148-0.0643)	0.1154 (0.0723 – 0.1634)	1.44 ± 0.2751
B. thuringiensis	48	2.1×10^{8} (3.3×10^{7} – 1×10^{11})	2.6×10^{10} (1.4×10^{9} – 8.7×10^{10})	0.321 ± 0.0982
	72	9.8×10^{7} (7×10^{5} – 7.2×10^{8})	2.5×10^{9} (2.4×10^{8} – 3.9×10^{12})	0.2802 ± 0.0830
	96	1.16×10^{5} (0.3×10^{2}- 1.3×10^{6})	7.9×10^{8} (1.1×10^{7} – 6.44×10^{9})	0.2380 ± 0.0756
	168	3.1×10^{3} ($0.5 - 4.2 \times 10^{4}$)	1.5×10^{5} (2.9×10^{3} – 7×10^{5})	0.3980 ± 0.1149

CL: Confidence limit. SE: Standard error.

Also, antibiotic activity of metaflumizone in combination with amitraz was reported on *Malasseziapachydermatis* (Weidman) yeast (Tarallo et al., 2009). Furthermore, Camargo (1983) observed that *M. anisopliae* is inhibited by different concentrations of pyrethroid insecticides. Deltamethrin had the highest inhibitory action.

Efficacy of some synthetic insecticides and *B. Thuringiensis* spores against the 2nd larval instar of *S. littoralis*:

The toxicity of the tested compounds

(chloropyrofos methyl, lambda cyhalothrin, methomyl, emamectin benzoate, flufenoxuron and B. thuringiensis spores) against the 2nd larval instar of *S. littoralis* at different times of exposure are shown in Table (3).

Amongst all the tested synthetic insecticides, emamectin benzoate was the most effective compound after 48h of treatment as showed the lowest LC_{50} value (0.0503ppm), followed by lambda cyhalothrin and flufenoxuron with LC_{50} values = of 0.3508 and 0.5035 ppm, respectively. While chloropyrofos-methyl and methomyl showed the least effective insecticides with LC_{50} value = of 4.82 and 7.42 ppm, respectively. The bioagent, *B. thuringiensis* spores recorded LC_{50} value of 1.5 x 10^5 ppm after 7days of treatment.

There was a negative correlation between the time elapsed from treatment and the LC_{50} values of all tested compounds, as the toxicity increased with increasing period of exposure (Table 3). .

Use of Integrated pest management (IPM) protocols is important in achieving effective protection against pests and preventing their spread

In this respect, the comparative efficacy of some insecticides belong to different groups with different mode of action was investigated in the current study to detect the most effective compound against the Egyptian cotton leafworm. Results confirm that the newer insecticides, emamectin benzoate and flufenoxuron have potentiating effects with low concentration against the larval instar of *S. littoralis*.

The present data are similar to that reported by other researchers (Khan et al., 2011; Bhatti et al., 2013; Rashwan et al., 2013; El- Sheikh, 2015; Metayi et al., 2015 and Maqsood et al., 2017) who compared the efficacy of emamectin benzoate with different types of insecticides and found that emamectin benzoate was the superior insecticides by recorded the lowest LC_{50} among the tested insecticides (deltamethrin, bifenthrin, chlorfluazuron, flubendamide, chlorpyrifos, profenofos, spinosad, indoxacarb, methoxyfenozide and lufenuron against *S. littoralis* larvae under laboratory condition. The toxicity of emamectin benzoate was studied on different insect species and showed high toxic effects against a wide variety of lepidopterans (Argentine et al., 2002; Firake and Pande, 2009 and El-Sheikh, 2015). In addition Abd-El-Aziz (2014); Saleh et al. (2015); EL-Dewy (2017) and Ismail et al. (2017) reported that emamectin benzoate LC_{50} value ranged from 0.007 to 1.35 ppm against 4th larval instar of *S. littoralis* laboratory strain.

Flufenoxuron in the present study showed high toxicity to 2nd instar larvae of *S. littoralis*. This toxicity was less than that of emamectin benzoate based on LC_{50} value. These results agree with that obtained by Saad et al. (2011) who revealed that emamectin benzoate was more toxic than lufenuron and flufenoxuron against 2nd, 3rd and 4th instar larvae of *S. littoralis*. Furthermore Ishtiaq et al. (2012) found that populations of *S. exigua* are more susceptible to emamectin benzoate and lufenuron compared to pyrethroid and organophosphorous insecticides. S. exigua developed no to moderate resistance to emamectin benzoate and lufenuron (as CSIs) and thus these compounds are environmentally safe and could be used in IPM and in pesticide resistance management programmes (Ishtiaq et al. (2012).

Interaction between *B. thuringiensis* spores suspensions and some synthetic insecticides:

Potentiation, antagonistic and additive interaction effects were observed upon application of LC_{25} of the tested insecticides in combination with LC_{50} of *Bt* or after 24h from treating the 2nd instar larvae with *Bt* as shown in Tables (4 and 5).

Results indicated that emamectin benzoate exhibited the highest potentiation effect, where Co-Toxicity Factor (CTF) value was 50 when applied in combination with *Bt* at zero time and was 22.73 after 24 h from treating the larvae with *Bt*. On the other hand, chloropyrofos-methyl and methomyl gave remarkable antagonistic effect (-43.48 and -25), respectively when applied in combination with *Bt* at zero time. In contrary, the potentiation effect (31.58) was observed when chloropyrofos-methyl applied after 24 h from treating the larvae with *Bt*, while treatment with methomyl caused additive effect (11.77).

Table (4): Observed percentage mortality of some synthetic insecticides and *B. thuringiensis* against 2nd larval instar of *S. littoralis* at different times after treatment.

Tested compounds	Concentration level	Observed (%) mortality				
		After 24 hr.	After 48 hr.	After 72 hr.	After 96 hr.	After 168 hr.
Chloropyrofos-	LC_{25}	13.33	26.67	30	43.33	-

methyl						
Lambda-cyhalothrin	**LC_{25}**	**16.67**	**30**	**33.33**	**43.33**	**-**
Methomyl	**LC_{25}**	**10**	**23.33**	**23.33**	**33.33**	**-**
Emamectin benzoate	**LC_{25}**	**6.67**	**13.33**	**26.67**	**40**	**-**
Flufenoxuron	**LC_{25}**	**3.33**	**10**	**30**	**53.33**	**-**
B. thuringiensis	**LC_{50}**	**6.67**	**10**	**23.33**	**33.33**	**56.66**

In case of lambada-cyhalothrin and flufenoxuron treatments, additive effect was noticed with CTFs values ranged from 18.18 - 15.79 at the two time intervals.

Application of *Bt* at LC_{50} in combination with LC_{25} of tested insecticides at (zero time) showed positive effect (Table 5) than when applied separately (Table 4) by increasing the larval mortality thereby causing potentiation effect with emamectin benzoate treatment and additive effect with lambada- cyhalothrin and flufenoxuron treatments. While, antagonistic effect was observed in case of methomyl and Chloropyrofos-methyl treatments.

Similar finding was reported by Salama et al., 1984 who found that mixtures of pyrethroid-based insecticides have been shown to potentiate the activity of the microbial, *B. thuringiensis* Berliner subsp. *galleriae* against the cotton leafworm, *S. littoralis***,** and *B. thuringiensis* subsp. *kurstaki* against the fall armyworm, *S. frugiperda* (J. E. Smith) (Habib and Garcia 1981). Also Luo et al. (1986) reported that a small amount of fenvelerate along with *B. thuringiensis* resulted in an increased lint yield in cotton when used against *Pectinophora gossypiella* Saunders. Moreover, Amizadeh et al. (2015) observed an antagonistic effect between *Bt* (at LC_{50}) with abamectin, azadirachtin, indoxacarb, chlorantraniliprole, dichlorovos and metaflumizone (at LC_{10} and LC_{25}) for control of *Tuta absoluta*, where *Bt* was applied immediately after the chemical insecticides. Also, antagonism was observed when treatment with *Bt* was done 12 h after azadirachtin and metaflumizone applications Amizadeh et al. (2015). Farooq and Freed (2016) found that the insecticides acetamiprid, emamectin benzoate, imidacloprid and lufenuron in combination with insect pathogenic fungi showed higher mortality than expected with significant synergistic interactions when tested as a bait against *M. domestica*. Furthermore, the combination of entomopathogenic fungi and synthetic insecticides can decrease the concentrations of the active ingredient required.

Khalifa et al. (2015) investigated the effect of applying mixtures of chlorantraniliprole (LC_{50}, LC_{25} and $LC_{12.5}$) with *Bt* (LC_{50}, LC_{25} and $LC_{12.5}$) against the 4th larval instar of *Spodoptera littoralis*. They reported that the mixture of chlorantraniliprole (at LC_{50} and LC_{25}) with *Bt* (at LC_{50} and LC_{25}) resulted in an antagonistic effect while, the mixture of chlorantraniliprole (at $LC_{12.5}$) with *Bt* (at $LC_{12.5}$) resulted in an additive effect. Also, the antagonistic effect that was observed in the present study when applied methomyl in combination with *Bt* at zero time was confirmed with results obtained by Abdel-Aal and El- Shikh (2012) who recorded an antagonistic effect with Co-Toxicity factor -29.98 after treatment the 2nd instar larvae of *S. littoralis* with mixture of *Bt* and methomyl at LC_{25} level.

On the other side, using all the tested insecticides at (LC_{25}) after 24 h of exposure to LC_{50} of *Bt* exhibited an additive and potentiation effects in the present study. It seems that, *Bt* might be acting as a stressor; making the larvae more susceptible to death, and leading to a final positive effect. Similarly, Amizadeh et al. (2015) reported that, applying *Bt* 12 and 24 h after treatment with LC_{25} of chlorantraniliprole, dichlorovos and abamectin resulted in synergism. Also, synergism with LC_{10} of dichlorovos and abamectinwas observed only after 12 h.

Table (5): Interaction between LC_{50} of *B. thuringiensis* with LC_{25} of some synthetic insecticides on 2nd larval instar of *S. littoralis*.

Tested insecticides	Intervals (hours)	Observed (%) mortality			Expected (%) mortality			Co toxicity factor			Interaction After 96h
		After 48 h	After 72 h	After 96 h	After 48 h	After 72 h	After 96 h	After 48 h	After 72 h	After 96 h	
Chloropyrofos-methyl	0	30	36.67	43.33	36.67	53.33	76.66	-1817	-31.26	- 43.48	Antagonistic
Lambda-cyhalothrin	0	36.67	56.67	83.33	40	56.67	76.66	-8.35	0	8.70	Additive
Methomyl	0	33.33	40	50	33.33	46.67	66.67	0	-14.27	- 25.00	Antagonistic
Emamectin benzoate	0	30	66.6.7	90	23.33	50	73.33	28.59	33.32	22.73	Potentiation
Flufenoxuron	0	26.67	60	83.33	20	53.33	86.67	33.3	12.5	- 3.84	Additive
Chloropyrofos-methyl	24	30	66.67	83.33	23.33	50	63.33	28.59	33.32	31.58	Potentiation
Lambda-cyhalothrin	24	36.67	73.33	96.67	26.67	53.33	66.67	37.51	37.50	45.00	Potentiation
Methomyl	24	23.33	43.33	63.33	20	46.67	56.67	16.65	-7.14	11.77	Additive
Emamectin benzoate	24	20	56.67	86.67	16.67	26.67	60	0	54.56	50	Potentiation
Flufenoxuron	24	10	46.67	73.33	13.33	33.33	63.33	-24.98	40.00	15.79	Additive

Peters and Ehlers (1994) reported that, bacterial infestation could cause a loss of defense reactions like suppression of encapsulation against the invading EPNs.

Collectively, results indicated that, combined application of *Bt* with lambada-cyhalothrin or emamectin benzoate or flufenoxuron at low concentrations may improve the efficacy of *Bt* to control 2nd instar larvae of *S. littoralis*, reduce the amounts of synthetic insecticides and thus reduce environmental pollution and cause less harm to natural enemies and human. While mixing of methomyl and chloropyrofos-methyl with *Bt* is not useful for controlling this insect and reduced the efficacy of these insecticides. In additions, to achieve additive or potentiation effects, the larvae should be exposed to *Bt* for 24 h before the addition of the tested insecticide at (LC_{25}), because of *Bt* may be acting as a stressor.

The use of reduced application rates is also in line with IPM programmes (Georghiou, 1994). Lower rates of insecticides, would decrease the harm to natural enemies present in the ecosystem. This leads to effective control of the pest and also delays the development of insecticides resistance. Moreover, the sequence of using *Bt* as stressing the insect resulting in enhanced the efficacy of the synthetic insecticides at low rates which in turn controls the insects with resistance alleles not controlled by the insecticides. Variation in capability and the nature of interaction depends the species and strain of the entomopathogen, host species, application timing and the type of insecticides used (Anderson et al., 1989 and Mannion et al., 2000).

CONCLUSION

Combination of *Bt* with synthetic insecticides increased the efficacy of some insecticides but not at all cases and at all time intervals. There is a need to screen all group of synthetic insecticides on field level to quantify insecticide performance at the farm level. If the additive or potentiation effect of combinations of the synthetic insecticides with *Bt* confirmed in the field level, this approach may be a useful tool of integrated management of *S. littoralis*.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

All authors contributed equally in all parts of this study. All authors read and approved the final version.

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