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The First Field Evaluation of *Bacillus thuriengiensis* Bt407 against *Cassida vittata* and Its Natural Enemies In Egyptian Sugar Beet Fields.

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ABSTRACT

The scientific community working in the field of insect pathology is experiencing an increasing academic and industrial interest in the discovery and development of new bioinsecticides as environmentally friendly pest control tools to be integrated with chemicals in pest management programs. Therefore, field studies were conducted at the experimental farm of Sakha Agricultural Research Station during the 2019/2020 and 2020/2021 seasons for estimating the efficiency of the new strain, *Bacillus thuriengiensis* Bt407 (identified in Germany, GATC company) against *Cassida vittata* Vill larvae and their predators. Moreover, calculating the percentages of parasitism on *C. vittata* eggs and on *C. vittata* larvae-pupal compared to some conventional insecticides; Penadryl 10% EC. (Pyridalyl) and Coragen 20% SC. (Chlorantraniliprole). The overall average of reductions in this insect were 69.45 and 75.21% by *Bacillus*, 78.96 and 81.52% by Penadryl, and 79.06 and 81.31% by Coragen during the two seasons, respectively. On the other hand, the overall average reductions in *P. alfierii* were 48.88 and 47.51% by *Bacillus*, 94.18 and 88.65% by Penadryl, and 90.60 and 84.52% by Coragen. *Coccinella undecimpunctata* decreased by 38.13 and 34.99% by *Bacillus*, 90.27 and 88.16% by Penadryl, and 92.63 and 79.47% by Coragen. *Chrysoperla carnea* dropped by 38.45 and 49.7% by *Bacillus*, 92.24 and 91.50% by Penadryl, and 90.39 and 84.17% by Coragen during the two seasons, respectively. Further, the mean number of emerged parasitoids and parasitism percentages were higher in *Bacillus*-treated plots than in conventional insecticide-treated plots through the two seasons. Lastly, these findings demonstrate that this bacterium strain is effective in reducing *C. vittata* larvae populations, but not of their predators and parasitoids compared to conventional insecticides. Thus, it could be recommended to enroll this strain in the IPM of sugar beet insects.

INTRODUCTION

Numerous species of insects attack the sugar beet crop (*Beta vulgaris* L., Family: Chenopodiaceae), and cause considerable reduction in its root and sugar yield (Hawila, 2021). The tortoise beetle, *Cassida vittata* Vill. (Coleoptera: Chrysomelidae) is one of the most devastating insects in sugar beet fields, especially the second (mid-September) and third (mid-October) (El- Dessouki, 2019). The larvae and adults of this insect feed upon sugar beet leaves causing reduction in sugar content and sugar root yield by 40.10 and

56.20%, respectively (Bazazo and Besheit, 2019). Many authors showed the importance of this insect pest on sugar beet crops since 1982 - until now e.g., Bassyouny and Beleih (1996) reported that the initial rate of infestation of *C. vittata* appeared on sugar beet plants late in December or on mid - January for all plantation. The population density of this insect gradually increased as the sugar beet plants has become older. Abd El- Kareim and Awadalla (1998) indicated that all developmental stages of *C. vittata* gradually increased from March towards the end sugar beet growing season. They recorded two peaks of immature stages; the first in March and the second in May. The highest peak of the adult stage was found in April and sharply declined in May and June. Also, Bassyouny (1998) recorded high numbers of *C. vittata* on sugar beet plants of both mid - September and late - October plantations, while the lowest numbers were recorded in August plantations. Moreover, Abo El-Naga (2004) noticed the appearance of *C. vittata* adults by late January, since three peaks were displayed by late March, mid - April and late May. The larval population appeared by late February and exhibited three peaks by late March, early and late May. Mousa (2005) recorded high populations of *C. vittata* during the November plantation, while it occurred in low numbers during the August plantation. El- Khouly (1998) studied the population fluctuations of *C. vittata* larvae and recorded that the initial appearance of this insect was in January and reached its peak in April. In addition, Amin et al. (2008) indicated that the infestation by *C. vittata* appeared in the last week of March and extended until the second week of May. The population density of this insect recorded three peaks during the last week of March, mid - April and mid-May. In another study, Sherief *et al.* (2013) recorded two peaks of *C. vittata* larvae and adults that represented 587, 695 larvae/ 50 plants and 243, 240 adults/ 50 plants, respectively on mid - March and mid - April. Finally, El- Dessouki (2014) mentioned that larvae of *C. vittata* showed two peaks of activity which were during late March and late April. Concerning insect predators and parasitoids, sugar beet fields have several species that should be conserved to keep the natural balance in the fields, mainly for *C. vittata* populations (El- Dessouki, 2019 and Hawila 2021). Youssef and Abou- Attia (2001) showed that *C. carnea* plays an important role in managing major sugar beet insects; e.g., *C. vittata*. *Chrysoperla carnea* fed upon eggs and larvae of *C. vittata*. Further, Talha (2001) recorded that *C, undecimpunctata* is a vital predator of *C. vittata* larvae. Al- Habashy (2013) indicated that *C. carnea* + *C. undecimpunctata* + *P. Alfierii* are effective predators against *C. vittata* larvae. On the other hand, Awadalla (1993) recorded two parasitoid species of *C. vittata*: the egg parasitoid, *Monorthochaeta nigra* Blood, and larval - the pupal parasitoid, *Tetrastichus* sp. The mean rate of parasitism ranged between 8.3 to 37.1%. Zawarh (2000) found that the mean of parasitism ranged between 16.4 to 40%.

Because of several problems of massive applications of conventional insecticides against insects. Further attention has been given to other safe compounds (Abuldahab et al., 2011). Biopesticides are one of the most promising alternatives over conventional insecticides, offering less or minimum harm to natural enemies and the environment (Jisha *et al.,* 2013). Sheppard *et al.* (2013) clarified that *Bacillus thuriengiensis* produces crystalline proteins during its stationary phase of growth which are lethal to Lepidoptera, Coleoptera and Diptera insects. Moreover, Bazazo *et al.* (2015) and (2016) isolated a new strain of *B. thuriengiensis* from *Pegomyia mixta* Vill. and *Scrobipalpa ocellatella* Boyd larvae, as a first record in Egyptian sugar beet fields. This strain was identified with the aid of GATC company, Germany as *B. thuriengiensis* Bt407. The reduction in populations of *P. mixta* and *S. ocellatella* by this strain was 18.64 and 69.83%, respectively. Therefore, this study was planned to evaluate the new strain of *B. thuriengiensis* Bt407 against *C. vittata* larvae as compared to conventional insecticides and its side - effects on insect predators and parasitoids.

MATERIALS AND METHODS

This experiment was performed at the Experimental farm of Sakha Agricultural Research Station, Kafr El-Sheikh Governorate, Northern Delta, Egypt during 2019/ 2020 and 2020/ 2021 seasons. Sahr variety was planted on 15th October and 17th October during the two seasons, respectively. Three compounds (Table 1) were applied, one of them was biocide and the others were traditional insecticides; Pyridalyl and Chlorantraniliprole. Each insecticide was replicated four times (3 × 4 = 12 replicate). Moreover, four replicates were used as a check. Each replicate was measured at 42 m^2. A completely randomized block design (CRBD) was assigned. A Knapsack sprayer (20L. volumes) was used for spraying these compounds. The Knapsack sprayer was washed with water after every insecticide. The date of spraying was on the 1st and 5th of April throughout the two seasons, respectively. 10 plots/ replicate were inspected just before spraying and after 1, 7 and 10 days post-treatment for conventional insecticides. Whereas, were after 3, 7 and 10 days after the application for biocide according to Anonymous (2021). Numbers of *C. vittata* larvae and their predators were counted by visual examination in the field. Reductions in *C. vittata* larvae and predators were calculated through Henderson and Tilton (1955) formula as follow:

$$\textbf{Reduction}\ (\%) = 1 - \left(\frac{\text{No. in check before}}{\text{No. in check after}} \times \frac{\text{No. in treated after}}{\text{No. in treated before}}\right) \times 100$$

Calculating the Percentage of Parasitism:

Eggs, larvae and pupae of *C. vittata* were cut by scissors with pieces of plants from the previous experiment. Eggs were enclosed individually into Petri dishes (9cm diameter). In addition, larvae were provided with small portions of sugar beet leaves under laboratory conditions (25 ± 2˚C and 60 - 70% R.H.). The emerged parasitoids were collected and counted and percentages of parasitism were calculated. The samples were taken from the treated plots with the insecticides after 1, 3, 7 and 10 days post-treatment.

$$\textbf{Parasitism}\ \%\ (\textbf{eggs}) = \frac{\text{Total number of parasitoid species}}{\text{Total number of eggs}} \times 100$$

$$\textbf{Parasitism}\ \%\ (\textbf{pupae}) = \frac{\text{Total number of parasitoid species}}{\text{Total number of pupae}} \times 100$$

Table 1: Insecticides applied against *C. vittata* larvae during 2019/ 2020 and 2020/ 2021 seasons.

Common name	Trade name	Category	Rate/ Feddan
Bacillus thuriengiensis Bt407	-	Alternative	10^8 cfu/ ml water
Pyridalyl	Penadryl 10% EC	Conventional	250 cm^3
Chlorantraniliprole	Coragen 20% Sc	Conventional	60 cm^3

Statistical Analysis:

Statistical analysis was performed using one-way ANOVA. The means were compared using Duncan's Multiple Range Test (Duncan, 1955) at a 5% probability level. All analyses were conducted by “SPSS” computer software package

RESULTS AND DISCUSSION

Effectiveness of *Bacillus* and Conventional Insecticides on *C. vittata* larvae, Associated Predators And Percentages Of Parasitism During The Two Seasons:

1. *Cassida vittata* Larvae: Data in Table (2) and Figure (1) show that the overall average reduction in *C. vittata* larvae population was 69.45 and 75.21% by biocide application during the two seasons, respectively. Whereas, the conventional insecticides eliminated these insect numbers with 78.96 and 81.52% for Penadryl® 10% EC. through the two seasons, respectively. Also, the conventional insecticide, Coragen 20% decreased the insect populations by 79.06 and 81.31% in the two seasons, respectively. Statistical analysis proved that there were significant differences between the three treatments throughout the two seasons. These results demonstrate that biocide (*B. thuriengiensis* Bt407) induced a reduction in *C. vittata* larvae numbers satisfactorily as compared to conventional insecticides.

Table 2: Impact of different insecticides on *C. vittata* larvae under field conditions during 2019/ 2020 and 2020/ 2021 seasons.

Compounds	Before M.	After 1 M.	1 Red.	3 M.	3 Red.	7 M.	7 Red.	10 M.	10 Red.	Overall average
2019/ 2020										
Bacillus thuriengiensis Bt407	20.00	0.00	0.00	10.50	43.75	5.75	78.30	4.00 a	86.32	69.45
Penadryl 10% EC	20.25	7.75	63.97	0.00	0.00	5.00	81.36	2.50 b	91.55	78.96
Coragen 20% Sc	20.75	8.00	63.71	0.00	0.00	5.25	80.90	2.25 b	92.58	79.06
Check	20.00	21.25	-	24.00	-	26.50	-	29.25	-	-
2020/ 2021										
Bacillus thuriengiensis Bt407	19.75	0.00	0.00	9.50	58.31	5.25	79.26	3.50 a	88.08	75.21
Penadryl 10% EC	19.25	6.50	68.64	0.00	0.00	4.00	83.79	2.25 b	92.14	81.52
Coragen 20% Sc	19.50	6.75	67.85	0.00	0.00	4.25	83.00	2.00 b	93.10	81.31
Check	19.50	21.00	-	22.50	-	25.00	-	29.00	-	-

*Means followed by the same letters in a column are not significantly different at 0.05 probability level (Duncan's Multiple Range Test).

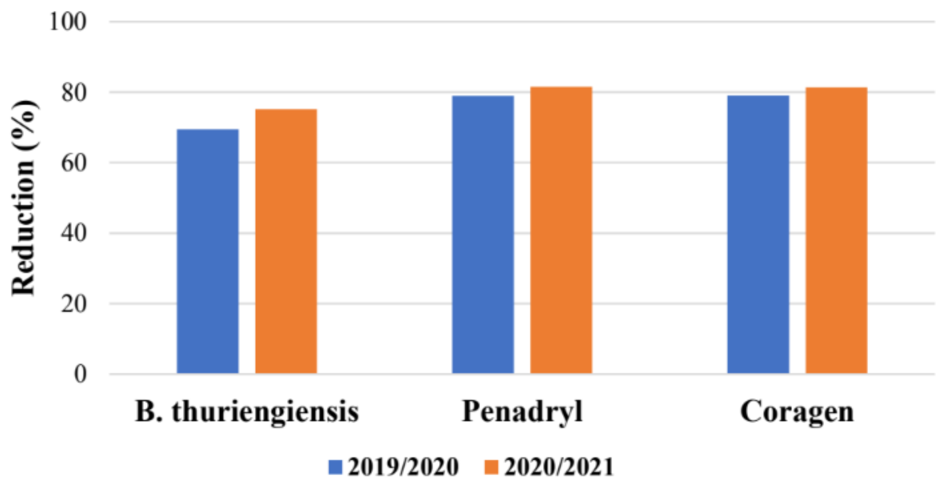

Fig. 1: Overall average reduction in *C. vittata* larvae due to biocides and conventional insecticides during 2019/ 2020 and 2020/ 2021 seasons.

Concerning the insect predators, Table (3) and Figure (2) indicate that biocide induced reduction in *P. alfierii* populations with 48.88 and 47.51% during the two seasons, respectively. While, Penadryl® decreased this predator by 94.18 and 88.65% throughout the two seasons, respectively. Further, Coragen® decreased the same predator by 90.60 and 84.52% in the two seasons, respectively. Statistical analysis proved that there were significant differences between the treatments through the two seasons.

Table 3: Influence of various insecticides on *P. Alfierii* under field conditions during 2019/ 2020 and 2020/ 2021 seasons.

Compounds	Before M.	After								Overall average
		1		3		7		10		
		M.	Red.	M.	Red.	M.	Red.	M.	Red.	
2019/ 2020										
Bacillus thuriengiensis Bt407	2.25	0.00	0.00	2.00	46.66	2.00	50.00	2.25 a	50.00	48.88
Penadryl 10% EC	2.25	0.25	99.92	0.00	0.00	0.25	93.75	0.50 b	88.88	94.18
Coragen 20% Sc	2.00	0.25	91.34	0.00	0.00	0.25	92.96	0.50 b	87.50	90.60
Check	2.25	3.25	-	3.75	-	4.00	-	4.50	-	-
2020/ 2021										
Bacillus thuriengiensis Bt407	2.50	0.00	0.00	2.00	42.85	2.25	47.05	2.25 a	52.63	47.51
Penadryl 10% EC	2.25	0.25	90.74	0.00	0.00	0.50	86.92	0.50 b	88.30	88.65
Coragen 20% Sc	2.25	0.25	90.74	0.00	0.00	0.75	80.39	0.75 b	82.45	84.52
Check	2.50	3.00	-	3.50	-	4.25	-	4.75	-	-

* Means followed by the same letters in a column are not significantly different at 0.05 probability level (Duncan's Multiple Range Test).

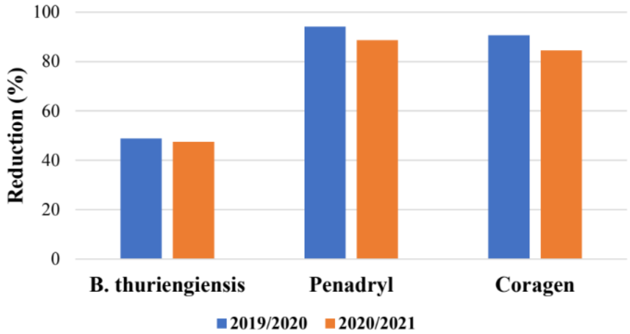

Fig. 2: Overall average reduction in *P. alfierii* larvae due to biocides and conventional insecticides during 2019/ 2020 and 2020/ 2021 seasons.

Table (4) and Figure (3) clarify that biocide induced reduction in *C. undecimpunctata* numbers with - 38.13 and 34.99%., with90.27 and 88.16% with Penadryl, and 92.36 and 79.47% with Coragen in the two seasons, respectively. Concerning, *C. carnea* individuals, Table (5) and Figure (4) noticed that biocide reduced this predator with 38.45 and 49.78%, 92.24 and 91.50% with Penadryl, and 90.39 and 84.17% with Coragen in the two seasons, respectively. Regarding insect predator species, Table (6) and Figure (5) elucidate biocide-induced reduction in all of the previous insect predator numbers with 41.16 and 43.40%, 91.27 and 91.04% with Penadryl, and 91.48 and 83.53% with Coragen during the two seasons, respectively.

Table 4: Overall average reduction in *C. undecimpunctata* due to certain insecticides throughout 2019/ 2020 and 2020/ 2021 seasons.

Compounds	Before M.	After								Overall average
		1		3		7		10		
		M.	Red.	M.	Red.	M.	Red.	M.	Red.	
2019/ 2020										
Bacillus thuriengiensis Bt407	5.00	0.00	0.00	4.50	29.56	4.50	40.00	4.75 a	44.83	38.13
Penadryl 10% EC	4.75	0.50	90.52	0.00	0.00	0.75	89.47	0.75 b	90.83	90.72
Coragen 20% Sc	4.50	0.25	95.00	0.00	0.00	0.50	92.59	0.75 b	90.32	92.63
Check	4.50	5.00	-	5.75	-	6.75	-	7.75	-	-
2020/ 2021										
Bacillus thuriengiensis Bt407	4.25	0.00	0.00	3.75	25.00	4.00	33.33	4.00 a	46.66	34.99
Penadryl 10% EC	4.25	0.25	94.73	0.00	0.00	0.75	87.50	1.33 b	82.26	88.16
Coragen 20% Sc	4.00	0.75	83.22	0.00	0.00	1.33	76.44	1.50 b	78.75	79.47
Check	4.25	4.75	-	5.00	-	6.00	-	7.50	-	-

* Means followed by the same letter in a column are not significantly different at 0.05 probability level (Duncan's Multiple Range Test).

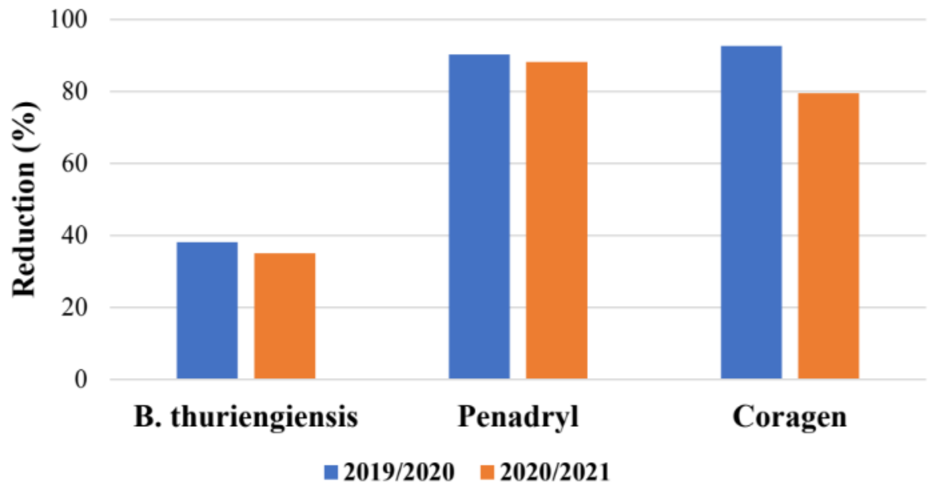

Fig. 3: Overall average reduction in *C. undecimpunctata* larvae due to biocide and conventional insecticides during 2019/ 2020 and 2020/ 2021 seasons.

Table 5: Overall mean of reduction in *C. carnea* individuals due to some insecticides during 2019/ 2020 and 2020/ 2021 seasons.

Compounds	Before M.	After 1 M.	1 Red.	3 M.	3 Red.	7 M.	7 Red.	10 M.	10 Red.	Overall average
2019/ 2020										
Bacillus thuriengiensis Bt407	3.25	0.00	0.00	2.75	32.30	3.00	38.46	3.00 a	44.61	38.45
Penadryl 10% EC	3.00	0.25	92.30	0.00	0.00	0.25	94.44	0.50 b	90.00	92.24
Coragen 20% Sc	3.00	0.25	92.30	0.00	0.00	0.50	88.88	0.50 b	90.00	90.39
Check	3.00	3.25	-	3.75	-	4.50	-	5.00	-	-
2020/ 2021										
Bacillus thuriengiensis Bt407	2.75	0.00	0.00	2.25	43.56	2.50	48.86	2.5 a	56.93	49.78
Penadryl 10% EC	2.75	0.25	92.56	0.00	0.00	0.25	94.88	0.75 b	87.08	91.50
Coragen 20% Sc	2.50	0.50	83.63	0.00	0.00	0.75	83.12	0.75 b	85.78	84.17
Check	2.25	2.75	-	3.25	-	4.00	-	4.75	-	-

*. Means followed by the same letters in a column are not significantly different at a 0.05 probability level (Duncan's Multiple Range Test).

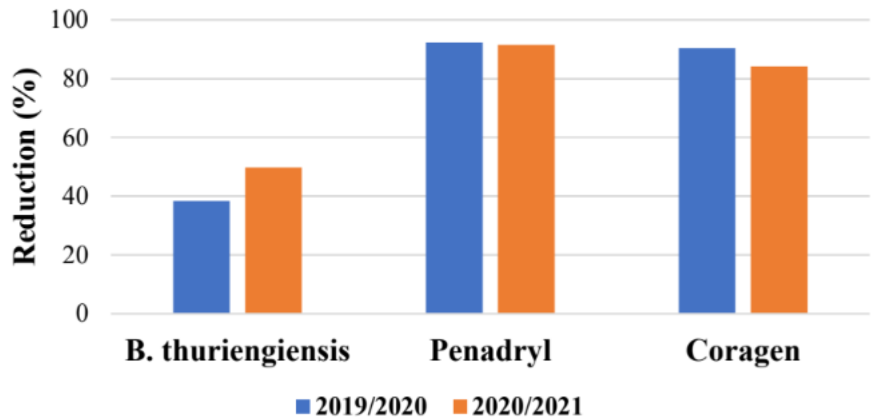

Fig. 4: Overall mean of reduction in *C. carnea* individuals due to some insecticides during 2019/ 2020 and 2020/ 2021 seasons.

Table 6: Overall average reduction in all of the previous insect predator populations due to biocide and conventional insecticides throughout 2019/ 2020 and 2020/ 2021 seasons.

2019/ 2020										
Compounds	Before	After								Overall average
		1		3		7		10		
	M.	M.	Red.	M.	Red.	M.	Red.	M.	Red.	
Bacillus thuriengiensis Bt407	10.50	0.00	0.00	9.25	35.17	9.50	42.15	10.00 a	46.16	41.16
Penadryl 10% EC	10.00	1.00	91.52	0.00	0.00	1.25	92.20	1.75 b	90.10	91.27
Coragen 20% Sc	9.50	0.75	93.30	0.00	0.00	1.25	91.58	1.75 b	89.58	91.48
Check	9.75	11.50	-	13.25	-	15.25	-	17.25	-	-
2020/ 2021										
Bacillus thuriengiensis Bt407	9.50	0.00	0.00	8.00	35.49	8.50	43.49	8.75 a	51.23	43.40
Penadryl 10% EC	9.25	0.75	93.05	0.00	0.00	1.50	90.10	1.75 b	89.98	91.04
Coragen 20% Sc	8.75	1.50	85.30	0.00	0.00	2.50	81.95	2.75 c	83.36	83.53
Check	9.00	10.50	-	11.75	-	14.25	-	17.00	-	-

*. Means followed by the same letters in a column are not significantly different at a 0.05 probability level (Duncan's Multiple Range Test).

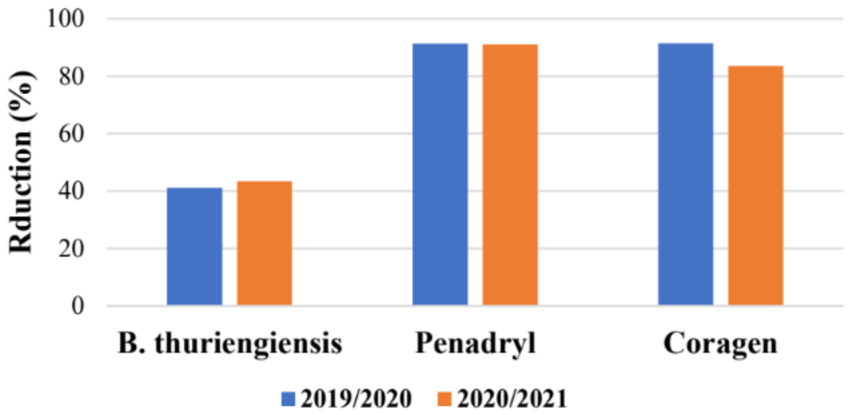

Fig. 5: Overall average reduction in all of the previous insect predator populations due to biocide and conventional insecticides throughout 2019/ 2020 and 2020/ 2021 seasons.

2. Percentages of Parasitism:

2.1. Egg Parasitoid, *Monorthochatea nigra*:

Data in Table (7) indicate that the mean number of emerged parasitoids and parasitism percentage to *C. vittata* eggs were higher in biocide-treated plots than in conventional-treated plots. The mean number of emerged parasitoids was 3.80±1.10 in plots treated with biocide, 0.562±0.10 in those treated with Penadryl, and 0.375±0.20 in plots treated with Coragen. Concerning, parasitism percentage values were 77.50±4.10 in plots treated with biocide, 11.25±1.20 in plots treated with Penadryl, and 7.5±1.13 in those treated with Coragen during 2019/ 2020. The same trend was obtained during the second season. These results showed that conventional insecticides were harmful to parasitoids compared to the biocide.

2.2. Larval - pupal parasitoid, *Tetrastichus* sp.

The findings in Table (8) demonstrate that the mean number of emerged parasitoids and parasitism percentage to *C. vittata* larval-pupal parasitoid were higher in biocide-treated plots than in conventional-treated plots. The mean number of emerged parasitoids was 3.37±1.20 in biocide plots, and 0.06±0.01 for Penadryl, and 0.06±0.01 for Coragen. In such concern, parasitism percentage values were 84.37±7.10 for biocide, and 1.47±0.20 for Penadryl, also 1.38±0.30 for Coragen during 2019/ 2020. The same results were recorded during the second season.

Table 7: Effect of the same previous insecticides on numbers of emerged parasitoids and parasitism percentages to *C. vittata* egg parasitoid.

Sampling date post-spraying	*B. Thuriengiensis* Bt407		Penadryl		Coragen	
	M. nigra emerged parasitoid	Parasitism%	*M. nigra* emerged parasitoid	Parasitism%	*M. nigra* emerged parasitoid	Parasitism%
2019/ 2020						
1	3.25	65.00	0.25	5.00	0.00	0.00
3	3.50	70.00	0.50	10.00	0.00	0.00
7	4.25	85.00	0.75	15.00	0.50	10.00
10	4.50	90.00	0.75	15.00	1.00	20.00
Mean ± SE	3.81±1.10 a	77.50±4.10 a	0.562±0.10 b	11.25±1.20 b	0.375±0.20 b	7.50±1.13 c
2020/ 2021						
1	3.50	73.68	0.25	5.55	0.00	0.00
3	3.75	78.94	0.25	5.55	0.25	5.26
7	4.00	84.21	0.50	11.11	0.25	5.26
10	4.50	94.73	0.50	11.11	0.50	10.52
Mean ± SE	3.93±1.20 a	82.89±3.10 a	0.375±0.10 b	8.33±2.12 b	0.25±0.10 b	5.26±2.10 c

Means followed by the different letters in a raw are significantly different at 0.05 probability level (Duncan's Multiple Range Test).

Table 8: Effect of the same previous insecticides on numbers of emerged parasitoids and parasitism percentages to *C. vittata* larval - pupal parasitoid.

Sampling date post- spraying	*B. Thuriengiensis* Bt407		Penadryl		Coragen	
	M. nigra emerged parasitoid	Parasitism%	*M. nigra* emerged parasitoid	Parasitism%	*M. nigra* emerged parasitoid	Parasitism%
2019/ 2020						
1	3.25	81.25	0.00	0.00	0.00	0.00
3	3.25	81.25	0.00	0.00	0.00	0.00
7	3.50	87.50	0.00	0.00	0.00	0.00
10	3.50	87.50	0.25	5.88	0.25	5.55
Mean ± SE	3.37±1.20 a	84.37±7.10 a	0.06±0.01 b	1.47±0.20 b	0.06±0.01 b	1.38±0.30 b
2020/ 2021						
1	3.00	57.14	0.00	0.00	0.00	0.00
3	3.00	57.14	0.00	0.00	0.00	0.00
7	3.25	61.90	0.25	4.34	0.00	0.00
10	3.75	71.42	0.00	0.00	0.25	5.26
Mean ± SE	3.25±1.10 a	61.10±5.10 a	0.06±0.01 b	1.08±0.10 b	0.06±0.20 b	1.31±0.20 b

Means followed by the different letters in a raw are significantly different at 0.05 probability level (Duncan's Multiple Range Test).

Bazazo (2005 and 2010) reported that the sugar beet ecosystem has enormous predators and parasitoids that should be wisely conserved to keep the insect pests under the economic threshold levels. Kalyanasundaram and Kamala (2016) indicated that natural enemies have recently gained much interest because of the problems encountered by the use of pesticides and environmental concerns. Parasitoids are an important biological tool used widely in agriculture for the suppression of various insect pests. Also, Clarke *et al.* (2019) concluded that parasitoids are among arthropods that are most widely used in biological control against crop pests and thus are a significant component of Integrated Pest Management Systems (IPM).

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