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Impact of Chemical Composition and Molecular Diversity of Sugar Beet Cultivars on *Scrobipalpa ocellatella* Boyd (Lepidoptera: Gelechiidae) and *Cassida vittata* Vill (Coleoptera: Chrysomelidae)

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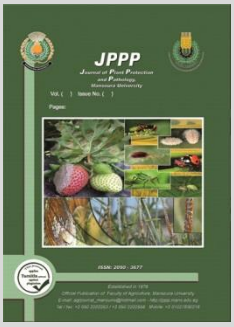

ABSTRACT

The field experiments were carried out at the experimental farm of Sakha Agricultural Research Station, Kafr El-Sheikh Governorate, Egypt to study the influence of both chemical composition and molecular variations of sugar beet cultivars on the population of *Scrobipalpa ocellatella* and *Cassida vittata* in 2020-2021 and 2021-2022 seasons. Results showed that Celnne cultivar harbored higher population of *S. ocellatella* (16.50 and 18.67 larvae/ 5 plants) in February and *C. vittata* (54.87 and 55.40 larvae and adults/ 5 plants) in March, with significant differences, than Heliospoly cultivar during the two investigated seasons, respectively. These differences may be due to the direct proportion between protein content and insect populations. A maximum standard of DNA polymorphism was found out by RAPD-PCR technique for both cultivars appearance certain positive and negative markers linked with plant afford to insect offensive. The RAPD-PCR technique of both cultivars revealed that Heliospoly was more resistant; while Celnne was more sensitive to these insects. The present study could be utilized by breeders and entomologists to develop sugar beet cultivars resistant to the two insects.

Keywords: *Beta vulgaris*; Sugar beet moth; Sugar beet beetle; Molecular markers.

INTRODUCTION

Sugar beet, *Beta vulgaris* plants are attacked by several insect pests that harm the plants by partial or complete defoliation from cultivation till harvest and reduce the final yield (Shalaby, 2001; El-Dessouki, 2014 & 2019 and Mansour *et al.*, 2021). The sugar beet moth, *Scrobipalpa ocellatella* (Boyd.) larvae feed on the sugar beet plants and reduce root quantity and sugar content (Al-Keridis, 2016). On the other hand, the feeding of the larval stage facilitates the invasion of fungi resulting in heavy harmfulness, causing most weak plants to yellow and wilt (Kheiri, 1991 and Bazazo and Mashaal, 2014). The maximum numbers of *S. ocellatella* larvae are noticed at harvest time (Ganji and Moharramipour, 2017 and El-Sheikh *et al.*, 2023). *S. ocellatella* injuries rate from 20 to 25% under field conditions and can minimize root yield by 2.3 to 3.8 tons/hectare with 0.5 to 1.15% sugar lack (Razini *et al.*, 2016). The sugar beet beetle, *Cassida vittate* (Vill.) larvae and adults appear in a high-density during March (Abd El-Kareim and Awadalla (1998)) and feed on the lower side of the sugar beet leaves, where they eat the lower epidermis and inner tissues, but the upper epidermis remains intact looking like a glass (El-Dessouki, 2014 and Kandil, 2016). Control of sugar beet insect pests could be achieved through optimizing the cultural practices such as adjusting planting dates and cultivation of resistant varieties (Shalaby 2001; Abo El-Naga, 2004; Abou-ElKassem, 2010; El-Dessouki, 2019 and Mansour *et al.* 2021).

Molecular biology is used in testing the effects of prospect markers for insect resistance genes in plant species, meantime gene term studies (Singh and Singh, 2005). Randomly Amplified Polymorphic DNA (RAPD) markers are useful for the assessment of genetic diversity because of their speed and relatively low cost compared to other molecular markers (Williams *et al.*, 1993). RAPD markers have been used extensively in sugar producing crops to detect DNA sequence polymorphism (Lorenz *et al.*, 1994), to analyze genetic relationships (Shen *et al.*, 1998). Few works have been conducted to estimate genetic diversity and relationship by using RAPD markers.

The current study aimed to investigate the impact of both chemical composition and molecular variations of sugar beet cultivars on the population of *S. ocellatella* and *C. vittata.*

MATERIALS AND METHODS

1. Experimental design

Field experiments were carried out at the experimental farm of Sakha Agricultural Research Station, Kafr El-Sheikh Governorate, Egypt (31°09´N latitude and 30°94´E longitude) during 2020-2021 and 2021-2022 seasons. The objective was to evaluate the susceptibility of two sugar beet cultivars (i.e. Celnne as a monogerm and Heliospoly as a polygerm) to *Scrobipalpa ocellatella* and *Cassida vittata* infestation. Three replicates of a randomized complete block design were used, with a plot size of 42 m^2. Each plot consists of ten rows of 60 cm width and 7 m

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length. The two sugar beet cultivars were planted on the 1st and 5th of October, with harvest dates on the 10th and 15th of May during 1st and 2nd seasons, respectively.

2. Sampling program

Five plants from each plot were visually and randomly taken weekly to count *S. ocellatella* larvae from December 17th to April 21st and *C. vittata* larvae and adults from January 7th to April 21st in 2020-2021 and 2021-2022 seasons, respectively according to period of activity of each insect.

3. Relationship between sugar beet leaf constituents and insect populations

Five plants from each plot were dried and milled to determine total protein and carbohydrates by electromagnetic spectrum, using the Near-Infrared (NIR) Spectroscopy apparatus, model DA1650, produced by FOSS Corporation according to AOAC, (2010) and Taha *et al.* (2016).

4. Molecular analysis of the tested sugar beet cultivars

Molecular genetic tests were carried out to detect the genetic markers associated with the tested insect pest tolerance genes. DNA isolation and RAPD-PCR fingerprinting methods were archived by Genetic Engineering and Biotechnology Research Institute, Sadat University, using Randomly Amplified Polymorphic DNA of the Polymerase Chain Reaction (RAPD-PCR) amplification.

Isolation, purification, and quantification of genomic DNA

The CTAB (Cetyl-tetramethyl ammonium bromide) method (Murray and Thompson, 1980) was used to isolate and purify DNA.

RAPD analysais

A total amount of 20 µl PCR was employed which is containing 1.0 µl (50 ng template DNA), 0.2 µldNTP,s (10 mM), 1.6 µl Mg Cl2 (25 mM), 2.0 µl 10X buffer (10 mM tris, pH 8.0, 50mM KCl and 50 mM ammonium sulphate), 4.0 µl primer (15 pmole), 0.1 µltaq polymerase (10u/ µl). Autoclaved double distilled H_2O was used to increase the volume to 20 l. The PCR cycling conditions included initial denaturation at 94°C for 5 minutes, followed by 35 cycles of amplification with the following parameters: template denaturation at 94°C for 1 minute, primer annealing at 36°C for 1.5 minutes, and primer extension at 72°C for 2 minutes, followed by storage at 4°C. T-Gradient thermo block PCR thermo cycler machines from Biometra (Germany) were used. Data presented in Table (1) indicated the 10 different 10-mer Oligonucleotide RAPD primers used for molecular profiling of sugar beet cultivars.

Table 1. The 10 different 10-mer Oligonucleotide RAPD primers were used for molecular profiling of sugar beet cultivars.

Primer	Sequence
1	AGG GGT CTT G
2	CAG GCC CTT C
3	GAA ACG GGT G
4	GTG ACG TAG G
5	GGG TAA CGC C
6	CTG CTG GGA C
7	GTA GAC CCG T
8	TTC GAG CCA G
9	GAT GAC CGC C
10	GAA CGG ACT C

Sodium dodecyl sulphate (SDS) Protein Electrophoresis:

SDS–Polyacrylamide gel electrophoresis (SDS-PAGE) was used according to Laemmli (1970).

5. Statistical analysis

The ″F″ Test was used to do an analysis of variance (ANOVA) on the data. To compare the variations in population of *S. ocellatella* and *C. vittata* in two sugar beet cultivars and the interaction between cultivars and inspection date, the least significant differences (L.S.D) at the 0.05 level were determined using a computer program (COSTAT software, 1988).

RESULTS AND DISCUSSION

Results

1. Susceptibility of sugar beet cultivars to sugar beet moth, *Scrobipalpa ocellatella*

Data in Table (2) show monthly average numbers of *S. ocellatella* larvae in Celnne and Heliospoly cultivars during 2020-2021 and 2021-2022 seasons. The monthly average number of *S. ocellatella* larvae/ 5 plants increased gradually from December to March with significant differences. The highest average (14.29 and 18.25 larvae/ 5 plants) was recorded in February in the 1st and 2nd seasons, respectively. On the other side, the two sugar beet cultivars significantly varied and Celnne cultivar achieved superiority on Heliospoly cultivar with an average number (10.54 and 8.82 larvae/ 5 plants) of *S. ocellatella* in 1st season and (12.02 and 10.66 larvae/ 5 plants) in 2nd season, respectively with significant differences. In general, the same data pointed out that the average number of *S. ocellatella* larvae were significantly differences by the interaction between observation date and cultivars during the two studied seasons. It is important to clear that, Celnne cultivar achieved the highest average number of *S. ocellatella* (16.50 and 18.67 larvae/ 5 plants) while Heliospoly achieved an average number of *S. ocellatella* (12.08 and 17.83 larvae/ 5 plants) in February in 1st and 2nd season, respectively, with significant differences.

2. Susceptibility of sugar beet cultivars to sugar beet beetle, *Cassida vittata*

Data listed in Table (3) show the average number of *C. vittata* larvae and adults on Celnne and Heliospoly cultivars during 2020-2021 and 2021-2022 seasons. The average number of *C. vittata* larvae and adults/ 5 plants increased gradually from January to March, and decreased in April with significant differences. The highest mean numbers (49.40 and 49.23 larvae and adults/ 5 plants) were recorded in March in 1st and 2nd seasons, respectively. On the other side, the two sugar beet cultivars were significantly varied, and Celnne cultivar performed superiority on Heliospoly cultivar with in average numbers of *C. vittata* infestation (27.28 and 18.46 larvae and adults/ 5 plants) in 1st season and (29.11 and 20.92 larvae and adults/ 5 plants) in 2nd season, respectively with significant differences. In general, the same data pointed out that the average number of *C. vittata* significantly differed by the interaction between check date × cultivars during the two studied seasons. Celnne cultivar had higher average number of *C. vittata* larvae and adults (54.87 and 55.40/ 5 plants) while Heliospoly achieved average numbers of *C. vittata* larvae and adults (43.93 and 43.07/ 5 plants) in March in 1st and 2nd seasons, respectively, with significant differences.

Table 2. Monthly average number of *Scrobipalpa ocellatella* larvae/ 5 sugar beet plants in 2020-2021 and 2021-2022 seasons.

Observation date (A)	Mean number of *S. ocellatella* larvae/ 5 plants					
	1st season (2020-2021)			2nd season (2021-2022)		
	Cultivar (B)		Mean (A)	Cultivar (B)		Mean (A)
	Celnne	Heliospoly		Celnne	Heliospoly	
December	2.22	2.56	2.39	3.22	2.56	2.89
January	9.17	8.92	9.04	14.17	9.42	11.79
February	16.50	12.08	14.29	18.67	17.83	18.25
March	15.80	11.53	13.67	17.80	16.60	17.20
April	9.00	9.00	9.00	6.22	6.89	6.56
Mean (B)	10.54	8.82	9.68	12.02	10.66	11.34
LSD 0.05						
A	1.02			A	2.04	
B	0.26			B	0.62	
AB	0.60			AB	1.38	

Table 3. Monthly average number of *C. vittata* individuals (larvae and adults)/ 5 sugar beet plants in 2020-2021 and 2021-2022 seasons.

Observation date (A)	Mean number ofindividuals (larvae and adults)/ 5 plants					
	1st season (2020-2021)			2nd season (2021-2022)		
	Cultivar (B)		Mean (A)	Cultivar (B)		Mean (A)
	Celnne	Heliospoly		Celnne	Heliospoly	
January	3.50	0.00	1.75	4.42	0.00	2.21
February	14.75	10.92	12.83	25.42	17.83	21.63
March	54.87	43.93	49.40	55.40	43.07	49.23
April	36.00	19.00	27.50	31.22	22.78	27.00
Mean (B)	27.28	18.46	22.87	29.11	20.92	25.02
LSD 0.05						
A	2.12			A	2.84	
B	1.92			B	1.18	
AB	3.70			AB	2.28	

3. Relationship between sugar beet leaf contents and insect populations

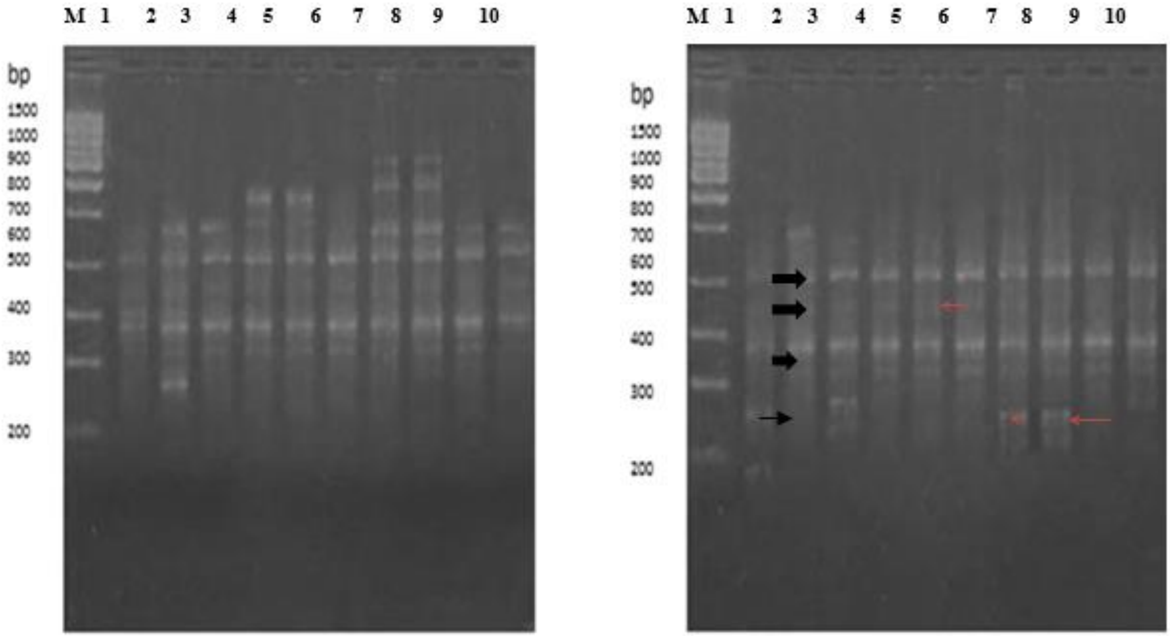
In the present work, population fluctuation of *S. ocellatella* and *C. vittata* was estimated in response to the changes in the total protein and carbohydrate contents of sugar beet leaves cultivars (Celnne and Heliospoly) during the growing seasons (2020-2021 and 2021-2022). As shown in Table (4), the obtained data showed that Celnne cultivar significantly harbored the highest population of *C. vittata* (27.28 and 29.11 individuals/ 5 plants) and *S. ocellatella* (10.54 and 12.02 larvae/ 5plants) as well as protein content in leaves (24.67±0.67% and 27.67±0.88%), in respectively compared with the Heliospoly cultivar during the two studied seasons. On the other hand, the Celnne cultivar gave the lowest of total carbohydrates (54.33±0.88% and 56.33±0.33%) compared with Heliospoly cultivar (56.33±0.88% and 58.67±0.33%) during the two investigated seasons, respectively. These differences may be due to the direct proportion between protein content and insect populations.

Table 4. Relationship between sugar beet leaf content and insect populations in 2020-2021 and 2021-2022 seasons.

Season	Cultivar	Mean no. of insects/ 5 plants		Total protein % ± Standard error	Total carbohydrates % ± Standard error
		C. vittata	*S. ocellatella*		
2020-2021	Celnne	27.28	10.54	24.67±0.67	54.33±0.88
	Heliospoly	18.46	8.82	22.33±0.33	56.33±0.88
	LSD 0.05	1.92	0.26	1.43	7.40
2021-2022	Celnne	29.11	12.02	27.67±0.88	56.33±0.33
	Heliospoly	20.92	10.66	19.67±0.33	58.67±0.33
	LSD 0.05	1.18	0.62	2.48	1.43

4. Molecular analysis of sugar beet cultivars

Each RAPD-PCR primer gave eleven bands ranging from 150-1500 base pairs (bp). Bands with MW 300 was present in Celnne cultivar at primer 3 and absent in primers 1, 2, 4, 5, 6, 7, 8, 9 and 10, respectively (Fig. 1), as for Heliospoly cultivar (Fig. 2) at the same MW 300 was present at primers 3, 7, 9 and absent in primers 1,2,4,5,6,8,10 respectively. The results in (Fig.1) Indicated that the bands with molecular size 500 bp was absent in all the tested primers for Celnne cultivar, whereas, for as Heliospoly cultivar was present at primer 2 and absent in other nine primers. Also, bands with MW 600 were appeared in Celnne cultivar at primers 7 and 8 and absent at primers 1, 2, 3, 4, 5, 6, 9 and 10, respectively (Fig. 1), whereas in Heliospoly cultivar in the same MW 600 was absent in all the tested primers. The previous results in (Tables 2 and 3) indicated that sugar beet variety Heliospoly was resistance to *S. ocellatell*a and *C. vittata* insects, whereas Celnne cultivar was more susceptible to insect infestation. The listed results in (Fig.1) indicated that the present or absent molecular bands in tested cultivars showed a different resistance level in these cultivars to the infestation of the tested insects.



Cellne **Heliospoly**

Figure 1. RAPD-PCR primers amplified polymorphic bands of Cellne and Heliospoly sugar beet cultivars, Lan 1:10 primer numbers.

5. SDS protein electrophoresis

Water soluble leaf proteins extracted from Cellne and Heliospoly cultivars were evaluated by SDS-PAGE. Banding patterns of total soluble proteins are illustrated in Figure (2). The bands with MW 60 KDa were existent in susceptible cultivar (Cellne) but absent in the resistant one Heliospoly. On the other hand, the bands having molecular weights of 70 KDa were absent in the susceptible cultivar but appeared in the resistant cultivar Heliospoly.

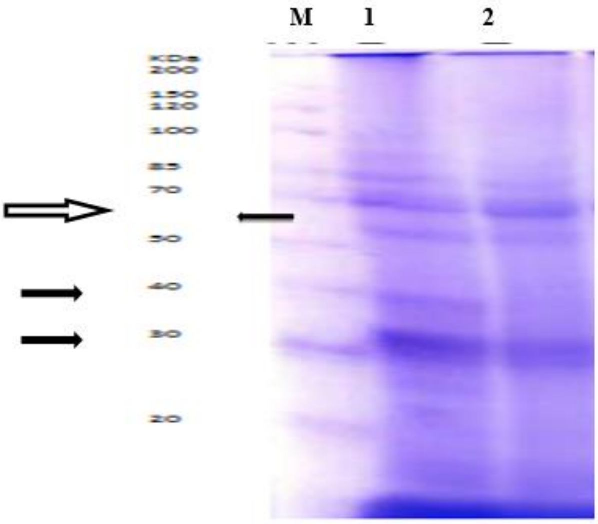


Figure 2. SDS-PAGE of protein banding patterns of two sugar beet cultivars (Lan 1: Cellne - Lan 2: Heliospoly).

Discussion

According to Mansour *et al.* (2021) the highest average number of *C. vittata* and *S. ocellatella* population was observed in April in the Kafr El-Sheikh region, in the present study, the highest average number of *S. ocellatella* population was in February, while it was in March for *C. vittate*. It is noted that the insect population has varied depending on the years in one region. This difference may be due to weather change. Based on the results of the current investigation, Heliospoly sugar beet cultivar received fewer populations of *S. ocellatell*a and *C. vittata* than Celnne cultivar. Chemical analysis indicated that Celnne leaves have the highest protein content in comparison with Heliospoly. However, the highest population correlated with the highest protein and less carbohydrates contents and the reverse were true. A similar conclusion was reported by El-Dessouki, 2019. These results are highly in agreement with those obtained by Berner *et al.*, 2005; Abo El Ftooh *et al.*, 2007; El-Rawy and Shalaby 2011; Abo El-Ftooh*et al.*, 2013; Ali *et al.*, 2014; Abbas, 2018; El-Dessouki, 2019; Awadalla *et al.*, 2020 and Khattab, 2021. The sugar beet varieties helsinki, sibel, and francesca had the least infestation by *S. ocellatella* insect, whereas two other varieties, maghrible and revel, had a moderate insect population (El-Rawy and Shalaby 2011). These findings may be due to the genetic variations between the two studied cultivars. Therefore, plants have a variety of constitutive and abettor defense mechanisms to protect them from attack; they include protein-based and chemical defenses as well as constitutional defenses like thorns and waxy cuticles (Lev-Yadun, 2016). The presence or absence molecular bands in tested cultivars showed a different resistance level in these cultivars to the infestation of the tested insects and certain molecular bands were sitting in Pyramids sugar beet variety while wanting in zinagri sugar beet cultivar which affects the resistance of the tested plants to insect infestation, Fayed *et al.* (2014). DNA (RAPD) primers of sugar beet genotypes were used, and the 3 different primers produced diverse banding, eight bands for OPAB10, five bands for S1155, and 18 bands for MHR-25. Thirty-one bands in total were determined to be polymorphic (96.77%), while one band was monomorphic (3.23%), Saidin, 2014. In the cultivars examined, RAPD provided greater genetic variety resolution than ISSR (Izzatullayeva e*t al.* 2014). For SDS protein electrophoresis, our results showed the bands have molecular weights 70 KDa was absent in the susceptible variety, whereas was appeared in the resistant variety Heliospoly (Fig. 3). This is consistent with the results reported by El-Mahalawy, 2011 who recorded that Mw 150 KDa bands can be used to lead a negative marker associated with plant vindication to insects, whereas Mw 11.5 KDa bands can be used to guide a positive marker connected with plant vindication to insects. For the *C. vittata* insect, the bands can serve as negative marks associated to plant damage (Fayed *et al.,* 2014).

CONCLUSION

Heliospoly sugar beet cultivar received less *S. ocellatell*a and *C. vittata* infestations than Celnne cultivar. The maximum total protein in Celnne cultivar was associated with the highest infestation by these insects. A maximum standard of DNA polymorphism was found out by RAPD-PCR technique for two sugar beet cultivars (Cellne and Heliospoly) appearance certain positive and negative markers linked with plant afford to insect offensive. The present study interprets the basis of resistance of sugar beet cultivars to the two abovementioned insects.

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تأثير التركيب الكيميائي والتنوع الجزيئي لأصناف بنجر السكر على فراشة البنجر وخنفساء البنجر السلحفائية

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الملخص

تم إجراء التجارب الحقلية في المزرعة البحثية لمحطة البحوث الزراعية بسخا، محافظة كفر الشيخ، مصر لدراسة تأثير التركيب الكيميائي والتنوع الجزيئي لأصناف بنجر السكر على الإصابة بفراشة البنجر وخنفساء البنجر السلحفائية خلال الموسمين 2020-2021 و2021-2022. أوضحت النتائج أن صنف Celnne احتوى على تعداد أعلى ليرقات فراشة البنجر (16.50 و18.67 يرقة/ 5 نباتات) فى فبراير واليرقات والحشرات الكاملة لخنفساء البنجر السلحفائية (54.87 و55.40 يرقة وحشرة كاملة/ 5 نباتات) فى مارس بالمقارنة بالصنف Heliospoly بإختلافات معنوية بينهما خلال موسمى الدراسة. ارتبط الحد الأقصى للبروتين الكلي في الصنف Celnne بأعلى إصابة بهذه الحشرات. أكتشف الحد الأقصى للشكل الظاهري DNA القياسي بواسطة تقنية RAPD-PCR وتبين وجود دلالات إيجابية وسلبية مرتبطة بقدرة النبات على مقاومة الحشرات. وبإستخدام تقنية RAPD-PCR لصنفى البنجر أظهرت النتائج أن الصنف Heliospoly كان أكثر مقاومة بينما كان الصنف Celnne حساساً للحشرتين. يمكن الإستفادة من هذه الدراسة الحالية من قبل المربين وعلماء الحشرات لتحسين مقاومة أصناف بنجر السكر لتلك الحشرتين.