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Micronutrients' Foliar Fertilization and Releasing Green Lacewing *Chrysoperla Carnea* (Stephens) Could Efficiently Suppress Sugar Beet Insect Pests

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Sugar beet is attacked by many mischievous insect pests. To avoid too much reliance on pesticides, many studies have been conducted to search alternative methods of sugar beet pest control. In the present study, we evaluated whether pant fertilization and biological control could be efficacious techniques to suppress insect pests of sugar beet. In two successive growing seasons of 2015–2016 and 2016–2017, we investigated the impacts of Nutrimix ® complete, a foliar fertilizer with concentrated micronutrients including nitrogen and sulfur, and also release of the common green lacewing *Chrysoperla carnea* larvae, a generalist predator of insect herbivores, on insect pest infestation in the sugar beet fields. Applications of the foliar fertilizer significantly decreased the percentages of plants infested by cotton leaf worms or sugar beet beetles, while the infestations by sugar beet flies and the sugar beet moths were less influenced. Releasing the predator *C. carne*a in sugar beet fields was found to effectively reduce pest populations though the effectiveness depended on the seasons. The plants in lacewing–released plots were attacked by fewer aphids, sugar beet moths, leafhoppers and sugar beet flies than those in control plots. Aphid populations were the most influenced, and the reduction percentages reached 100% in the 2015–2016 season and 97.08% in 2016–2017 after 14 days of release, while the sugar beet flies were the least influenced with 64.84% and 49.88% reduction in the first and second seasons, respectively. Our study demonstrated the usefulness of micronutrient application and *C. carnea* larvae for suppressing insect pest populations in sugar beet fields.

Key words: foliar fertilization, insect pest populations, insect predators, plant nutrition, common green lacewing, natural enemies

INTRODUCTION

Sugar beet, *Beta vulgaris* L., is grown for sugar production and occupies around twenty percent of sugar production in the world (FAO, 2009). In addition, sugar beet has recently been received much attention for its high potential to produce biofuel or ethanol (Maung and Gustafson, 2011). It is also an important crop in Egypt, playing a vital role in the crop rotation system in the Egyptian fields, and the cultivated area in 2017 was 236,732 hectare with total production reached 12,106,661 tons (Anonymous, 2019). The Egyptian government has been encouraging growers to increase sugar beet production because a gap between sugar production and consumption is large.

Numerous insect pests attack sugar beet throughout growth stages and harm directly and indirectly the taproot (Evaristo, 1983), often causing a great yield loss and quality decrease (Bassyouny, 1993). Under Egyptian ecosystems, the main insect pests of sugar beet are: cotton leafworm *Spodoptera littoralis* (Boisd.) and *S. exigua* Hübner, sugar beet fly *Pegomya mixta* Vill., sugar beet beetle *Cassida vittata* Vill., and sugar beet moth *Scrobipalpa ocellatella* Boyd.; these pests are commonly abundant in sugar beet plantations in Egypt throughout plant growth stages (Metwally *et al*., 1987; Abo–Aiana, 1991; Amin *et al*., 2008; Badawy and Shalaby, 2015). Although farmers rely strongly on synthesized chemical pesticides, growing attention has been paid to avoid overuse or misuse of pesticides. Then, insect pest control in sugar beet should be based on integrated pest management (IPM) programs, in which pesticides may be carefully used to avoid pernicious impacts on natural enemies, development of pesticide resistance, and environmental hazardousness (Ueno, 2006; Ueno and Tran, 2015). In this context, applying combinations of other practices, such as use of inter–cropping technique, plant extracts, resistant varieties, natural materials, is advantageous to minimize insect pest overrun and to the sustainable use of biodiversity (Gu *et al*., 2008; Scherr and McNeely, 2008; Ebadollahi and Mahboubi, 2011; Mousa *et al*., 2013; Badawy and Shalaby, 2015; Elsharkawy and Mousa, 2015; Mousa and Ueno, 2019).

Here, we first focus on a foliar fertilizer to evaluate whether it could be incorporated into IPM programs in sugar beet fields. Agricultural crops require macronutrients and micronutrients to grow normally, and the deficiency of such nutrients causes stunted growth and decreased plant resistance to insect herbivores and plant disease (Reddy, 2017). Fertilizers are, hence, applied commonly to improve the nutritional status of the crops by optimizing or maximizing crop growth. Crop growth is associated with nutritional or physiological state of the

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crops, which can affect pest severity. In fact, use of nitrogen fertilizers is known to increase pest severity whereas phosphate or potassium fertilizers may rather decrease pest damage (Lu *et al*., 2007; Reddy, 2017). Also, pest damage may depend on type of fertilizers (Rakshit, 2013). We therefore expect selection of appropriate fertilizers may help reduce pest damage in sugar beet fields.

Second, we focus on the usefulness of biological control in sugar beet fields. Biological control, *i.e*., use of natural enemies, has long been recognized as an effective method in regulating insect pest populations (Wang *et al*., 2001; Bassiony *et al*., 2017; Perez–Alvarez *et al*., 2019) and are useful for sustainable crop production (Bale *et al*., 2008). The common green lacewing *Chrysoperla carnea* (Stephens) is a common and widespread polyphagous predator belonging to the family Chrysopidae (Brooks, 1994; Wang and Nordlund, 1994; Tauber *et al*., 2000). Although the adults feed on nectar and pollen (Villenave *et al*., 2005), the larvae are active predators, preferentially feeding on aphids (Tauber *et al*., 2000), whitefly (Alghamdi *et al*., 2018), thrips (Khan and Mores, 1999), leafhoppers (Daane *et al*., 1996) and even soft–bodied caterpillars (Lopez *et al*., 1976). This predator is commercially available as a bio–control agent against multi pest species (Tauber *et al*., 2000).

No previous studies have examined whether *Chrysoperla carnea* could be effective in controlling sugar beet insect pests though the larvae of this green lacewing had been frequently used in augmentative bio–control programs (Hagley and Miles, 1987; Wang and Nordlund, 1994; Turquet *et al*., 2009). However, this predator is one of the major natural enemies widely found in agricultural fields of Egypt, and is also commonly observed on sugar beet plants. We thus expect that *C. carnea* can be incorporated for IPM programs in sugar beet fields.

Accordingly, the present study was carried out to examine the efficacies of foliar fertilization and *C. carnea* in suppressing the four main sugar beet insect pests. Field studies were therefore designed, and foliar spray of micronutrients and release of green lacewing larvae were made in sugar beet fields. Based on the results, we discuss usefulness of foliar fertilizers and biocontrol agents in sugar beet IPM.

MATERIALS AND METHODS

Micronutrient fertilization

Experimental setup

This experiment was carried out in the two successive growing seasons in 2015–2016 and 2016–2017 at the experimental farm of Sugar Crops Research Institute, Sakha, Kafer El–Sheikh, Egypt. An area of 700 m^2 was measured and wasdivided into two parts, each with three sampling plots. Then, the area was planted with the sugar beet variety Farida as multigerm seeds in mid–September. The area was uniformly fertilized with the recommended values of N–P–K, and no pest control practices, including insecticide applications, were made throughout the growing seasons (except the following experimental treatments).

Chemicals used

To study the influence of foliar fertilization with micro elements on the major sugar beet insect pests, Nutrimix ® complete (Table 1), obtained from Shoura Chemicals Co., Egypt, was sprayed (720 gm/ha) using a manual hand sprayer during the last third of October in both seasons. Experimental plots were treated with Nutrimix while the others for the control were sprayed with water.

Table 1. The chemical composition of the synthesis Nutrimix® complete

Element	Concentration (%)
Zinc	3
Iron	3
Manganese	4
Magnesium	1.8
Copper	3
Molybdenum	0.04
Sulfur	15
Nitrogen	3.5
EDTA	57

Data collection

Five days after application, ten plants from each plot were randomly examined to record damaged plants that were infested by the main insect pests, *i.e.* cotton leaf worm (= beet armyworm) *Spodoptera exigua* HÜbner, sugar beet fly *Pegomya mixta* Vill., sugar beet beetle (= tortoise beetle) *Cassida vittata* Vill., and sugar beet moth *Scrobipalpa ocellatella* Boyd. This sampling procedure was repeated on the weekly basis until harvest. Sugar beet taproot weight (ton/hectare) and sugar content (%) were calculated at the end of the experiment. The percentage of sugar in taproot was estimated with the aid of a refractometer.

Green lacewing releasing

Experiment setup

In order to examine the efficiency of the green lacewing *Chrysoperla carnea*, a field study was carried out in two successive seasons of 2015–2016 and 2016–2017 at two locations. The first location was at Sugar Crops Research Institute, Sakha Agricultural Research Station; this location was used for the releasing of the predator. Another location was at the experimental farm of the Faculty of Agriculture, Kafrelsheikh University and was used as a control area. Both fields were located in the same region with seven km apart from each other. The two study fields were similar in terms of soil structure and the atmospheric conditions. In this study, the variety Halawa was planted in mid–October, and both fields had an area of 700 m^2. In each study field, pest insects were sampled from four plots. No insecticides were applied during the study period.

C. carnea

Larvae of *C. carnea* were obtained from the Green Lacewing Mass Rearing Center, Faculty of Agriculture, Cairo University. In the last week of March, 2800 lacewing larvae in all (a mixture of first, second and third instars) were released in the first field by placing them on the plants using a fine brush.

Data collection

The first sampling was done before the release of the predator. Ten plants from each plot were randomly chosen and inspected directly in the field to count pest insects. Forty plants in all were thus sampled from each study field. Because the green lacewing requires 3 days to develop between 1st, 2nd and 3rd instars, subsequent sampling were made after 3, 7, 10 and 14 days after releasing. The numbers of aphid (nymphal and adult stages), *C. vittata* (larval stage), *S. ocellatella* (larval stage), leaf hoppers (nymphal and adult stages) and *P. mixta* (larval stage) were recorded.

The reduction percentage was calculated according to Henderson and Tilton's equation (1955) as follows:

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

where: Ta = population density in a treated plot after treatment, Tb = population density in a treated plot before treatment, Ca = population in control after treatment, and Cb = population density in control before treatment.

Statistical analyses

The collected data were statistically analyzed using COSTAT software version 6.4. Analyses of variance (ANOVA) were applied to examine significant differences in means, and, then, the means were compared using Tukey's HSD test at a significance level of 0.05.

RESULTS AND DISCUSSION

Micronutrients fertilization

In the current study, we examined the impact of a foliar fertilizer, Nutrimix® complete, on the infestation percentages of four main sugar beet pests, *i.e., S. exigua, P. mixta, C. vittata* and *S. ocellatella*, during the two growing seasons in 2015–2016 and 2016–2017. In the first season, after five days of foliar application with the synthesis micronutrients, the percentages of sugar beet plants infested by *S. exigua* and *C. vittata* were significantly lower in treated plots than in control plots, in the first season of 2015/2016 (F = 13.5; P = 0.021 for *S. exigua* and F = 36.57; P = 0.0038 for *C. vittata*), respectively (Fig. 1). Similar trends were detected for *P. mixta* and *S. ocellatella* though the differences were not significant or marginal (F = 4.5, P = 0.101 for *P. mixta*; F = 7.69; P = 0.050 for *S. ocellatella*) (Fig. 1). It appeared that that the foliar fertilizer most negatively

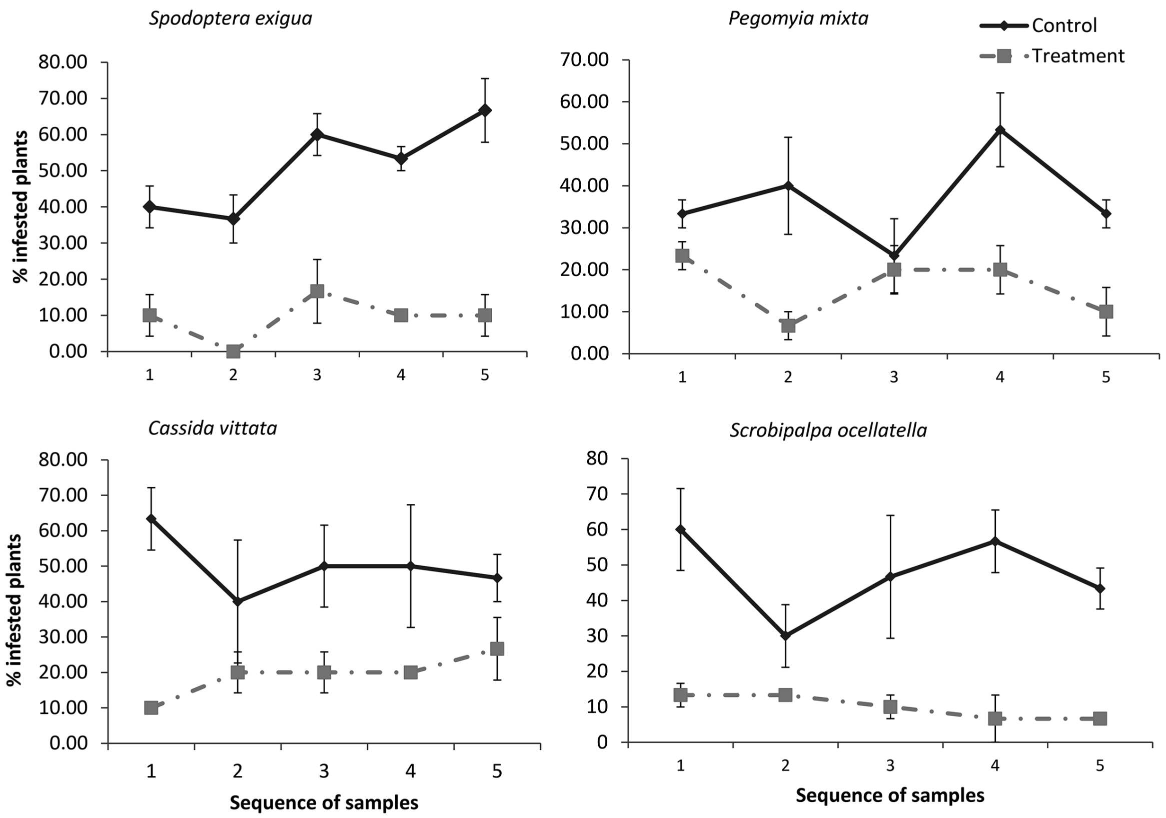

Fig. 1. Mean percentages of infested sugar beet plants treated with Nutrimix® complete or with water (control) in the first season 2015–2016. Sampling was made weekly from 5 days after the fertilizer treatment (from end of October till mid–November). Vertical lines indicate standard errors.

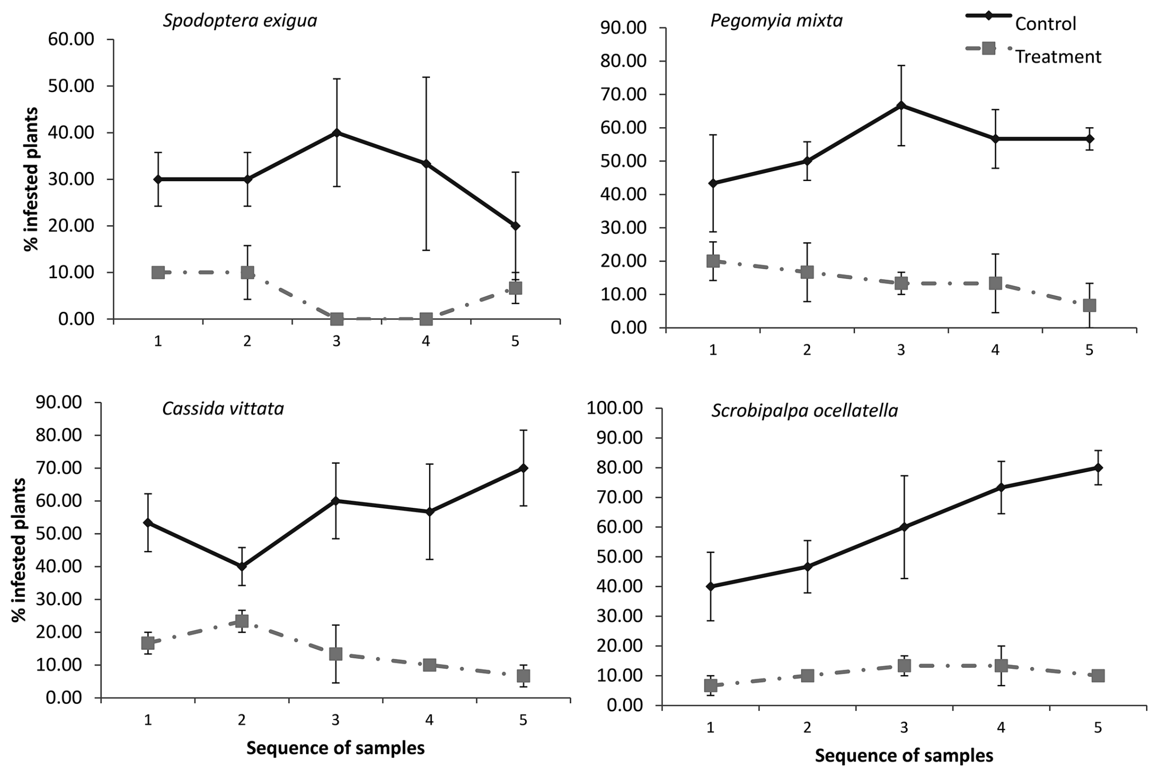

Fig. 2. Mean percentage of infested sugar beet plants treated with Nutrimix® complete or with water (control) in the second season 2016–2017. Sampling was made weekly from 5 days after the fertilizer treatment (from end of October till mid–November).

affected the infestation by *S. exigua* while it had a small, if any, impact on *C. vittata* ($F = 3.53$; $P = 0.039$). However, in the second season, in the first sampling date, *i.e.*, five days after application, *C. vittata* and *S. exigua* were the most influenced among the four pests ($F = 15.12$; $P = 0.017$ and $F = 12$; $P = 0.025$, respectively) (Fig. 2). The percentages of infested plants with the tortoise beetle dropped to 16.67 ± 3.33% in treated plants whereas it was 53.33 ± 8.82% in control; the infestation by beet armyworm was reduced from 30.00 ± 5.77% in untreated plants to 10.00 ± 0.00% in treated plants (Fig. 2).

The application with nutrients helps plants to produce more succulent and fresh leaves, reduce the deficiency symptoms and increase the yield of crops (Çelik *et al.*, 2010), which means that plant quality can change for herbivorous insects after fertilization. It is well documented that the quality of host plants plays an indispensable role in mediating the population dynamics, reproductive performance, growth, and foraging behavior of herbivorous insects (Awmack and Leather, 2002; Lu *et al.*, 2007; Shah, 2017). Some farming practices, *e.g.*, using mineral nutrients, could ensure that plants have appropriate growing conditions though insect damage may not be affected (Baidoo and Mochiah, 2011). In other case, it was recognized that plants receiving high level of nitrogen attracted more insect pests (Ma and Lee, 1996; Lu *et al.*, 2007; Kulagold *et al.*, 2011). Also, applications of micronutrients such as calcium, zinc and sulphur may negatively affect the pest populations (Rouhani and Samih, 2013). In the current study, we used Nutrimix® complete which is a mixture of several micronutrients, *e.g.*, zinc, iron, sulfur, manganese, and showed that foliar spray of this fertilizer could help reduce at least some of major pest species in sugar beet fields. We do not know the mechanism and process of how such a foliar application of micronutrients can lead to a reduction of pest populations. Further detailed studies are thus necessary to examine this mechanism and process. In any case, the foliar spray can be a good option for integrated pest management in sugar beet.

In the present study, we further examined the consequence of micronutrient applications on crop yields. The results evidently showed that the yield of taproots was influenced by the application. In the first season of 2015–2016, the mean taproot weight was 72.10 ± 1.66 ton/ha in treated plots while it was only 50.40 ± 0.98 ton/ha in untreated plots. The difference was highly significant (Fig. 3; $P = 0.0004$). In addition, even greater difference was found in the second season of 2016–2017, and the taproot weight in treated plants was 76.80 ± 1.16 ton/ha while it was 43.20 ± 1.13 ton/ha in untreated plants (Fig. 3; $P < 0.0001$). These results evidently demonstrated a great increase in crop yields with micronutrient applications.

The sugar concentration did not differ between control and treated sugar beet plants, both in the first season ($F = 0.571$; $P = 0.491$) and in the second one ($F = 0.825$; $P = 0.415$) (Fig. 4). In accordance with our results, Yarnia *et al.* (2008) reported that an application

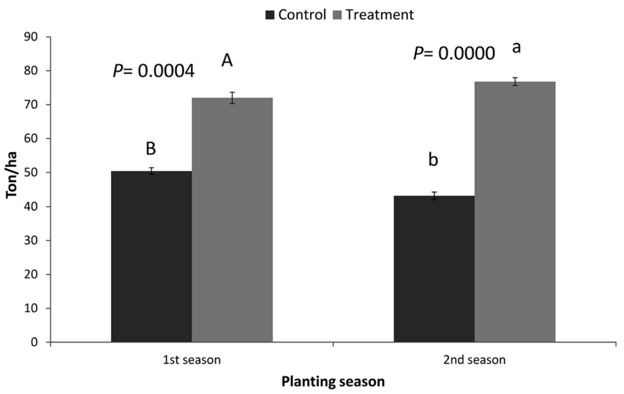

Fig. 3. Mean taproot yields of sugar beet treated with Nutrimix® complete or with water (control) in two successive seasons 2015–2016 and 2016–2017. Vertical lines indicate standard errors. Different letters above bars show a significant difference by the Tukey's HSD test ($P < 0.05$).

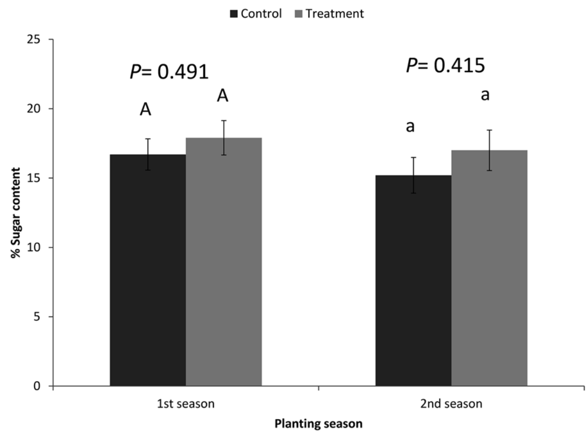

Fig. 4. Mean percentages of sugar content in taproots treated with Nutrimix® complete or with water (control) in two successive seasons 2015–2016 and 2016–2017. Vertical lines indicate standard errors. Significant differences are not detected between the groups with the Tukey's HSD test ($P > 0.05$).

of micronutrients to sugar beet plants resulted in high root yields; however, their study also showed the application caused low sucrose contents, which suggested that sugar beet plant quality was rather reduced. Mousavi *et al*. (2013) indicated that micronutrients such as Zn, Mn and Fe were essential trace elements that could enhance sugar beet growth and yield. However, such micronutrients themselves may not increase sugar production per plant individual, leading to a relative reduction of sugar concentration. In contrast, we used a different micronutrient product, which contains nitrogen, etc., other than Zn, Mn and Fe, and the difference may be the reason why sugar beet quality was not reduced in the present study though more detailed studies are needed to examine which combinations of micronutrients can be effective to balance sugar beet yield and quality.

Green lacewing releasing

Species in the genus *Chrysoperla* have long been considered as important naturally occurring predators in many vegetation systems, and *C. carnea* has been used for controlling a variety of vegetable pests (Brooks, 1994; Wang and Nordlund, 1994; Tauber *et al*., 2000). However, the control efficiency can depend on type of crops, and little information is available about its capability to control insect pests in sugar beet fields. In the present study, we evaluated the role of *C. carne*a larvae in suppressing sugar beet insect pest populations.

Our results showed that, before the releasing of the predator *C. carnea*, each sugar beet plant had a mean of 4.00, 10.75, 8.25, 6.50 and 17.75 individuals of aphid, tortoise beetle, sugar beet moth, leafhopper and sugar beet fly pests, respectively, but after three days of release, the mean numbers of such pests were reduced to 2.25, 9.25, 5.25, 4.25 and 16.00 individuals/plant, respectively. However, such reduction patterns may simply emerge if the pest density decreases as the plant growing stage or the season goes. In our study, therefore, we calculated reduction percentages of pests on the basis of the comparison with control plants in each sampling date (see the Materials and Methods). The analyses demonstrated that the pest densities decreased significantly on sugar beet plants on which green lacewing larvae had been released (Table 2). It was noticeable that, in the first season of 2015–2016, the population of aphids (F = 7.569; P = 0.0042), sugar beet moths (F = 15.121; P = 0.0002), leafhoppers (F = 7.988; P = 0.0034) and sugar beet flies (F = 3.706; P = 0.0426) decreased significantly with the time passage, while the tortoise beetle did not (Table 2). However, in the second season of 2016–2017, only aphid populations were reduced with the passage of time (F = 8.794; P = 0.002), and the other pests were not. On the other hand, the reduction percentages did not significantly differ among insect pests within the same sampling date, *i.e.* 3rd, 7th and 10th. However, on the last sampling date, *i.e.* 14th day, aphid populations reached the maximum reduction of 100.00 ± 0.00% and 97.08 ± 2.92% in the first and second seasons, respectively, while the reduction percentages of sugar beet flies were significantly less (Table 2).

The present results suggest that *C. carnea* larvae were an effective predator to reduce major pest populations in sugar beet fields. Lacewing predators are shown to be most effective when the larvae are released to control the target pests (Tauber *et al*., 2000). Our study is hence in accordance with previous studies. From the present results, it seemed that the lacewing larvae needed a period of time after being released so as to show significant influences on pest populations. Solangi *et al*. (2013) reported that young instar larvae of *C. carnea* was much less voracious against the target pest species than the final 3rd instars. In the present study, we released mixed staged larvae of lacewing predators, in which young instars, 1st and 2nd instars, have been included. We suspect that this can be an explanation for

Table 2. Reduction percentages (%) of selected sugar beet insect pests after releasing of *C. carnea*

Insect pests	Days after releasing									
	2015–16 season					2016–17 season				
	3 days	7 days	10 days	14 days	P–value	3 days	7 days	10 days	14 days	P–value
Aphids	48.45± 11.88Ab	82.83± 11.80Aab	95.72± 2.87Aa	100.00± 0.00Aa	0.0042	64.09± 5.33Ac	74.72± 6.17Abc	90.85± 5.29Aab	97.08± 2.92Aa	0.002
Tortoise Beetle	21.81± 19.24Aa	54.85± 14.21Aa	58.77± 23.72Aa	89.70± 5.95ABa	0.097	16.24± 27.82Aa	32.54± 17.68Aa	61.95± 23.33Aa	75.56± 9.88ABa	0.221
Beet moth	55.99± 2.62Ab	80.30± 3.51Aa	84.28± 3.19Aa	83.82± 4.36ABa	0.0002	37.97± 14.16Aa	63.47± 15.97Aa	70.38± 13.40Aa	80.97± 7.12ABa	0.175
Leafhoppers	33.07± 12.77Ab	65.53± 13.58Aab	87.71± 2.72Aa	91.88± 2.86ABa	0.0034	40.13± 23.73Aa	45.78± 21.51Aa	64.23± 18.84Aa	78.16± 10.22ABa	0.5088
Beet fly	23.73± 9.91Ab	40.55± 17.46Aab	76.91± 6.13Aa	64.84± 13.28Bab	0.0426	29.58± 13.96Aa	35.32± 16.73Aa	42.86± 16.13Aa	49.88± 13.11Ba	0.793
P–value	0.262	0.170	0.235	0.028		0.503	0.338	0.396	0.038	

Means ± SD are shown. Means followed by the same capital letters in a column and lower case letters in a row do not differ significantly by the Tukey's HSD test ($P < 0.05$).

the time lag between the predator release and pest reduction in our study.

AUTHORS' CONTRIBUTIONS

In the present study, H. M. Khattab conducted the field experiments. A. E. Youssef, A. S. Ibrahim, and K G. Bazazo designed the field study, prepared the first draft of the manuscript and molding the research concept. T. Ueno participated in manuscript preparation, discussed the results, and polished up the research concept and manuscript. K. M. Mousa analyzed the data, wrote the manuscript and participated in discussion.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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