Egypt. J. Plant Prot. Res. Inst. (2022), 5 (4): 432 – 439

Ministry of Agriculture and Land Reclamation
Agricultural Research Center

Egyptian Journal of Plant Protection Research Institute
www.ejppri.eg.net

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PLANT PROTECTION RESEARCH INSTITUTE

Effect of *Bacillus aryabhattai* B8W22 and two conventional insecticides on *Scrobipalpa ocellatella* (Lepidoptera: Gelechidae) larvae and their natural enemy populations

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ARTICLE INFO
Article History
Received: 31/10 /2022
Accepted:23/12 /2022

Keywords

Efficacy, *Bacillus aryabhattai*, *Scrobipalpa ocellatella*, natural enemies, sugar beet and productivity.

Abstract

The present study was undertaken at Sakha Agricultural Research Station, Kafr El-Sheikh Governorate throughout 2020/2021 and 2021/2022 to compare the efficiency of the new bacterial strain *Bacillus aryabhattai* B8W22 and conventional insecticides (Dora 48% Ec and goldbein 90% Sp.) in controlling Scrobipalpa ocellatella (Boyd.) (Lepidoptera: Gelechidae) larvae with particular emphasis on their side effect on natural enemies. Results clarified that the overall mean of reductions in *S. ocellatella* larvae by *B. aryabhattai* suspension was 80.17 and 71.91%, by dora were 98.19 and 90.72%, and by goldbein were 98.10 and 89.97% in the first and second seasons, respectively. The mean reductions in insect predators were 22.32 and 23.91% by *B. aryabhattai*; 98.17 and 100% by dora and 99.20 and 100% by goldbein, respectively, whereas in the case of insect parasitoids, these values were 56.48 and 47.94% by *B. aryabhattai*; 100 and 100% by dora and 100 and 100% by goldenbein. This study therefore, proved that the bacterial strain was very efficacious in reducing the densities of *S. ocellatella* larvae with less impact on natural enemies of *S. ocellatella* larvae than both conventional insecticides used. Thus, *B. aryabhattai* can be used as an effective biocontrol method in Integrated Pest Management programs (IPM) of *S. ocellatella*.

Introduction

The beet moth, *Scrobipalpa ocellatella* (Boyd.) (Lepidoptera: Gelechiidae) is a serious insect pest of sugar beet crop and negatively reduces crop foliage, roots and amount of extracted yield of sugar (%) (El-Khouly *et al*., 2011). Female lays 40-70 (Maximum of 200) eggs on the host leaves. The hatching larvae bore into the leaf midrib, petiole or root to feed. This feeding activity forms irregular mines that may be covered with silk. After completing development, the larvae form silken cocoons in which to pupate. Leaves may roll, become distorted and blackish, and damaged plants become yellow and wilt. Heavily infested sugar beets lose up to 24% of their sugar content and the plants may further be damaged by invading pathogens (Abuldahab *et al*., 2011). Bazazo *et al*. (2016) observed larvae of *S. ocellatella* occurred on young sugar beet plants in mid – November, and continued until June. The larva numbers gradually increased and reached a peak at harvest time. Severe infestations of this insect in sugar beet led to significant reductions in root weight and sugar content (%) with 38.20 and 52.40%, respectively.

Intensive use of conventional insecticides led to health and environmental problems and huge reductions in natural enemies (Awad *et al*., 2014). There are numerous studies about using natural enemies in the biological control of many sugar beet insects. Coccinellidae, Staphylinidae, Formicidae and others are some of the important families of insect predators and have been used effectively in combating insect pests (Follett *et al*., 2015).

However, the efficiency of natural enemies has decreased in the last few years since farmers have used wide-spectrum insecticides that have negative effects on natural enemies and the environment. With respect to insect parasitoids, Bazazo and Ibrahim (2019) recorded *Diadegma oranginator* Aubert as an important parasitoid of *S. ocellatella* larvae. The seasonal mean of parasitism ranged between 44.44 to 68.91%. Further, Bazazo and Hassan (2021) recorded *D. aegyptiator* as a vital parasitoid of *S. ocellatella* Boyd larvae. The seasonal mean of parasitism ranged between 24.52 and 31.03%. These parasitoid species were identified for the first time in Egypt.

In addition to insect predators and parasitoids, microbial agents are one of the ways to control this pest. Microbial pathogens of insects are increasingly being considered environmentally friendly alternatives to conventional insecticides. Many insect pathogens such as *Bacillus* can be mass-produced, formulated, and applied against pest populations in a similar manner to chemical insecticides (Bhattarai *et al*., 2016).

One of the pathogens that have been successfully used as *B. thuringiensis* (Ramanujam *et al*., 2014). *Bacillus thuringiensis* Kurstaki therefore is an important insect pathogen that causes mortality through its toxic action against insect species (Tikar *et al*., 2008). Jisha *et al*. (2013) proved that *B. thuringiensis* produces crystalline proteins during its growth stationary phase which are lethal to lepidopterous, coleopterous and dipterous insects. Moreover, *B. thuringiensis* is pathogenic to a wide range of insect and nematode species (Bazazo *et al*., 2015 and Sheppard *et al*., 2013).

B. thuringiensis Bt407 is an important microbial strain for controlling *Pegomyia mixta* Vill. and *S. ocellatella*, in Egyptian sugar beet fields. (Bazazo *et al*., 2016). *Bacillus aryabhattai* Shivaji was reported to enhance plant growth by producing phytohormones (Park *et al*., 2017). Further, Bazazo *et al.* (2019) isolated and identified *B. aryabhattai* B8W22 from *Cassida vittata* larvae for the first time in Egypt by the help of GATC Company, Germany. The mortality of larvae ranged between 20.00 to 45.00% in a laboratory test after 10 days with suspension of this strain.

For an effective IPM program for this insect pest, it is necessary to find biocontrol agents that can work together without negative effects. Therefore, the current study was designed to evaluate the new strain, *B. aryabhattai* B8W22 and two conventional insecticides against *S. ocellatella* larvae and their natural enemies' populations. Moreover, estimating the productivity of the sugar beet crop.

Materials and methods

1. Effect of certain insecticides on *Scrobipalpa ocellatella* and its natural enemies:

This trial was done at Sakha Agricultural Research Station, Kafr El-Sheikh Governorate. Sahr sugar beet cultivar was planted on 20th of October, 2020 and 25th October, 2021 during the two seasons, respectively. Three compounds (Table 1) were used, each one was replicated four times (3×4 = 12 plots), each plot measured 42 m^2, in addition to four plots were used as control (Check). A completely Randomized Block Design (CRBD) was designed. Reduction in larvae and natural enemies was calculated by Henderson and Tilton (1955). A knap sack sprayer (20 L. volume) was used in applying insecticides. There is space area of 5 m^2 between each one of replicate to avoid insecticide drifting. The numbers of insect predators [*Coccinella* ssp. (Coleoptera: Coccinellidae) + *Paederus alfierii* Koch. (Coleoptera, Staphylinidae)], and parasitoids [*Diadegma oringinator* Aubert + *Diadegma aegyptiator* Shaumer (Hymenoptera: Ichneumonidae)] were counted using sweep net method (50 sweeps per sample) every date of inspection. Then, the catch was placed into a paper bag in the field, and transferred to the laboratory. After that, a piece of cotton saturated with chloroform was inserted into the bag for 30 minutes to anaesthetize the confined insects. Then, the bag was opened and the catch was dropped onto a petri dish (9 cm) with 70% ethyl alcohol and some drops of glycerin. The insects were inspected using a stereo microscope. The samples were identified at Plant Protection Research Institute, Egypt. The numbers of *S. ocellatella* larvae were counted by the visual examination method. 40 plants were investigated/ treatment before spraying and after three, seven and ten days of spraying. The reduction in insects was calculated by Henderson and Tilton (1955). The date of spraying was 20th of March during the two seasons

Henderson and Tilton formula

$$\textbf{Reduction (\%)}= \left(\frac{\textbf{No.in check before}}{\textbf{No.in check after}} \times \frac{\textbf{No.in treated after}x}{\textbf{No.in treated before}L}\right) \times \mathbf{100}$$

Table (1): Microbial and chemical insecticides applied against *Scrobipalpa ocellatella* larvae.

Insecticides		**Category**	**Usage rate/ 8 liter water**
Common name	**Trade name**		
***Bacillus aryabhattai* B8w22**	-	Aternative	10^8 cfu/ ml
Chorpyrifos	Dora 48% EC	Conventional	42 cm^3
Methomyl	Goldbein 90% SP	Conventional	13 m.

2. Assessment of root and sugar yield:

The roots of treated plots which sprayed with previous insecticides and check ones were weighed after harvest to estimate the root yield and sugar yield (%) per faddan. Date of harvest was 20th May and 25th May for the two seasons, respectively. Concerning, sugar percent (%) was determined by sucrometer device according to AOAC (1990), at Sugar Crops Research Department, Sakha Agriculture Research Station.

3. Statistical analysis:

The statistical analysis was conducted using one-way ANOVA at probability of 5% and means separated Duncan Multiple Range test (Duncan, 1955).

Results and discussion

1. Effect of certain compounds on the larval population *Scrobipalpa ocellatella* :

Results in Tables (2, 3, 4, 5, 6 and 7) showed the mean reduction in the density of *S. ocellatella* larvae caused by *B. aryabhattai* B8W22 was 80.17 and 71.91 in seasons 1 and 2, respectively. While applications of the conventional insecticides resulted in

98.19 and 90.72% mean larval density reduction in season 1 and 2, respectively, for dora; and 98.10 and 89.97% mean larval density reduction in season 1 and 2, respectively for goldbein.

On the other hand, applications of the bacterium reduced the densities of insect predators by 22.32 and 23.91% and the densities of insect parasitoids by 56.48 and 47.94% in the two seasons, respectively. Concerning the conventional insecticides, in the first season, the density of insect predators in plots treated with these insecticides was reduced by 99.39% for dora and 99.20% for goldbein. In the second season, the reduction in the population of insect predators was 100% for both the dora and goldbein treated plots. No insect parasitoids were recovered from the plots treated with conventional insecticides suggesting that the parasitoids suffered 100% mortality in those plots.

According to larvae reduction percentages (Tables 2 and 5), there were significant differences among the means of the applied treatments. The overall reduction means of *S. ocellatella* larvae due to *B. aryabhattai* suspension were close to dora and goldbein insecticides, respectively in the present study. Goldbein eradicated all the larvae after 7 days; however, dora killed all the larvae after 10 days. As indicated in Tables (2 and 5); although the conventional insecticides are strong and faster, the reduction percentage of the tested microbial insecticides increased over time and reached a comparable level with the conventional ones in both seasons.

Table (2): Reduction in *Scrobipalpa ocellatella* numbers by different compounds during 2020/ 2021 season.

Insecticide	Before spray	After spray/ day						Overall mean of reduction
		3		7		10		
	Mean	Mean	Red.	Mean	Red.	Means*	Red.	
***Bacillus aryabhattai* B8w22**	10.00	5.25 a	58.21	2.25 a	85.84	0.75 a	96.47	80.17 a
Dora	10.25	0.50 b	96.11	0.25 b	98.46	0.00 b	100	98.19 b
Goldbein	10.50	0.75 b	94.31	0.00 b	100	0.00 b	100	98.10 b
Control	9.75	12.25	-	15.50	-	20.75	-	-

***Means followed by different letters are significantly different at level 5% of probability.**

Table (3): Reduction in insect predator numbers by different compounds during 2020/ 2021 season.

Insecticide	Before spray	After spray/ day						Overall mean of reduction
		3		7		10		
	Mean	M.	Red.	M.	Red.	M.*	Red.	
***Bacillus aryabhattai* B8w22**	7.50	7.00 a	15.15	7.00 a	24.32	7.25 a	27.50	22.32 a
Dora	7.75	0.00 b	100	0.00 b	100	0.25 b	98.17	99.39 b
Goldbein	7.50	0.00 b	100	0.25 b	97.61	0.00 b	100	99.20 b
Control	7.50	8.25	-	9.25	-	10.00	-	-

***Means followed by different letters are significantly different at level 5% of probability.**

Table (4): Reduction in insect parasitoids populations by different compounds during 2020/ 2021 season.

Insecticide	Before spray	After spray/ day						Overall mean of reduction
		3		7		10		
	Mean	M.	Red.	M.	Red.	M.*	Red.	
***Bacillus aryabhattai* B8w22**	2.75	2.00 a	34.54	1.50 a	55.37	0.75 a	79.54	56.48 a
Dora	2.50	0.00 b	100	0.00 b	100	0.00 b	100	100 b
Goldbein	2.50	0.00 b	100	0.00 b	100	0.00 b	100	100 b
Control	**2.25**	**2.50**	**-**	**2.75**	**-**	**3.00**	**-**	**-**

***Means followed by different letters are significantly different at level 5% of probability.**

Table (5): Reduction in *Scrobipalpa ocellatella* larval populations by different compounds during 2021/ 2022 season.

Insecticide	Before spray	After spray/ day						Overall mean of reduction
		3		7		10		
	Mean	M.	Red.	M.	Red.	M.*	Red.	
***Bacillus aryabhattai* B8w22**	12.75	8.25 a	38.88	3.00 a	83.33	1.50 a	93.54	71.91 a
Dora	12.50	2.50 b	81.15	1.00 b	94.33	0.75 b	96.70	90.72 b
Goldbein	12.50	2.75 b	79.22	1.25 b	92.91	0.50 b	97.80	89.97 b
Control	12.75	13.50	-	18.00	-	23.25	-	-

***Means followed by different letters are significantly different at level 5% of probability.**

Table (6): Reduction in insect predator numbers by different compounds 2021/ 2022 season.

Insecticide	Before spray	After spray/ day						Overall mean of reduction
		3		7		10		
	Mean	M.	Red.	M.	Red.	M.*	Red.	
***Bacillus aryabhattai* B8w22**	6.25	6.00 a	16.38	6.25 a	22.85	6.25 a	32.50	23.91 a
Dora	6.50	0.00 b	100	0.00 b	100	0.00 b	100	100 b
Goldbein	6.50	0.00 b	100	0.00 b	100	0.00 b	100	100 b
Control	6.75	7.75	-	8.75	-	10.00	-	-

***Means followed by different letters are significantly different at level 5% of probability.**

Table (7): Reduction in insect parasitoids populations by different compounds during 2021/ 2022 season.

Insecticide	Before spray	After spray/ day						Overall mean of reduction
		3		7		10		
	Mean	M.	Red.	M.	Red.	M.*	Red.	
***Bacillus aryabhattai* B8w22**	3.25	2.50 a	28.20	2.00 a	42.56	1.00 a	73.07	47.94 a
Dora	3.25	0.00 b	100	0.00 b	100	0.00 b	100	100 b
Goldbein	3.50	0.00 b	100	0.00 b	100	0.00 b	100	100 b
Control	3.50	3.75	-	3.75	-	4.00	-	-

***Means followed by different letters are significantly different at level**

B. aryabhattai is toxic to *Cassida vittata* larvae (Bazazo *et al.*, 2019), to *Brooks dentate* (Blibech *et al.*, 2012), to *Spodoptera littoralis* (Sakr, 2017), and to *Halymorpha halys* (Tozlu et al., 2019). *Bacillus* species are some of the most important pathogens used in the management of insects. The species include *B. thuringiensis*, *B. brevis*, *B. cereus*, *B. circulans*, *B. megaterium*, *B. subtilis* (Mazrou *et al.*, 2020), and *B. aryabhattai* (Xu and Cote, 2003 and Rooney *et al.*, 2009). Insect pathogens are one of the most effective factors in controlling insect pests invading plant crops. Worldwide, various biopesticides are widely used in greenhouse products, ornamental plants, stocked products, forest products, garden products, vegetables and fruits as biological pest control (Rishad *et al.*, 2017).

As shown in this study, the efficacy of the microbial insecticide increased over time while maintaining natural enemies within the treated area. By, maintaining the natural enemies in an area, plant health may be improved by providing some resistance of the area to invading pests because of the presence of the natural enemies. Biological control using *Bacillus* species isolates promotes plant growth and enhances plant resistance (Park *et al.*, 2017 and Mazrou *et al.*, 2020) against invading pests (Rishad *et al.*, 2017) where *Bacillus* strains produced different antimicrobial products

(Aunpad and Na-Bangchang, 2007; Song *et al*., 2012 and Che *et al*., 2015). Therefore, it has antimicrobial effects against soil- borne pathogens fungi (Ahmed and Omar, 2014; Minghui *et al*., 2015 and Rooney *et al*., 2009).

2. Impact of previous insecticide groups against *Scrobipalpa ocellatella* on root and sugar yield of sugar beet: Table (8) showed that in 2020/ 2021 season root yield of sugar beet in plots treated with insecticides compared with the check ones. The values of yield were 35.714, 35.833 and 35.904 tons/ fad. for *Bacillus* strain, dora and goldbein, respectively, while 32.619 tons/ fad. in check. Concerning, sugar yield the values were 6.482, 6.507 and 5.254 tons/ fad. for the previous insecticides, respectively, as compared with 5.254 tons/ fad. in the untreated plots.

In 2021/ 2022 season, indicate that root yield was 35.666, 35.738 and 35.690 tons/ fad. for the above mentioned insecticides, respectively, while 30.00 tons/ fad. in check plots. Also, the corresponding values of sugar yield were 6.419, 6.436 and 6.424 tons sugar/ fad. in check. Statistical analysis proved significant differences among the treated plots and untreated ones. Whereas, insignificant differences between the three insecticides in root yield and sugar yield during the two seasons.

The results of this investigation indicated that *B. aryabhattai* B8w22 reduces larval densities of *S. ocellatella*, while not significantly reducing densities of insect predators + parasitoids, as seen in plots treated with conventional insecticides, in addition, to yield of roots and sugar of sugar beet for conventional insecticides are similar to *B. aryabhattai* B8w22.

Table (8): Root and sugar yield and sucrose (%) in treated and untreated plots, 2020/2021and 2021/ 2022 seasons.

Treatment	Root weight (Kg. /168m^2)		Root yield (Ton / Fed.) *		Sucrose (%)*		Sugar yield (Ton / / Fed.) *	
	2020/ 2021	2021/ 2022	2020/ 2021	2021/ 2022	2020/ 2021	2021/ 2022	2020/ 2021	2021/ 2022
Bacillus aryabhattai **B8w22**	1500 a	1498 a	35.714 a	35.666 a	18.15 a	18.00 a	6.482 a	6.419 a
Dora	1505 a	1501 a	35.833 a	35.738 a	18.16 a	18.01 a	6.507 a	6.436 a
Goldbein	1508 a	1499 a	35.904 a	35.690 a	18.20 a	18.00 a	6.534 a	6.424 a
Control	1370 b	1260 b	32.619 b	30.00 b	16.11	16.61	5.254 b	4.983 b

***Means followed by different letters are significantly differences at level 5% of probability.**

Acknowledgments

The authors would like to express their deep gratitude and sincere appreciation to prof. Dr. Kamal Bazazo, Sugar Crops Research Institute for giving us a suspension of this bacteria. Also, thanks to Plant Pathology Research Institute (Prof. Dr. M. Saleh) to prepare this strain.

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