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Ecological studies on *Spodoptera exigua* (Hubner) and its biological control by the novel parasitoid, *Cotesia ruficrus* (Haliday) (Hymenoptera: Braconida) on sugar beet

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ABSTRACT

Spodoptera exigua (Hubner) (Lepidoptera: Noctuidae) is one of the polyphagous insects destroying crops which included sugar beet global. It causes a highly reduction in sugar percentage and roots weight. parasitoids are the most element used around the world in biocontrol of insects. The current inquiry was conducted during the two succeeding growing seasons of 2019/2020 and 2020/2021 at the Experimental Farm of the Sakha Agricultural Research Station. The results obtained showed that seasonal parasitism percentage of *Cotesia ruficrus* on *S. exigua* larvae were 24.23, 22.86 and 34.33% during the first season 2019/2020. While was 21.48, 20.39 and 27.58% in the second season 2020/2021 throughout the three cultivations, respectively. This is the first report about parasitoid on *S. exigua* and their occurrence in Egyptian sugar beet fields, highly significant correlation was recorded between *S. exigua* larvae and parasitoid in three cultivations respectively. Biocide (Protecto) reduced *S. exigua* numbers with 51.51 and 52.26%, and *C. ruficrus* with 18.60 and 22.67%. While, conventional insecticide (Chlorpyrifos), reduce *S. exigua* with (88.50 and 92.11% and *C. ruficrus* with 99.06 and 100%) in the two seasons, respectively. Insignificant differences between sprayed plots with Protecto and Chlorpyrifos in both root and sugar yield of sugar beet were observed. In addition, *C. ruficrus* and protecto are consider as vital elements in the integrated pest management (IPM) Program against *S. exigua.* The sensible and justifiable application of biological control is the primary factor in the control of the insect pest complex throughout the sugar beet crop.

Keywords: *Cotesia ruficrus*: protecto: *Spodoptera exigua*: sugar beet: productivity

1. Introduction

Sugar beet (*Beta vulgaris* L.) has been commercially introduced in Egypt since 1982 with planted area about 20000 feddans and increased gradually to be 620000 feddan in 2020 (Anonymous, 2021). “Sugar” is a central nutrient in Europe and is extracted from sugarcane (72%) and sugar beet (28%) (Özgür, 2015). It is traditionally used for sugar extraction, and recently for biofuel production. Sugar beet plants attract numerous insect species during growing season, which cause economic loss in sugar yield (Bassyouny, 1993) *Spodoptera exigua* (Hübner) is an adverse insect pest of sugar beet globally. Sugar beet plants are subject to attack by numerous insect pests since pit growth up to produce (Saleh *et al.*, 2009; Abou El-Kassem, 2010; Khalifa, 2018; Bazazo and Hassan, 2021 and Mohsena, *et al*, 2021). Among most important of them is *S. exigua* (Talae *et al.,* 2017). It is a periodic pest attacking the roots as well as the foliage of sugar beet (Bazazo 2010). It became a destructive pest for sugar beet causing high economic damage (Hussein, 2001 and Mahmoud *et al.,* 2011). *S. exigua* deposits its eggs in patches that are coated with a mat of scales from the female's abdomen (Sertkaya *et al.,* 2004). According to (Bassyouny *et al.,* 1991), *S. exigua* severely damages sugar beet roots and leaves, which

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results in a significant drop in sugar percentages. Additionally, (Chi *et al.,* 2013) discovered that *S. exigua* may severely harm sugar beet seedlings, resulting in extensive fields of barren plants and significant financial losses. Widespread use of chemical insecticides as the main manipulate coverage against *S. exigua* brought about resistance to an extensive range of insecticides including organophosphates, carbamates, pyrethroids and some novel insecticides. This has brought about the pest outbreaks and harvest loss (El-Mahalawy, 2011). Insecticide resistance is a main trouble inside the control of this insect as it has developed resistance to many insecticides (Su and Sun, 2014). (Silva *et al.,* 2014) established that parasitoids one of the most critical components of biological control of lepidopterous larvae. Because of its efficiency and specificity to their hosts. Therefore, this study become done to recognize the larval parasitoid of this pest and its impact on beet armyworm populations in the three cultivations of sugar beet during the two seasons. Also, the effect of biocide and conventional one on *S. exigua*, *C. ruficrus*, root and sugar yield were studied.

2. Materials and Methods

This study was done at the experimental farm of the Sakha Agricultural Research Station in the Kafr El–Sheikh Governorate during 2019/2020 and 2020/2021 seasons. The experimental area is around 1/2 feddan. For each of the three cultivations throughout the two seasons, the Farida variety was sown on the 15th of August, the 15th of September, and the 15th of October. In each sample, fifth and sixth *S. exigua* larvae instars were collected from 30 plants/ sample. Every larva put into glass flask (5 X 10cm), covered with fine gauze to prevent parasitoids to go through it. The larvae were fed with pieces of sugar beet leaves under laboratory conditions (25 ± 2 C°, 60–70% RH.), and the food was changed every day until pupation or parasitoid emergence. Daily records and counts of the parasitoids were made, and they were kept in 75% ethyl alcohol. Every time a sample was taken, the percentages of parasitism were calculated using the following formula:

$$\% \text{ Parasitisme} = \frac{\text{No.Parasitoids}}{\text{No.Larvae of S.exiuga}} \text{ x100}$$

Parasitoids were identified by department of insect taxonomy and surveys at The Plant Protection Research Institute, Agricultural Research Center in Egypt. Parasitoid was recorded for the first time in sugar beet fields in Egypt, the samples were recognized as *Cotesia ruficrus* Fig (1).

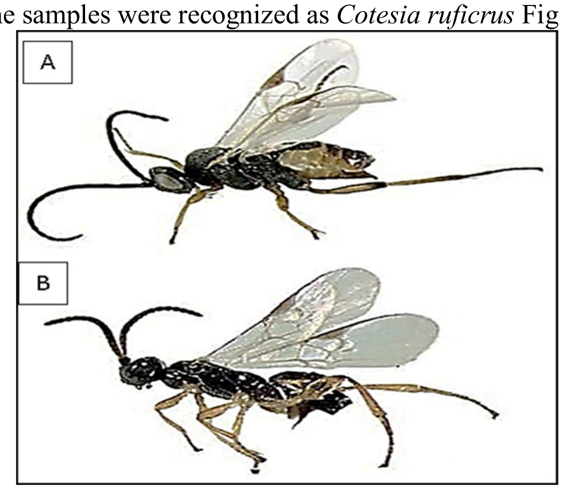

Fig. 1: *C. ruficrus* adult (A) male; (B) female (the size of adult about 2.0-2.5mm in length, the body was mostly black and legs were dark- yellow)

2.1. Effect of some biocides and insecticides on *S. exigua* population, *C. ruficrus*, root and Sugar yield

This experiment was carried out at a separate sugar beet field planted with Farida Variety on 15 August during the 2019–2020 and 2020–2021 seasons, in the experimental farm of Sakha Agricultural Research Station. In this study, two of the insecticides listed in Table [1] were used. Each compound to four replications (2 X 4 = 8 plots), each plot area was 42m^2. Also, four plots as control. Completely randomized block design was applied. Knap sac sprayer (20 L volume) was used in spraying on 15th

and 16th November during the two seasons. Numbers of parasitoids were recorded by sweep net method (50 double strikes per sample), larvae (by visual record) were counted 3, 7 and 10 days after spraying. Reduction percentage in larvae and its parasitoid was calculated according to (Henderson and Tilton1955).

$$\text{Reduction (\%)} = 1\text{-}\left(\frac{\text{No.Control Before}}{\text{No.Control After}}\ x\ \frac{\text{No.Treated After}}{\text{No.Treated Before}}\right) x100$$

To estimate roots of treated and untreated plots were weighed after the harvesting the root yield and sugar percentage (%). Sugar percent was determined at Sakha Sugar Crops Research department laboratory by saccharometer according to, (AOAC 1990).

Table 1: Certain biocide and insecticides sprayed against *S. exigua* larvae during 2019/2020 and 2020/2021 Seasons.

Compound	Chemical Class	Common Name	Rate
Chlorpyrifos 48% EC	Organophasphate	Chlorpyrifos	1000 ml /fad.
Protecto 9.4% WP	*Bacillus thuringiensis* Sub sp. Kurstaki	*Bacillus thuringiensis* Sub sp. Kurstaki	400 gm /fad.

2.2. Statistical analysis

Using SPSS statistics, the correlation coefficient values between *S. exigua* larvae and its parasitoid were computed. Software bundle 16.0 according to (SPSS. 2006) (Snedecor and Cochran, 1989). Means were. Analyzed according to (Duncan, 1955).

3. Results and Discussion

Results in Table (2) showed that parasitism percentages for the three cultivations throughout the 2019/2020 season varied from 0.00% to 46.15%, 0.00% to 40.0%, and 0.00% to 55.55%, respectively. A total of 132, 79, and 57 larvae were collected, of which 38, 21, and 22 were parasitized by each of the three Cultivations. (Fig.2) For the three seasonal average of parasitism was 24.23, 22.86, and 34.33%. for each of the three cultivations, respectively. Statistical analysis showed that significant differences between the three cultivations.

Table 2: Parasitism Percentages of *C. ruficrus* on *S. exigua* larvae during season 2019/2020, every 10 days intervals.

Date	1 st Cultivation			2nd Cultivation			3rd Cultivation		
	No. Larvae	No. Parasitoid	% Parasitism	No. Larvae	No. Parasitoid	% Parasitism	No. Larvae	No. Parasitoid	% Parasitism
15/9	3	1	33.33	-	-	-	-	-	-
25/9	6	1	16.66	-	-	-	-	-	-
5/19	7	0	0.00	-	-	-	-	-	-
15/10	8	2	25.00	2	0	0.00	-	-	-
25/10	11	0	0.00	4	0	0.00	-	-	-
5/11	13	3	23.07	4	1	25.00	-	-	-
15/11	15	5	33.33	5	2	40.00	1	0	0.00
25/11	20	6	30.00	6	2	33.33	2	1	50.00
5/12	23	8	34.78	8	2	25.00	3	1	33.33
15/12	26	12	46.15	9	3	33.33	5	2	40.00
25/12	-	-	.	12	0	0.00	6	0	0.00
5/1	-	-	-	13	5	34.64	7	2	28.57
15/1	-	-	-	16	6	37.50	7	3	42.50
25/1	-	.	.	.	-	.	8	3	37.50
5/_2	-	.	-	.	-	-	9	5	55.55
15/2	-	-	-	-	-	-	9	5	55.55
Total	132	38	28.78	79	21	26.58	57	22	38.59
Seasonal parasitism %		24.23b			22.86c			34.33a	
L.S. D 0.05 %					1.23				

Means with the same letters are not significantly different at 0.05 probability level

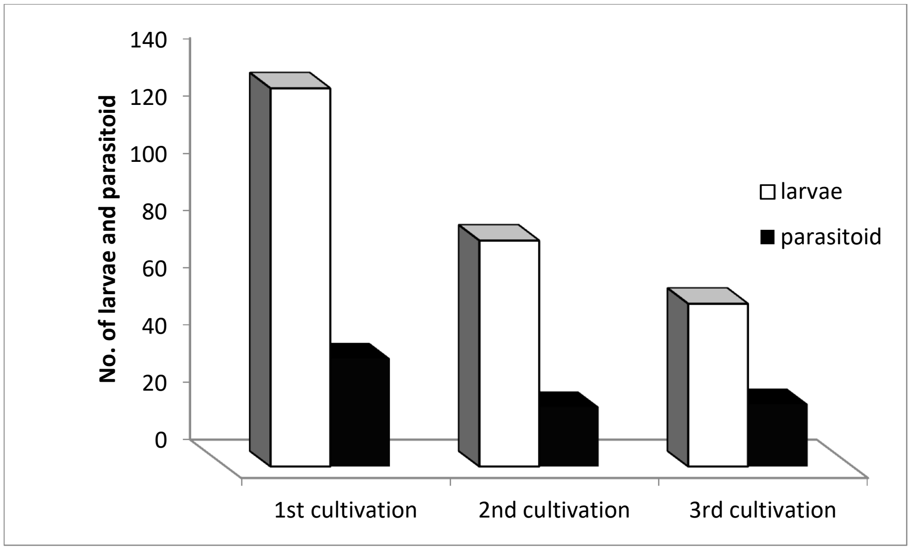

Fig. 2: Total Numbers of *S. exigua* larvae and parasitoid during season2019/2020

In the second season 2020/2021 Table (3), parasitism percentages ranged between (0.00 to 36.36%), (0.00 to 38.46%) and (0.00 to 60.00) for the three cultivations, respectively. The total number of larvae were 108, 80 and 71 larvae were collected while the total number of parasitoids were 30, 19 and 19 of which were collected from three cultivations, respectively.

Table 3: Parasitism Percentages of *C. ruficrus* on *S. exigua* larvae during season 2020/2021, every 10 days intervals.

Date	1st Cultivation			2nd Cultivation			3rd Cultivation		
	No. Larvae	No. Parasitoid	% Parasitism	No. Larvae	No. Parasitoid	% Parasitism	No. Larvae	No. Parasitoid	% Parasitism
16/9	2	0	0.00	-	-	-	-	-	-
26/9	5	0	0.00	-	-	-	-	-	-
6/10	6	0	0.00	-	-	-	-	-	-
16/10	6	2	33.33	1	0	0.00	-	-	-
26/10	9	2	22.22	3	1	33.33	-	-	-
6/11	12	3	25.00	5	0	0.00	-	-	-
16/11	12	4	33.33	5	1	20.00	2	1	50.00
26/11	16	5	31.25	8	2	25.00	2	1	50.00
6/12	18	6	33.33	10	2	20.00	3	0	0.00
16/12	22	8	36.36	11	0	0.00	4	0	0.00
26/12	-	-	-	11	4	36.36	5	0	0.00
6/1	-	-	-	13	4	30.76	5	3	60.00
16/1	-	-	-	13	5	38.46	8	3	37.50
26/1	-	-	-	-	-	-	12	3	25.00
6/2	-	-	-	-	-	-	15	4	26.66
16/2	-	-	-	-	-	-	15	4	26.66
Total	108	30	27.77	80	19	23.75	71	19	26.76
Seasonal parasitism%	21.48b			20.39b			27.58a		
L.S. D 0.05%				2.01					

Means with the same letters are not significantly different at 0.05 probability level

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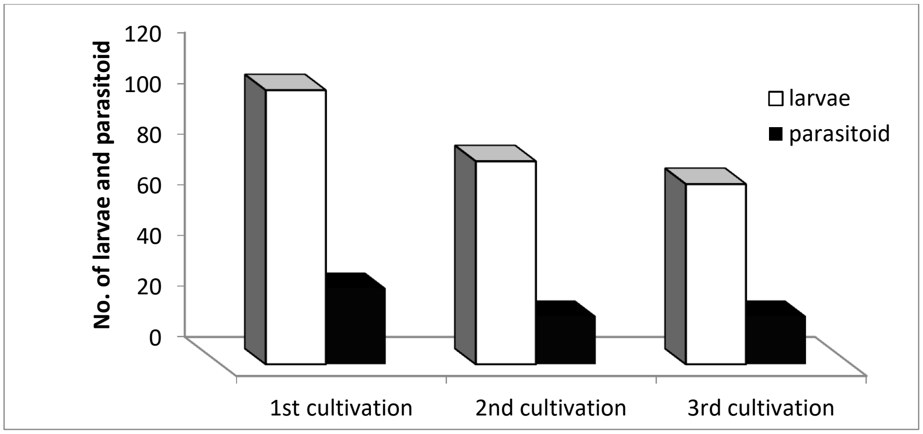

Fig 3: Total Numbers of *S. exigua* larvae and parasitoids during season 2020/2021

Fig. 3 shows seasonal average of parasitism were 21.48, 20.39 and 27.58% for the three cultivations, respectively. Statistical analysis proved that significant differences between the third cultivations and first & second cultivation. (Inanc and Beyarslan 2001) they recorded numerous hymenopterans and dipteran (Chlorpyrifos) parasitoids from *S. exigua*. Average of parasitism ranging between 21–42%. In Germany, (Belokobylskij *et al.,* 2003) reported that *Microplitis fulvicornis* as an important parasitoid on *S. exigua* in China, (Li Jian and Xiaofeng 2004) indicated that several species of the genus *Microplitis forster* are Known as important biocontrol agents such as *Microplitis mediator* (Haliday) and *Microplitis croceipes* (Cresson) on noctuid insects.

Also, (Karimi-Malati *et al.,* 2014) identified the parasitoid, *M. fulvicornis* on *S. exigua* as the first report. The parasitized host larvae did not feed and died within 7-8 days after emerging of the parasitoid larvae. In such concern, Khalifa (2018) indicated that sugar beet fields have enormous parasitoids that should be wisely conserved to keep the insect pests beyond the economic threshold levels.

Table (4) elucidate that highly significant differences were found between *S.exigua* and its parasitoid population. Values of “r” were 0.612**, 0.611**and 0. 621** for the first, second and third cultivations, respectively. During 2019/2020. While value of "r" was 0.622**, 0.613** and 0.631** for the three cultivations, respectively during season 2020/2021. These results demonstrate the close relationship between this insect's and its parasitoid and the results decline in population According to (Demers and Weatherhead 2000), parasitoid are crucial in maintaining the balance of many insect populations. They can occasionally stop the development of their larval hosts, (such as caterpillars), until the parasitoid is fully formed. They are quite selective, only going after a certain stage of life in one or more closely related species. In Costa Rica, (Blanco-Metzler *et al.,* 2009) discovered that the parasitoids play an important role in the reduction of the *G. aurantianum* population. Parasitoids play an important role in the reduction of insects. Also, (Pere *et al.,* 2013) demonstrated those parasitoids play an essential role in regulating Insect populations and preventing pest outbreaks.

Table 4: Correlation coefficient values between the number of *S. exigua* larvae and its parasitoid *C. ruficrus* during 2019/2020 and 2020/2021.

Cultivation	2019/2020	2020/2021
	r	r
First	0.612**	0.622**
Second	0.611**	0.613**
Third	0.621**	0.631**

r = Correlation coefficient *Significant ** high Significant

3.1. Effect of two insecticides on *S. exigua* larvae and its parasitoid:

Data presented in Table (5) showed that mean reduction percentages of *S. exigua* larvae were 51.51 and 88.50% for protecto and Chlorpyrifos, respectively in 2019/2020. In addition, the mean reduction percentages were 52.26 and 92.11% for the two insecticides, respectively in 2020/2021. Data in Table (6) showed that the biocide induced reduction in this parasitoid with 18.60 and 22.67% in the two seasons, respectively. As, the conventional insecticide (Chlorpyrifos) caused reduction in this Parasitoid population with 99.06 and 100% in two seasons, respectively.

Table 5: Reduction percentages of *S. exigua* larvae as affected by insecticides during the cultivation seasons.

Time treatment of insecticides								
2019/2020								
Treatment	**Before Spray**	**After 3 days**		**After 7 days**		**After 10 days**		**Mean of Reduction%**
	Mean	**Mean**	**Red%.**	**Mean**	**Red%.**	**Mean**	**Red%.**	
Protecto	20.00	12.75	38.58	10.50	51.77	8.25	64.18	51.51a
Chlorpyrifos	20.25	3.75	82.15	2.25	89.79	1.50	96.56	88.50b
Control	19.75	20.50	-	21.50	-	27.75	-	-
2020/2021								
Treatment	**Before Spray**	**After 3 days**		**After 7 days**		**After 10 days**		**Mean of Reduction%**
	Mean	**Mean**	**Red%.**	**Mean**	**Red%.**	**Mean**	**Red%.**	
Protecto	18.00	11.50	36.88	9.50	52.95	7.25	66.97	52.26a
Chlorpyrifos	17.25	2.50	86.08	1.25	93.72	0.75	96.53	92.11b
Control	20.50	20.75	-	23.00	-	25.00	-	-

Means with the same letters are not significantly different at 0.05 probability level

Many investigators proved that biocides are efficient in reducing insect pests, and at the same time are safer to parasitoids than conventional insecticides. Several problems of massive applications of hazardous insecticides against pests, attention has been given to other safer compounds, such biocides. Which are one of the most promising alternatives to conventional pesticides, while offer less or minimum harm to the environment and Parasitoids (Jisha *et al.,* 2013). Also, (El-Husseini *et al.,* 2008) concluded that biocides were able to reduce 50 – 60% of the cotton leafworm populations in Egyptian Sugar beet fields.

Table 6: Reduction in *C. ruficrus* population due to certain insecticides application.

Time treatment of insecticides								
Treatment	**Before Spray**	**After 3 days**		**After 7 days**		**After 10 days**		**Mean of Reduction**
	Mean	**Mean**	**Red %.**	**Mean**	**Red %.**	**Mean**	**Red %.**	
2019/2020								
Protecto	7.50	7.25	6.91	6.75	19.31	6.50	29.58	18.60a
Chlorpyrifos	7.25	0.00	100	0.00	100	0.25	97.19	99.06b
Control	6.50	6.75	-	7.25	-	8.00	-	-
2020/2021								
Treatment	**Before Spray**	**After 3 days**		**After 7 days**		**After 10 days**		**Mean of Reduction**
	Mean	**Mean**	**Red.**	**Mean**	**Red.**	**Mean**	**Red.**	
Protecto	4.75	4.25	14.25	4.00	25.50	4.00	28.26	22.67a
Chlorpyrifos	5.25	0.00	100	0.00	100	0.00	100	100b
Control	5.75	6.00	-	6.50	-	6.75	-	-

Means with the same letters are not significantly different at 0.05 probability level

These results indicate that biocide provided an acceptable reduction in *S.exigua* larvae numbers, and at the same time maintain *C.ruficrus* populations in comparison with conventional ones.

Table (7) clarify that insignificant differences between treated plots with protecto and Chlorpyrifos insecticides in root and sugar yield of sugar beet while significant with control. These insignificant differences because biocide protecto killed the larvae in reasonable Percentages. Also, Protecto did not affect the population of parasitoid in high Percentages. Therefore, *C.ruficrus* parasitoid beside biocide protecto were able to suppress *S. exigua* outbreaks. (El-Husseini *et al.,* 2008**)** concluded that biocides were able to reduce 50-60% of the cotton leaf worm populations in Egyptian sugar beet fields.

Table 7: Effect of application of certain biocides and Insecticide against *S. exigua* on root weight, yield, sucrose and sugar yield of sugar beet during two seasons.

Treatment	**Root weight (Kg /168m^2)**		**Root yield**	**Sucrose**	**Sugar yield**
	Total	**Mean**	**(ton /fad.)**	**(%)**	**(ton /fad.)**
			2019/2020		
Protecto	908	227.0a	22.700	17.51	3.974a
Chlorpyrifos	913	228.25a	22.825	17.90	4.085a
Control	500	125.0b	12.500	12.00	1.50b
			2020/2021		
Protecto	910	227.50a	22.750	18.00	4.095a
Chlorpyrifos	921	230.25a	23.025	18.75	4.317a
Control	430	107.50b	10.750	11.14	1.197b

Means with the same letters are not significantly different at 0.05 probability level

4. Conclusion

In this study, the incidence of the biological control of *Spodoptera exigua* (Hubner) were examined at the Experimental Farm of the Sakha Agricultural Research Station during two subsequent seasons 2019/2020 and 2020/2021. it is suggested that *Cotesia ruficrus* based biological control and Biocide (Protecto) can be conservation during the two seasons in the three cultivations. While, conventional insecticide (Chlorpyrifos), reduced it. To knowledge the authors, this is the first report about parasitoid on *S. exigua* and their occurrence in Egyptian sugar beet fields. In addition, *C. ruficrus* and protecto are consider as vital elements depends on wise and justified in the integrated pest management (IPM) Program for evolving effective and efficient strategies of *S. exigua* suppression**.**

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