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Efficacy of *Bacillus thuringiensis* Strain 407 versus Synthetic Pesticides in Controlling Sugar Beet Pests under Open Field Conditions

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In sugar beet fields of West Eurasia and North Africa, including Egypt, the beet moth *Scrobipalpa ocellatella*, cotton leafworm *Spodoptera littoralis* and beet armyworm *Spodoptera exigua* are commonly major and serious pests causing irreparable damage to beet plantation. However, frequent use of synthetic pesticides can harm beneficial insects in the agroecosystem and should preferably be avoided in terms of environmental protection and safety. This study aimed to evaluate the impact of *Bacillus thuringiensis* (*Bt*) strain 407 in comparison to major chemical insecticides, chlorfenapyr (Pestpyr) and methomyl (Goldben), on the populations of the above–mentioned pests and their associated natural enemies in the field. Our results showed that the densities of *S. ocellatella* were remarkably higher than those of *S. littoralis* and *S. exigua* in two successive growing seasons. Within the first week of application with both chemical pesticides, numbers of pests were dropped and its population size reduced by 95 – 100% though reduction percentage tended to decline with time. *Bt* application was equally effective in reducing the three pest species after 7 or 10 days of application though it had less effectiveness in the earlier days due to a time–delayed impact. The major natural enemy predators, *i.e.*, the green lacewing *Chrysoperla carnea*, *Coccinella septempunctata* and *C. undecimpunctata*, disappeared completely three days after chemical application and remained few during the study period. By contrast, reduction of the predators compared to control plots was markedly small in study plots with *Bt* application, indicating use of *Bt* conserved the predator populations. Thus, multiple sprays of *Bt* may be useful in sugar beet production, which can solve the dilemma between pest control and beneficial natural enemy conservation.

Key words: Bio-insecticides, conservation biological control, natural enemies, IPM

INTRODUCTION

In Egypt, sugar beet, *Beta vulgaris* L., is one of the most important cultivated crops. Beside it plays an indispensable role in the crop rotation system, sugar beet has long been utilized for sugar extraction to meet the country's need for sugar (Youssef *et al.*, 2020). Sugar beet is a modern sugar crop in the Egyptian fields, and the industrial demand for sugar beets has been steadily increasing. Therefore, the Egptian government encourages farmers by offering a high price, thus incentivizing many farmers to plant more sugar beets. Accordingly, the harvested area in 2021/22 showed an increase of 10,000 hectares, compared to the previous year with total cultivated area of 265,000 ha (USDA, 2021).

Like other crops, sugar beet is attacked by numerous insect pests throughout growth stages, which directly or indirectly lead to yield and quality reduction (Bassyouny, 1993; El–Dessouki *et al.*, 2014; Evaristo, 1983; Youssef *et al.*, 2020). Among the insect pests, cotton leaf worm *Spodoptera littoralis* (Boisd.), beet armyworm *Spodoptera exigua* (Hübner) and sugar beet moth *Scrobipalpa ocellatella* Boyd. are commonly abundant and are destructive in sugar beet fields of Egypt, resulting in serious economic loss (Al–Keridis, 2016; Amin *et al.*, 2008; Shalaby and El–Samahy, 2010; Talaee *et al.*, 2016; Zheng *et al.*, 2011). Pest management is thus a key practice for stable production of sugar beet.

Although use of synthetic chemical pesticides is a main pest control practice, the frequent use or overuse often causes development of pesticide resistance, leading to the outbreak or resurgence of pests (El–Agamy *et al.*, 2021; Ishtiaq and Saleem, 2011; Su and Sun, 2014). Also, the excessive use of pesticides has pernicious impact on natural enemies or natural control and may result in the environmental hazardousness (Mousa *et al.*, 2013; Ueno and Tran, 2015). Therefore, combination and integration of other practices, such as use of resistant varieties, plant extracts, inter–cropping, natural materials and entomopathogenic micro–organisms, etc., are favorable to suppress insect pest overrun and promote environmental protection (Elkhateeb *et al.*, 2021; Elsharkawy and Mousa, 2015; Talaee *et al.*, 2016; Mousa and Ueno, 2019; Mousa, 2020). *Bacillus* is a well–known bio–insecticide widely used to control insect pests with a high level of specificity against different lepidopteran species (Daquila *et al.*, 2021; Mousa *et al.*, 2014; Mannu *et al.*, 2020). The advantage of such a bio–insecticide is safety to non–targeted beneficial organisms, enhancing conservation biological control of insect pests by reducing a negative impact on beneficial insects, *i.e.*, parasitoids and predators, in agricultural ecosystems (González–Zamora *et al.*, 2007; Zhao *et al.*, 2016). The use of bio–insecticides is also supposed to contribute in

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reducing production costs, ameliorates the product quality, and slowdown the development of pesticide resistance (De Bortoli *et al*., 2017). The genus *Bacillus* includes multiple species and/or strains, which in turn differ in their mode of action and efficacy to target insect pests. For example, the endophytic bacterium *Bacillus aryabhattai* can promote plant growth by inducing important molecular pathways (Park *et al*., 2017) while the bacterium *Bacillus thuringiensis* is infectious to insects, including agricultural pests, and produces delta–endotoxins (Bravo *et al*., 2007), which has been used to control various insect pests including Lepidoptera (Mousa *et al*., 2014).

From this perspective, we investigated the efficacy of applying the bacterium *Bacillus thuringiensis Bt* 407 in suppressing three main lepidopteran sugar beet pests. To compare its usefulness, the efficacies of two commercial chemical insecticides were tested. We also examined their impact on associated natural enemies to evaluate the compatibility with natural enemies for conservational biological control or integrated pest management. Field studies were therefore designed, and foliar spray of tested compounds were applied in sugar beet fields. Based on the results, we discuss usefulness of *Bt* for pest management in sugar beet fields.

MATERIALS AND METHODS

Experiment setup

The experiment was carried out in two successive beet growing seasons in 2019–2020 and 2020–2021 at the experimental farm of the Sugar Crops Research Institute, Sakha, Kafr El–Sheikh, Egypt. Beet was planted twice a year; first planting time was in the beginning of August to examine the presence of *Spodoptera littoralis* (Boisd.) and *S. exigua* (Hüb.) (Lepidoptera: Noctuidae), while the second planting time was in mid of October to check *Scrobipalpa ocellatella* (Boyd) (Lepidoptera: Gelechiidae) and its associated natural enemies. An area of 1020 m^2 was measured and divided into 16 plots; the area of each plot was 42 m^2 (6×7 m). Each treatment mentioned below was represented by four of these plots as replicates in a randomized complete block design. Two meters between the plots was left without planting, and, then, the plots *i.e*. replicates were planted with the sugar beet variety HUSAM as multigerm seeds. The area was uniformly fertilized and irrigated with the recommended values.

Pesticides and bio–pesticides

Two commercial pesticides that have been widely used in sugar beet fields were selected for use in this study. Formulations of the pesticides were: Pestpyr SC 36% (4–Bromo–2–(4–chlorophenyl)–1–(ethoxymethyl)–5 –(trifluoromethyl)–1H–pyrrole–3 carbonitrile) (Shandong Weifang Shuangxing Pesticide Co., Ltd., China) applied at 357 ml/hectare (= 150 ml/feddan) and Goldben SP 90% (S–methyl n–(methylcarbamoyl oxy) thioacetamide) (Shoura Chemicals, Egypt) was dissolved in water and was applied at 711 gm/hectare (= 300 gm/feddan, 600 liter) (Table 1). Spore suspension of *Bacillus thuringiensis* isolated from larvae of beet fly *Pegomya mixta* (Diptera: Anthomyiidae) was also tested as bio–pesticide. The isolation was identified by GATC Biotech Company, Germany as *Bt* strain 407 (Table 1), was kept at Sugar Crops Research Institute, Sakha, Kafer El–Sheikh, Egypt and was used in the present study.

Data collection

The first sampling was done before the spraying of the test pesticides in order to calculate the percentage of reduction of target insects (see below). Ten plants from each plot were randomly chosen and inspected directly in the field to count pest insects. Thus, forty plants in all were sampled for each experimental group. Sampling was made 1, 3, 7 and 10 days after spraying of test pesticides. Because the *Bt* requires two or three days to kill lepidopteran larvae (Nawrot–Esposito *et al*., 2020), we did not count the numbers of pest insects in *Bt* applied plots on the first day after application. The numbers of *S. ocellatella*, *S. littoralis* and *S. exigua* and the associated predators, *Chrysoperla carnea* (Steph.) (Neuroptera: Chrysopidae), *Coccinella septempunctata* L. and *C. undecimpunctata* L. (Coleoptera: Coccinellidae) were recorded. Percentage reduction of the insect populations was calculated according to the equation of formula given by Henderson and Tilton,1955 as follows:

$$\text{Population reduction \%} = 100\times\left[1-\frac{Ta\times Cb}{Tb\times Ca}\right]$$

where: Ta= Population in treated plots after treatment, Tb= Population in treated plots before treatment, Ca= Population in control after treatment, and Cb= Population in control before treatment. Tb was obtained as mentioned above.

Data analysis

The data were statistically analyzed using COSTAT software version 6.4. Analyses of variance (ANOVA)

Table 1. Pesticides and bio–pesticides used for testing

Common name	Trade name	Formulation	Conc.	Chemical group	Application Rate
Chlorfenapyr	Pestpyr	SC	36%	Chlorinated pyrrole	150 cm/feddan
Methomyl	Goldben	SP	90%	Oxim Carbamate	300 gm/ feddan
Bt	–	spores suspension	10^8 spores/ml	–	5 L/feddan

were applied to examine an overall difference among the groups, and, then, the means were compared using Tukey's HSD test at a significance level of 0.05. When necessary, percentage acrsine–square root transformation was made before statistical treatments.

RESULTS

S. ocellatella

Sugar beet plants had a mean of 18.25, 19.00 and 18.75 *S. ocellatella* /10 plants/plot before the treatment with *Bt*, Pestpyr and Goldben, respectively, but after three days of application, the mean numbers were dropped to 11.75, 0.00, and 0.00, compared to 23.50 in the control plot (Table 2). With the time passage, the mean number of *S. ocellatella* treated with *Bt* was significantly decreased to 6.25 and 2.50 individuals/ 10 plants (F = 31.80, P < 0.0001) in the 7th and 10th days of application, respectively. Conversely, the number of the pest tended to increase in the plots treated with the synthetic pesticides after it had fallen to 0 in the 3rd day though the tendency did not significantly differ within the same sampling date, *i.e.*, 7th and 10th. Similar trends were detected in the second season of 2020–2021 (Table 2).

S. littoralis and *S. exigua*

In our sugar beet fields, *S. littoralis* and *S. exigua* commonly coexisted causing irreparable damage to beet plantation; the densities were high (Table 3 and 4) though the density of *S. ocellatella* was remarkably higher than that of *S. littoralis* or *S. exigua*. The densities of both *S. littoralis* and *S. exigua* remained high in control plots but was significantly lower in plots treated with the synthetic pesticides (F = 7.55, P < 0.011 for *Bt*; F = 5.44, P < 0.013 for Pestpyr and F = 10.46; P < 0.001 for Goldben) for *S. littoralis* and (F = 4.07, P < 0.054 for *Bt*; F = 6.61, P < 0.006 for Pestpyr and F = 16.86; P < 0.001 for Goldben) for *S. exigua* (Table 3 and 4). In the third day of the application, the numbers were significantly lower in chemical pesticides plots than *Bt* plots (F = 49.116, P < 0.001 for *S. littoralis*; F = 82.904, P < 0.001 for *S. exigua*) and (F = 76.952, P < 0.001 for *S. littoralis*; F = 81.081, P < 0.001 for *S. exigua*) in the first and second seasons respectively. After the third day of the application, the decline in *S. littoralis* and *S. exigua* numbers continued in plots treated with *Bt*, whereas, the numbers of both pests tend to increase in plots treated with chemical pesticides in both seasons.

Pest reduction

Mean percentages of population reduction of examined pests are summarized in Table 2, 3 and 4. The data showed superiority of synthetic pesticides in controlling the targeted insect pests in comparison to *Bt*. In the first season, the highest population reduction of the three targeted pests was recorded on the 3rd day of application. *S. ocellatella* recorded 100% reduction when treated with Pestpyr and Goldben (Table 2), and the percentages were slightly lower for *S. littoralis* and *S. exigua* but still the results indicated high level of efficacy of the pesticides though the reduction percentages tended to decline in the seventh and tenth days after application (Table 3 and 4). However, *Bt* application resulted in the least in reduction percentage on the 3rd day but it significantly increased the efficacy of control in the following days (F = 6.814, P < 0.015 for *S. ocellatella*; F = 8.43, P < 0.008 for *S. littoralis* and F = 8.615; P < 0.012 for *S. exigua*, respectively), the values of the percentages almost the same with those of two synthetic pesticides, which indicated that *Bt* worked well in suppressing the three main pests of sugar beet. Similar trends were detected in the second season.

Natural enemies

In our study fields, a variety of arthropod natural enemies were detected, several species of insect predators were associated with the examined pests on sugar beet plants. Amongst them, the most frequently observed predators were larval *C. carnea* and larval ladybird beetles, *i.e*,. *C. septempunctata* and *C. undecimpunctata*.

Table 2. Density and reduction percentage of *Scrobipalpa ocellatella* larvae 1 – 10 days after spraying with two synthesized pesticides and *Bt*

Season	Treatment	No. before treatment	No. after treatment					% Population reduction				
			1st day	3rd days	7th days	10th days	*P*–value	1st day	3rd days	7th days	10th days	*P*–value
1st	***Bt***	18.25±0.75^{a}	– *	11.75±1.11^{Ab}	6.25±0.63^{Bb}	2.50±0.65^{Cb}	0.0001	– *	44.03±13.13^{Bb}	67.27±6.05^{ABa}	88.98±3.65^{Aa}	0.015
	Pestpyr	19.00±0.71^{a}	7.50±0.29^{Ab}	0.00±0.00^{Cc}	5.25±0.63^{Bb}	3.25±0.75^{Bb}	0.0001	63.82±3.06^{Ca}	100±0.00^{Aa}	73.22±6.24^{BCa}	86.69±2.80^{ABa}	0.0001
	Goldben	18.75±0.85^{a}	8.50±0.69^{Ab}	0.00±0.00^{Cc}	4.50±0.65^{Bb}	2.75±0.48^{Bb}	0.0001	58.34±5.87^{Ca}	100±0.00^{Aa}	77.80±3.84^{Ba}	88.45±2.02^{ABa}	0.0001
	Control	19.00±1.08^{a}	21.00±1.47^{Aa}	23.50±2.25^{Aa}	21.00±2.04^{Aa}	24.25±1.03^{Aa}	0.4636	–	–	–	–	
	***P*–value**	**0.9152**	**0.0001**	**0.0001**	**0.0001**	**0.0001**		**0.4387**	**0.0007**	**0.4309**	**0.846**	
2nd	***Bt***	21.50±1.71^{a}	– *	14.50±0.05^{Ab}	6.50±0.29^{Bb}	3.25±0.48^{Cb}	0.0001	– *	30.14±6.89^{Bb}	74.38±0.98^{Aa}	87.86±3.07^{Aa}	0.0001
	Pestpyr	20.25±1.65^{a}	6.00±041^{Ab}	0.00±0.00^{Cc}	3.50±0.87^{Bb}	3.50±0.65^{Bb}	0.0001	67.25±4.67^{Ba}	100±0.00^{Aa}	85.86±2.34^{Aa}	85.71±4.37^{Aa}	0.0002
	Goldben	21.00±1.35^{a}	6.00±0.71^{Ab}	0.00±0.00^{Bc}	3.50±1.04^{Ab}	3.50±0.50^{Ab}	0.0004	67.94±5.79^{Ba}	100±0.00^{Aa}	84.80±5.25^{ABa}	87.41±2.15^{Aa}	0.0011
	Control	20.50±1.32^{a}	19.25±1.80^{Ba}	20.25±1.03^{Ba}	24.50±1.85^{ABa}	27.75±1.31^{Aa}	0.0071	–	–	–	–	
	***P*–value**	**0.9384**	**0.0001**	**0.0001**	**0.0001**	**0.0001**		**0.9288**	**0.0001**	**0.0727**	**0.8915**	

*The first day was excluded due to delay in *Bt* symptoms appearance. Means ± SE are shown. Means followed by the same capital letters in a row and lower case letters in a column do not differ significantly by the Tukey's HSD test (P < 0.05).

Table 3. Density and reduction percentage of *Spodoptera littoralis* larvae 1 – 10 days after spraying with two synthesized pesticides and *Bt*

Season	Treatment	No. before treatment	No. after treatment					% Population reduction				
			1 day	3 days	7 days	10 days	*P*–value	1 day	3 days	7 days	10 days	*P*–value
1st	***Bt***	10.25±0.63^{a}	– *	6.25±1.11^{Ab}	4.50±0.65^{ABb}	2.00±0.41^{Bb}	0.0119	– *	53.32±8.49^{Bb}	73.00±4.64^{ABa}	87.25±3.12^{Aa}	0.0087
	Pestpyr	10.50±1.94^{a}	3.75±0.85^{Ab}	0.25±0.25^{Bc}	2.75±0.63^{ABb}	2.50±0.65^{ABb}	0.0135	67.93±6.01^{Ba}	96.94±3.06^{Aa}	80.74±6.09^{ABa}	82.39±7.76^{ABa}	0.0358
	Goldben	12.75±1.38^{a}	4.50±0.65^{Ab}	0.25±0.25^{Bc}	3.50±0.87^{Ab}	3.50±0.29^{Ab}	0.0011	67.27±6.59^{Ba}	99.03±0.97^{Aa}	82.47±5.15^{ABa}	81.88±3.56^{ABa}	0.0033
	Control	11.00±1.41^{a}	12.75±2.06^{Aa}	14.25±1.49^{Aa}	18.00±1.41^{Aa}	17.50±2.10^{Aa}	0.1678	–	–	–	–	
	***P*–value**	**0.6083**	**0.0018**	**0.0001**	**0.0001**	**0.0001**		**0.9439**	**0.0002**	**0.4417**	**0.7343**	
2nd	***Bt***	13.25±1.31^{a}	– *	7.25±0.95^{Ab}	4.75±0.48^{ABb}	3.00±0.41^{Bb}	0.0043	– *	48.90±13.60^{Bb}	72.27±5.78^{Aa}	83.39±4.43^{Aa}	0.0088
	Pestpyr	12.50±1.94^{a}	3.50±0.65^{Ab}	0.25±0.25^{Bc}	3.00±0.41^{Ab}	3.50±0.65^{Ab}	0.0020	70.74±9.88^{Aa}	98.61±1.39^{Aa}	78.49±8.19^{Aa}	79.15±5.34^{Aa}	0.0794
	Goldben	11.75±0.85^{a}	4.25±0.63^{Ab}	0.50±0.29^{Bc}	3.75±0.85^{Ab}	4.00±0.41^{Ab}	0.0021	69.66±2.41^{Ba}	96.80±1.85^{Aa}	76.81±5.01^{Ba}	77.00±1.67^{Ba}	0.0003
	Control	13.00±0.41^{a}	15.50±1.55^{Aa}	15.25±1.25^{Aa}	18.25±2.02^{Aa}	19.50±1.94^{Aa}	0.2703	–	–	–	–	
	***P*–value**	**0.8427**	**0.0001**	**0.0001**	**0.0001**	**0.0001**		**0.9189**	**0.0025**	**0.7860**	**0.5581**	

*The first day was excluded due to delay in *Bt* symptoms appearance. Means ± SE are shown. Means followed by the same capital letters in a row and lower–case letters in a column do not differ significantly by the Tukey's HSD test ($P < 0.05$).

Table 4. Density and reduction percentage of *Spodoptera exigua* larvae 1 – 10 days after spraying with two synthesized pesticides and *Bt*

Season	Treatment	No. before treatment	No. after treatment					% population reduction				
			1 day	3 days	7 days	10 days	*P*–value	1 day	3 days	7 days	10 days	*P*–value
1st	***Bt***	13.00±1.22^{a}	– *	7.50±1.32^{Ab}	4.50±0.65^{Ab}	3.75±0.85^{Ab}	0.0548	– *	55.12±9.65^{Bb}	74.03±7.45^{Aa}	81.78±2.85^{Aa}	0.0122
	Pestpyr	11.25±2.06^{a}	4.25±0.63^{Ab}	0.25±0.25^{Bc}	2.75±0.75^{ABb}	3.25±0.85^{Ab}	0.0069	65.49±6.36^{Aa}	95.83±4.17^{Aa}	76.28±10.86^{Aa}	75.37±8.04^{Aa}	0.0936
	Goldben	13.50±1.19^{a}	5.00±0.71^{Ab}	0.25±0.25^{Bc}	3.00±0.41^{Ab}	3.75±0.48^{Ab}	0.0001	67.36±6.54^{Ca}	98.33±1.67^{Aa}	85.01±2.52^{ABa}	80.51±4.06^{BCa}	0.0017
	Control	13.00±1.47^{a}	15.75±2.29^{Aa}	17.00±1.08^{Aa}	19.50±1.04^{Aa}	20.00±2.27^{Aa}	0.3206	–	–	–	–	
	***P*–value**	**0.7431**	**0.0005**	**0.0001**	**0.0001**	**0.0001**		**0.8442**	**0.0012**	**0.5892**	**0.6896**	
2nd	***Bt***	16.75±1.03^{a}	– *	6.50±0.29^{Ab}	5.00±0.41^{Bb}	2.75±0.63^{Bb}	0.0009	– *	62.79±0.94^{Ab}	79.35±2.17^{Aa}	87.32±3.61^{Aa}	0.0002
	Pestpyr	16.00±1.83^{a}	4.50±0.96^{Ab}	0.25±0.25^{Bc}	3.25±0.48^{Bb}	3.75±0.48^{Bb}	0.0003	67.66±6.78^{Ca}	98.93±1.07^{Aa}	84.40±1.66^{ABa}	82.46±3.02^{BCa}	0.0009
	Goldben	13.00±0.91^{a}	5.50±0.96^{Ab}	0.50±0.29^{Bc}	3.50±0.65^{Ab}	4.50±0.65^{Ab}	0.0022	61.79±4.13^{Ba}	94.82±1.89^{Aa}	73.95±7.63Ba	75.67±2.71^{ABa}	0.0025
	Control	15.75±0.63^{a}	17.25±1.38^{Aa}	17.00±1.47^{Aa}	20.50±2.63^{Aa}	22.50±1.94^{Aa}	0.1844	–	–	–	–	
	***P*–value**	**0.1577**	**0.0001**	**0.0001**	**0.0001**	**0.0001**		**0.4874**	**0.0001**	**0.3327**	**0.0758**	

*The first day was excluded due to delay in *Bt* symptoms appearance. Means ± SE are shown. Means followed by the same capital letters in a row and lower–case letters in a column do not differ significantly by the Tukey's HSD test ($P < 0.05$).

The green lacewing *C. carnea* was detected in both planting times, and was more abundant in the first planting time in August when *S. littoralis* and *S. exigua* were commonly found. Likewise, the two *Coccinella* species were commonly found but the numbers were higher in the second planting time when *S. ocellatella* was abundant. For both predator groups, a 100% reduction was observed 3 days after applying the chemical pesticides in both seasons (Fig. 1 and 2). By contrast, decrease of the predator populations was much smaller when *Bt* was used. For example, in the first season, after 10 days of the application, only 15% of *C. carnea* reduction was detected when treated with *Bt*, whereas this predator greatly declined ($F = 92.700$, $P < 0.001$) to 13.57% and 8.50% when treated with Pestpyr and Goldben, respectively (Fig. 1a). This was also the case in the second season (Fig. 1b). Similarly, *Bt* showed the least negative infuence to ladybird beetles, compared with the two synthetic pesticides in both seasons (Fig. 2a, b).

DISCUSSION

The present study demonstrated that synthetic chemical pesticides, in terms of rapid potency, were more effective in prompt controlling of sugar beet insect pests than an entomopathogenic pesticide, *Bt*, was. From the current observation, the beet moth *S. ocellatella* was reduced by 100% immediately after 3 days of spraying in both seasons, whereas *Bt* achieved 44.03% and 30.14% reduction in the first and second season, respectively. Also, the population size of *S. littoralis* and *S. exigua* were sharply reduced in the third day wtih chemical pesticide application. A strong advantage of synthetic chemical insecticides is that they can swiftly kill insect pests (Heckel, 2020) but they may cause multiple environmental issues including their negative impacts on beneficial organisms in the agroecosystem, such as natural enemies and pollinators. On the other side, it is often suggested that the use of bio-pesticides such as *Bt* can provide control of pest insects while it is safe for non–target organisms. This is a strong advan-

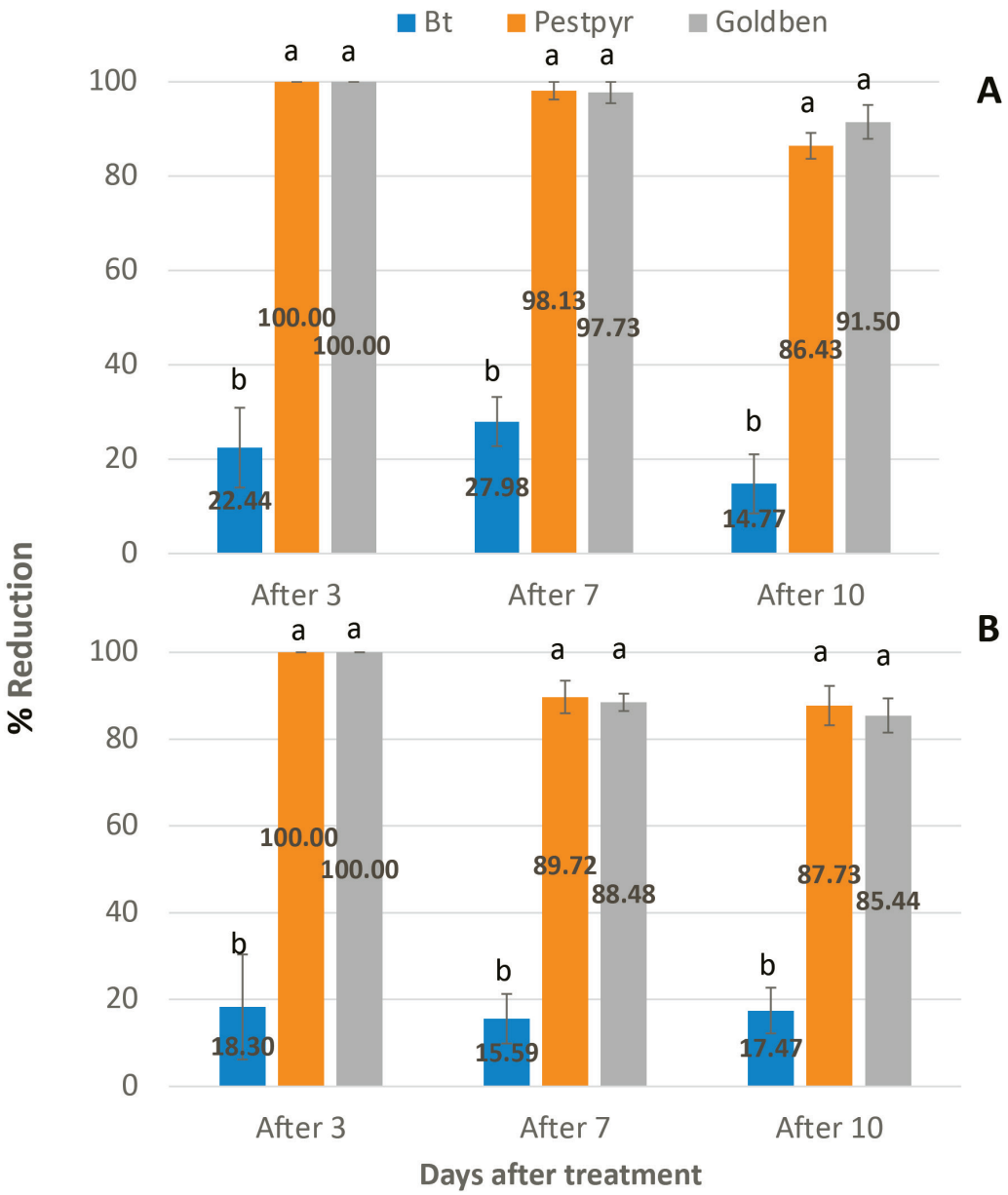

Fig. 1. The reduction percentages of the green lacewing *Chrysoperla carnea* (relative to the control) after application of *Bacillus thuringiensis* (*Bt*) and two other synthetic pesticides in (A) first season and (B) second season. Lines above bars indicate standard errors. Different letters above bars show a significant difference in the same inspection day by the Tukey's HSD test ($P < 0.05$).

tage of bio–pesticdes like *Bt* though *Bt* requires two or three days to begin to show its fatal effect (Nawrot–Esposito *et al.*, 2020); this held true in our study. We demonstrated that the influence of *Bt* application on three lepidopteran pests increased with the passage of time (Tables 2, 3 and 4). Under greenhouse condition, Legwaila *et al.* (2015) found that application of *Bt* significantly increased mortality of diamond–back moth *Plutella xylostella*, and that the greatest mortality occurred six days after its application. Following the ingestion of *Bt* by larval insects, the active toxins bind to the midgut epithelial cells and destroy the midgut epithelium, causing rapid osmotic cell lysis, and as the result, infected larvae stop to feed within hours leading to death from starvation within several days (Bravo *et al.*, 2007). Hence, *Bt* requires time to show a high reduction percentage of pests. This is indeed the case observed in our study. *Bt*, nevertheless, appears to be highly effective in suppressing the main lepidopteran pests examined here; the reduction percentages obtained were nearly the same with the two synthetic pesticides after 7 or 10 days of application. We thus conclude that *Bt* is enoughly useful in sugar beet pest management.

In the present study, we selected two synthetic pesticides for comparison. Chlorfenapyr (Pestpyr) is a N–substituted and halogenated pyrrole insecticide with a broad spectrum and has been used for controlling all stages of various insect pests (Dekeyser, 2005). It disrupts the respiratory chain and proton gradients and,

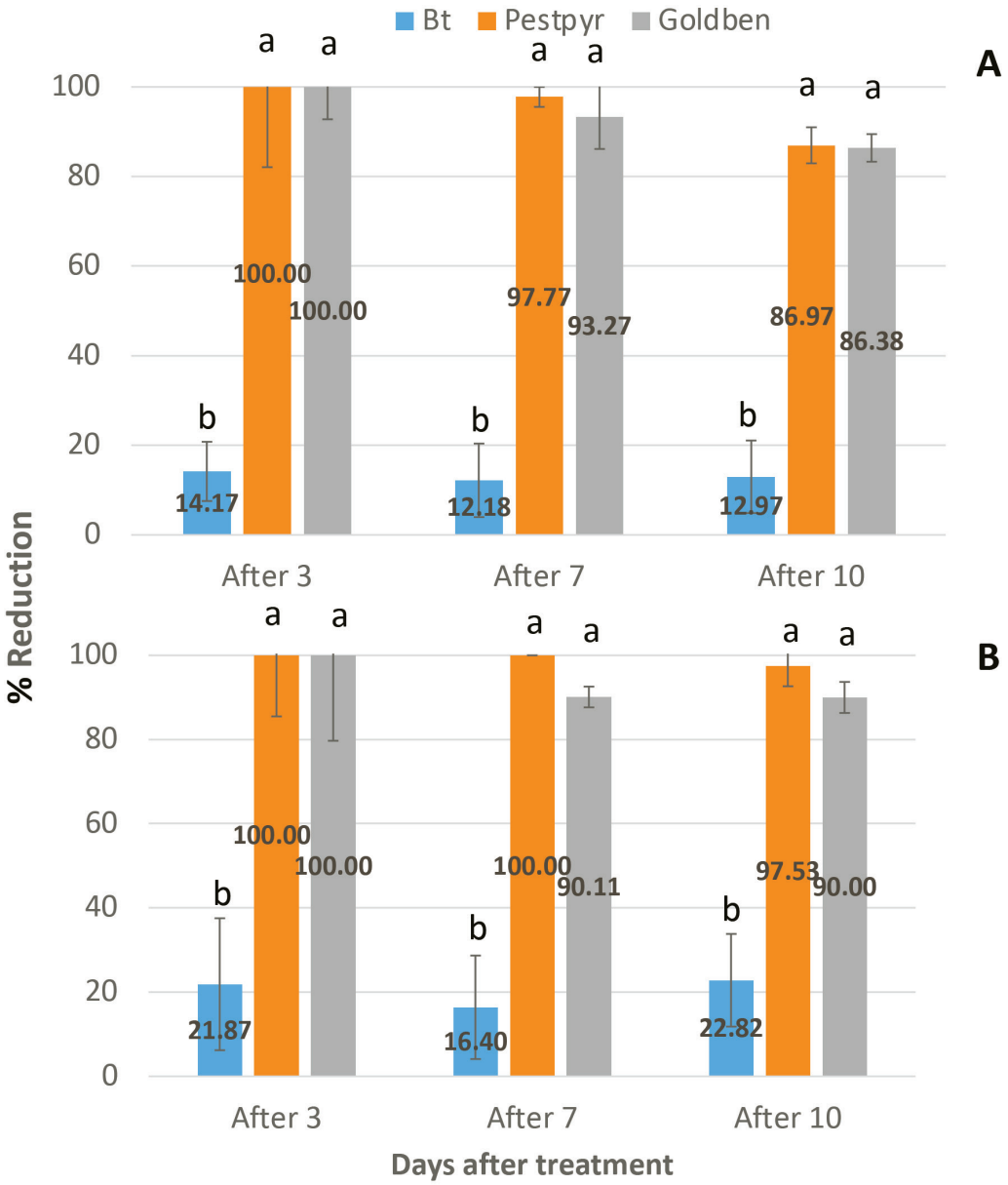

Fig. 2. The reduction percentages of ladybird beetle *Coccinella* spp. (relative to the control) after application of *Bacillus thuringiensis* (*Bt*) and two other synthetic pesticides in (A) first season and (B) second season. Lines above bars indicate standard errors. Different letters above bars show a significant difference in the same inspection day by the Tukey's HSD test ($P < 0.05$).

thus, stops ATP production in mitochondria resulting in cell dysfunction and eventually causing death of the targeted organism (Black *et al.*, 1994; Raghavendra *et al.*, 2011). Methomyl (Goldben) is a systemic, anticholinesterase broad–spectrum carbamate insecticide and has widely been used against many insect pests. Chemical pesticides including Chlorfenapyr and Methomyl generally show rapid potency against many insect pests including lepidopterans (Darabian and Yarahmadi, 2017; Desaeger *et al.*, 2011; Sallam *et al.*, 2015). Likewise, in our study, both Chlorfenapyr and Methomyl appear to be highly effective against *S. ocellatella*, *S. littoralis* and *S. exigua*. The last two lepidopterans are often known to have a high level of resistance to many conventional chemical pesticides (the Arthropod Pesticide Resistance Database, 2023). Nevertheless, the two tested pesticides can work well, at least, against the pest populations in our field. These pesticides should be useful in sugar beet pest management. It is, however, likely that frequent use of those pesticides can cause development of resistance, as in the previous case for other conventional pesticides, and their efficacy may greatly decrease in near future. To avoid this, or, at least, to slow or weaken the development, combined use of other pesticide or measure should be recommended (De Bortoli *et al.*, 2017). The present study, thus, highlights usefulness of *Bt* bio–pesticide in managing sugar beet pests that can easily develop resistance. Also, use of *Bt* would not disturb native natural enemies, as shown in the present study (Fig. 1 and 2), maintaining biological or natu-

ral control in sugar beet fields. This bio–control function may also lead to preventing development of resistance.

Further, because of current public demand, frequent use of synthetic pesticides should not be recommended, and reducing such pesticide use is ideal to promote environmental protection and safety. In fact, use of ecologically friendly pesticides, such as biological and botanical pesticides, have been increasing (Tran *et al*., 2017; Wen and Ueno, 2022a, b). At least, some of them are relatively cheap with low toxicity to vertebrates and non–target organisms (Tran *et al*., 2016; 2017). It is commonly observed that chemical pesticides negatively affect non–target invertebrate species including natural enemies of pests, such as insect parasitoids and predators, causing a decrease of natural enemy abundance (Mousa *et al*., 2013; Wilson *et al*., 1998). The present results indeed showed 100% reduction in predators' populations 3 days after the chemical pesticides had been sprayed (Fig. 1 and 2). Both chlorfenapyr and methomyl have a broad insecticidal spectrum, meaning that they are nonselective pesticides. The advantage of these pesticides is that they can effectively control a wide range of pest species. However, they can cause pest outbreaks or resurgence because they may eliminate beneficial natural enemies of target pests if pesticide resistance has once developed (Mousa *et al*., 2013; Pimentel, 2013). In the current study, we observed that the numbers of the three main predators immediately and sharply decreased after the synthetic pesticide application. In contrast, *Bt* had the least negative effects on both lacewings (Fig. 1) and ladybird beetles (Fig. 2). Such a benefit of using bio–pesticides like *Bt*, *i.e*., least non–target effects has often been mentioned (Carvalho *et al*., 2012; Kalha *et al*., 2013).

CONCLUSIONS

It is obvious that the use of synthetic pesticides achieved fast protection of sugar beet plants from insect pest attack. *Bt* is almost equally useful in controlling sugar beet pests though there is a time–delayed efficacy. The present study therefore recommends application of chemical pesticides once in the beginning of the season and subsequent multiple sprays of *Bt* suspension during the rest of the season. Reducing the application of broad–spectrum chemical pesticides would minimize the harmful impact on insect natural enemies, and combination use with Bt–bioinsecticides allows conservation of the agroecosystem while ensuring the safety of food production for human consumption.

AUTHORS' CONTRIBUTIONS

M. R. Mansour and K. M. Mousa designed the study, conducted the field experiments and prepared the first draft of the manuscript. K. M. Mousa also analyzed the data and interpreted the results. T. Ueno wrote the manuscript and polished up the research concept and manuscript.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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