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Bio-efficacy of eco-friendly insecticides against *Spodoptera littoralis* (Lepidoptera: Noctuidae) in sugar beetroot ecosystem

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Abstract

The field study conducted at Sakha Agricultural Research Station over two consecutive sugar beet growing seasons (2021-2022) aimed to assess the effectiveness of three eco-friendly insecticides in controlling *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae) infestations while considering their impact on associated predator populations. The tested insecticides included Abhold® 36% SC (Spinetoram 6% + Methoxyfenozide 30%), Robek® WP 50% (Acetamiprid 22.7% + Bifenthrin 27.3%), and Pyridalyl 50% EC. Results revealed significant efficacy of all three insecticides in reducing *S. littoralis* larval populations, with Abhold®) demonstrating sustained efficacy over time causing an overall reduction of 93.05 and 97.34% during the 1st and 2nd seasons. However, Robek®) and Pleo®) treatments led to rapid reductions in associated predator populations, while Abhold®) maintained a more balanced approach, preserving predator populations. These findings highlight the importance of considering not only pest control efficacy but also the potential impacts on predator populations for long-term ecosystem stability in agricultural settings. Further research is warranted to comprehensively assess the ecological implications of these insecticides.

Introduction

Sugar beetroot plays a crucial role in the sugar industry, agriculture, and various related sectors, contributing to economic growth, food production, and sustainable farming practices (El-Fergani, 2019). The cotton leafworm *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae) is recognized as a highly destructive pest that infests sugar beet crops at all growth stages, from seedling to harvest, resulting in significant and substantial yield losses (Fergani *et al.*, 2023). The maintenance of sustainable sugar beet cultivation and, consequently, sugar production can be achieved by effectively implementing a suitable pest management plan for sugar beet (Tomlin, 2000).

The extensive utilization of traditional insecticides for chemical control has been employed to control *S. littoralis*, leading to the development of resistance and causing environmental contamination (USDA, 2022). Alternative substances that exhibit efficacy against this pest while being safe for humans, environmentally sustainable, and compliant with appropriate integrated pest management (IPM) practices (Korrat *et al*., 2012). Utilizing a combination of novel chemical insecticides presents the most effective approach for managing insect pest populations that have developed resistance (Attique *et al*., 2006).

A combination of different groups of insecticides has the potential to enhance the toxicity and achieve more effective control of insect pests in both laboratory and field settings. Mixing insecticides could offer interesting opportunities for pest control, especially if there are interactions that enhance their effectiveness when combined (All *et al*., 1977 and Yu, 2008) and provide superior control of insect pests in both laboratory and field settings (Bhatti *et al*., 2013). Ahmad (2004) suggests that mixing pesticides with varying modes of action could potentially slow down the development of resistance in pest populations. This is because the resistance mechanisms needed for each pesticide in the combination might not be prevalent or present across insect populations.

The sugar beet pest ecosystem is accompanied by a range of predatory species including the Hymenoptera (Formicidae), and Neuroptera (Chrysopidae) (Stelzl and Devetak, 1999). Green lacewings, such as *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae), serve as significant generalist predators against a wide range of insect pests, including *S. littoralis*, owing to their polyphagous feeding habits (Hegazy, 2018).

The key to maintaining the stability of predator populations in field crops, ensuring their self-sustaining and sustainable presence, lies in coordinating the predator population with the pest population. So, it is essential to minimize the adverse effects of insecticides on non-target natural enemy species. Therefore, optimizing the dosage of application to reduce the pest population below the economic injury threshold while preserving the natural enemy (Bažok *et al*., 2016).

In light of these considerations, this study seeks to evaluate the effectiveness of Spinetoram6% + Methoxyfenozide30% (Abhold®), in combination with other eco-friendly insecticides Acetamiprid22.7% + Bifenthrin27.3% ((Robek ®) and Pyridalyl (Pleo®), in controlling *S. littoralis* infestations in sugar beet fields while ensuring the preservation of associated predator populations. Such investigations hold promise for advancing sustainable pest management practices and safeguarding the resilience of agricultural ecosystems.

Materials and methods

1. Tested insecticides:

1.1. Spinetoram6% + Methoxyfenozide30%: (Abhold® 36% SC), ecdysone agonist, applied at the rate of 125 cm / Feddan.

1.2. Acetamiprid22.7% + Bifenthrin27.3%: (Robek ® WP 50%) application rate of 25 gm /100L.

1.3. Pyridalyl: (Pleo® 50% EC) was produced by Sumitomo Chemical Co., with an application rate of 50 cm/100L.

2. Field studies:

This study was conducted over the course of two consecutive planting seasons, especially in 2021 and 2022, at the Sakha Agricultural Research Station, Kafr El-Sheikh Governorate, Egypt. Using a fully randomized block design for the two seasons, the local sugar beet variety known as (BLKIS) was planted on 15th August in an experimental area measuring roughly 168 m^2. Each of the equal-sized plots that made up the entire trial area was 42 m^2. Each treatment was assigned to four plots in the treated and untreated areas.

Between each plot, two plant rows were left unsprayed to assess the tested insecticides' impact on the related predators. Just before September 15th, the tested compounds were applied, once a season. The recommended dose of all insecticides was made as an aqueous solution. Using a motorized 20-liter backpack sprayer, the treatments were applied. Standard agricultural procedures with recommended field rates for all tested insecticides were adhered to Agricultural Pesticide Committee (http://www.apc.gov.eg/ar/APCReleases.aspx) . Water was the only ingredient used in the control group treatment.

The applied dose was the same for each replicate/ season. Ten plants/plots/ treatments were randomly chosen for insect sampling a few hours before the first application, as well as three, seven, and ten days after the application of the ecdysone agonist (Abhold®), Similarly, one, seven, and ten days after the application of the conventional insecticides (Robek® and Pleo®). The percentage of reduction in the larval population density of *S. littoralis* was calculated. Before the treatment as well as three, seven, and ten days later, the population densities of the associated predators, *C. Carnae* (Eggs and larvae), and formicide (Adults), were also assessed.

3. Statistical analysis:

Infestation reduction percentages for each treatment in both seasons were calculated using the formula outlined by Henderson and Tilton (1955).

$$\textbf{Reduction } \% = \left\{ \mathbf{1} - \frac{n\ in\ Co\ before\ treatment\ x\ n\ in\ T\ after\ treatment}{n\ in\ Co\ after\ treatment\ x\ n\ in\ T\ before\ treatment} \right\} \times \mathbf{100.}$$

n: Insect population, C: control, T: treated.

Insect population data were statistically compared using statistically significant differences determined by one-way analysis of variance (ANOVA) (SPSS, 2004).

Results and discussion

The effectiveness of Abhold® 36% SC Spinetoram6% + Methoxyfenozide30%, ecdysone agonist, Robek ® WP 50% (Acetamiprid22.7% + Bifenthrin27.3%), and

Following three and seven days of treatment, a significant rise in the percentage of pest reduction was observed ($P \leq 0.05$), compared to the untreated area, ranging from 96.11%to 97.32 %, respectively. A significant residual impact was observed reaching 97.32 % of pest infestation for up to 10 days following treatment. In the second growing season (2022), the natural infestation pattern in the untreated areas remained statistically indistinguishable from that observed during the initial growing season (2021) throughout the duration of the study. A high initial reduction was inferred as Abhold®) caused a 100% reduction in pest infestation.

A gradual decrease in population reduction was achieved reaching 94.50 % and 97.52 % seven and ten days post-treatment, Pleo® (50% EC) in controlling early infestations of *S. littoralis* in sugar beet fields was investigated across two consecutive seasons (2021–2022). At the same time, the ability of key insect predators, including *C. carnea* (Eggs and adult), and Formicidae ants, to withstand the treatments was indirectly assessed by monitoring their population densities throughout the experiment. Population densities of both *S. littoralis* larvae and the predators were measured before and after treatments, and the resulting percentage reduction in population was determined in (Tables 1, 2, 3, 4, and 5).

1. Effect of tested insecticides on the incidence of *Spodoptera littoralis* infestation:

The daily decline rate of *S. littoralis* larval population density was evaluated in sugar beet fields after the application of tested insecticides during the 2021 growing season. Before any treatment, the larval population exhibited a natural infestation pattern. Just a day after treatment, the recommended dose of Robic® and Pleo® proved highly effective against *S. littoralis* larvae compared to the control group, leading to a 95.58 % and 97.89 %, respectively reduction in their numbers. Furthermore, the Abhold®) treatment showed a pest population reduction of 90% three days after treatment, indicating a strong efficacy.

respectively. On the other hand, Robic® and Pleo® reduced the *S. littoralis* larval population by more than 97% before ten days post-treatment. Compared to the 2022 season, the overall reduction in *S. littoralis* larvae was higher than what was observed in 2021. The total reduction of the larval population after treatment with Abhold®), Robic®), and Pleo®) was 93.05%, 95.58%, and 96.92%, respectively, in the initial season (2021). However, the reduction in pest population increased to 97.34%, 96.88%, and 97.51%, respectively in the second season (2022).

The results indicate that all three tested insecticides - Abhold® 36% SC (Spinetoram 6% + Methoxyfenozide 30%), Robek® WP 50% (Acetamiprid 22.7% + Bifenthrin 27.3%), and Pyridalyl 50% EC - exhibited significant efficacy in controlling S. *littoralis* larvae populations. Shortly after treatment, Robek®) and Pleo®) achieved substantial reductions of 95.58% and 97.89%, respectively, in larval numbers, highlighting their rapid action. Abhold also demonstrated strong efficacy with a 90% reduction in three days post-treatment, which further increased to 97.32% by the seventh day.

These results were in coincidence with Yeligar *et al*. (2020) who studied the bio-efficacy of different concentrations of Spinetoram 6% w/v (5.66%w/w) + Methoxyfenozide 30% w/v (28.3%w/w) SC in comparison with other insecticides for the management of S. *littoralis* in rice. These results suggest that all three insecticides effectively suppressed *S. littoralis* infestations, with Abhold®) exhibiting a residual impact lasting up to ten days post-application.

In the second growing season (2022), the efficacy of the insecticides remained consistent, with Abhold®), Robek®), and Pleo®) achieving high reductions in *S. littoralis* larval populations. Notably, the overall reduction in *S. littoralis* larvae was even higher in the second season compared to the first, indicating sustained efficacy over time.

In our research, it was found that bifenthrin demonstrated the highest effectiveness when combined with Acetamiprid insecticides. Our findings align with those of previous studies by Attique *et al*. (2006), who mentioned that emamectin benzoate exhibited a synergistic effect when combined with bifenthrin for application against *Plutella xylostella*. An antagonistic effect was noted between chlorfluazuron, spinoteram, and fenpropathrin insecticides against *S. litura* (Ramzan *et al*., 2021) while our results proved effective in controlling *S. littoralis*. This antagonistic effect may result from the high doses of insecticides that counteract each other's effects.

2. Reduction in the population of the associated predators:

Investigating the influence of the tested insecticides on the population of pest-associated predators in sugar beet fields is a crucial aspect of assessing insecticide safety for non-target organisms. Concerning the treatment's effect on the population of associated arthropod predators (*C. carnea* and formicide ants), the actual numbers of predators were documented during the 2021 season (Table 3) and the 2022 season (Table 4).

Unexpectedly, in the initial season (2021), a total reduction of *C. carnea* and Formicidae ants' population density was observed just one day after treatment, suggesting an adverse effect of the recommended dose of Robic®) and Pleo®) on these predators. However, before the tenth day post-treatment, only 3% of the *C. carnea* and formicide ants population managed to recover from the toxic effects of the insecticide. On the contrary, using Abhold®) decreased the predator population without eliminating them. However, it maintained enough predators to ensure they could continue playing a role in controlling pest populations sustainably.

Within just three days of treatment during the first season, nearly all the predators were wiped out, which is seen as a drawback compared to using Robic®) and Pleo®). The following season (2022), a similar significant reduction was recorded, with Robic®) and Pleo®) treatment resulting in a complete reduction of *C. carnea* and formicid ants. Furthermore, treatment with Abhold®) aligned with the earlier hypothesis by moderately affecting predator numbers, thus preserving a consistent population in a dynamically stable pattern.

Regarding the general reduction in predator population, the population of formicide ants decreased by 41.67% and 41.67% when treated with Abhold®) during the initial and subsequent seasons, respectively (Tables 3 and 4). In contrast a reduction of its population by 98.07% and 98.26% in the case of Robic®) and 98.8% and 100% in the case of Pleo®) treatment during the first and second seasons, respectively. The population of *C. carnae* (Eggs, larvae) decreased by 48.21%and 36.74% when treated with Abhold®) during the initial and following seasons, respectively (Table 5, 6). On the contrary, a reduction of its population by 99% and 98.72% in the case of Robic®). *C. carnae* (Eggs, larvae) showed the highest susceptibility to the recommended dose of Pleo®), with their population being eliminated, reaching 100% reduction.

The study also investigated the effects of the insecticides on the population densities of *C. carnea* and formicide ants, important predators in sugar beet fields. Unexpectedly, the recommended doses of Robek®) and Pleo®) resulted in a rapid and significant reduction in predator populations within one day of treatment in the initial season (2021). However, Abhold®) treatment led to a gradual decrease in predator populations without complete elimination, suggesting a more sustainable approach.

In the second season (2022), similar reductions in predator populations were observed with Robek®) and Pleo®) treatments, while Abhold®) maintained a consistent population of predators. This suggests that Abhold®) may have a less severe impact on predator populations compared to Robek®) and Pleo®). Our obtained results were in line with Shah *et al*. (2015) and Schneider (2004) who proved that Methoxyfenozide is a compound known for its eco-friendly properties, causing fewer harmful effects on mammals, birds, fish, and natural enemies.

Also, Srinivasan and Shanthi (2021) applied a mixture of Spinetoram 6% w/v and Methoxyfenozide 30% w/v SC at rates of 126, 135, and 144 g a.i/ha and found no lethality to the coccinellid population in green gram when used for controlling pod borers. Methoxyfenozide is categorized as a diacylhydrazine compound, primarily employed for managing a range of insect pests, particularly lepidopteran insects and associated predators. Its mode of action involves functioning as an ecdysone agonist, which interferes with the insect molting process and results in the demise or disruption of growth and development of the targeted pests while causing minimal harm to non-target organisms.

Rahaman and Stout (2019) studied the adverse effects of insecticides that led to a decrease in the populations of various predators, including ladybird beetles, wolf spiders, carabid beetles, earwigs, green mirid bugs, and damselflies. Additionally, the numbers of adult egg parasitoids, including *Trichogramma* sp., *Telenomus* sp., and *Tetrastichus* sp., were significantly lower in plots treated with insecticides compared to untreated control plots.

Overall, the study demonstrates that mixing insecticides could offer interesting opportunities for pest control, especially if there are synergistic interactions among them. The efficacy of Abhold, Robek, and Pleo in controlling *S. littoralis* infestations in sugar beet fields. While all three insecticides effectively suppressed pest populations, Abhold showed a more balanced approach by maintaining predator populations, potentially promoting long-term ecosystem stability. However, further research is needed to understand the ecological implications of these insecticides comprehensively.

Table (1): Reduction percentage of *Spodoptera littoralis* larvae in sugar beet field after treatment with the tested insecticides during the (2021) season.

Treatment	**Before treatment**	**Days after treatments**								**Total reduction**
	Mean ±SE*	**1 DAY**		**3 DAYS**		**7 DAYS**		**10 DAYS**		
		Mean ±SE	**Reduction%**	**Mean ±SE**	**Reduction%**	**Mean ±SE**	**Reduction%**	**Mean ±SE**	**Reduction%**	
Abhold	**20.75±1.25**			**3 ±0.81**	**90%**	**1.25±0.95**	**96.11%**	**1**	**97.32 %**	**93.05%**
Robic	**20.5±1.91**	**.025±0.5**	**95.58 %**			**1.5±1.29**	**95.27 %**	**2±0.81**	**94.57 %**	**95.58%**
Pleo	**21 ±2.58**	**0.5 ±0.57**	**97.89 %**			**1**	**96.92 %**	**1.5±0.57**	**96.03 %**	**96.92%**
Untreated area	**21 ±2.70**	**23.75±2.62**		**28.25±1.25**		**32.5±1.25**		**37.75±2.06**		

In a column, means followed by the same letters are non-significantly different, P≥0.05.

Table (2): Reduction percentage of *Spodoptera littoralis* larvae in sugar beet field after treatment with tested insecticides during the (2022) season.

Treatment	**Before treatment**	**Days after treatments**								**Total reduction**
	Mean ±SE*	**1 DAY**		**3 DAYS**		**7 DAYS**		**10 DAYS**		
		Mean ±SE	**Reduction %**	**Mean ±SE**	**Reduction %**	**Mean ±SE**	**Reduction %**	**Mean ±SE**	**Reduction %**	
Abhold	**22.5±1.73**			**2.5± 1.29**	**100**	**1.5 ±1**	**94.50 %**	**0.75±0.5**	**97.52 %**	**97.34%**
Robic	**22.5±2.64**	**1**	**95.88 %**			**0.75±0.95**	**97.25 %**	**0.75±0.95**	**97.52 %**	**96.88%**
Pleo	**21.5±1.73**	**0.5**	**97.89 %**			**0.75±0.95**	**97.25 %**	**0.75±0.95**	**97.41 %**	**97.51%**
Untreated area	**22.5±1.91**	**24.25±1.70**		**27.25±1.25**		**27.25±1.25**		**30.25±1.5**		

In a column, means followed by the same letters are non-significantly different, P≥0.05.

Table (3): Reduction percentage of formicid in sugar beet field after treatment with the tested insecticides during the (2021) season.

Treatment	Before treatment	Days after treatments								Total reduction
	Mean ±SE*	1 DAY		3 DAYS		7 DAYS		10 DAYS		
		Mean ±SE	Reduction %	Mean ±SE	Reduction %	Mean ±SE	Reduction %	Mean ±SE	Reduction %	
Abhold	**9±0.81**			**8±0.81**	**28.89 %**	**7.25±0.5**	**39.92^{ab}%**	**8.75±0.5**	**35.19^{a} %**	**34.66^{ab}%**
Robic	**9±0.5**	**0**	**100%**			**0.25±0.5**	**97.93^{a} %**	**0.5±0.57**	**96.30^{ab} %**	**98.07^{a}%**
Pleo	**9.25±1.7**	**0**	**100%**			**0**	**100^{b}%**	**0.5±1**	**40^{b} %**	**98.8^{b}%**
Untreated area	**11±0.81**	**12.25±0.95**		**13.75±1.25**		**14.75±1.5**		**16.5±2.88**		

In a column, means followed by the same letters are non-significantly different, P≥0.05

Table (4): Reduction percentage of formicid in sugar beet field after treatment with the tested insecticides during the (2022) season.

Treatment	Before treatment	Days after treatments								Total reduction
	Mean ±SE*	1 DAY		3 DAYS		7 DAYS		10 days		
		Mean ±SE	Reduction %	Mean ±SE	Reduction %	Mean ±SE	Reduction%	Mean ±SE	Reduction %	
Abhold	**8.25±0.5**			**7.25±0.95**	**38.09 %**	**8±0.81**	**44.33^{ab} %**	**8.25±0.95**	**42.59 ^{ab}%**	**41.67 ^{ab}%**
Robic	**8.25±2.06**	**0**	**100%**			**0.25±0.5**	**98.26^{a} %**	**0.5±1**	**96.52 ^{a}%**	**98.26 ^{a}%**
Pleo	**8±1.41**	**0**	**100%**			**0**	**100^{b}%**	**0**	**100 ^{b}%**	**100 ^{b}%**
Untreated area	**7.75±1.5**	**9.25±1.5**		**11±1.41**		**13.5±1.91**		**13.5±1.91**		

In a column, means followed by the same letters are non-significantly different, P≥0.05.

Table (5): Reduction percentage of *Chrysoperla carnea* (Eggs, larvae) in sugar beet field after treatment with the tested insecticides during the (2021) season.

Treatment	Before treatment	Days after treatments								Total reduction
		1 DAY		3 DAYS		7 DAYS		10 DAYS		
	Mean ±SE*	Mean ±SE	Reduction %	Mean ±SE	Reduction %	Mean ±SE	Reduction %	Mean ±SE	Reduction %	
Abhold	**4.25±0.5**			**3.75±0.5**	**40.00 %**	**4±0.81**	**48.39^{ab}%**	**3.5±0.75**	**56.25 %**	**48.21^{ab}%**
Robic	**4.75±1.5**	**0**	**100%**			**0**	**100^{a}%**	**0.25±0.5**	**97.20 %**	**99^{a}%**
Pleo	**4.25±1.5**	**0**	**100%**			**0**	**100^{b}%**	**0**	**100%**	**100^{b}%**
Untreated area	**4.25±0.95**	**5.25±0.95**		**6.25±0.95**		**7.75±0.5**		**8±0.95**		

In a column, means followed by the same letters are non-significantly different, P≥0.05.

Table (6): Reduction percentage of *Chrysoperla carnea* (Eggs, larvae) in sugar beet field after treatment with the tested insecticides during the (2022) season.

Treatment	Before treatment	Days after treatments								Total reduction
		1 DAY		3 DAYS		7 DAYS		10 DAYS		
	Mean ±SE*	Mean ±SE	Reduction%	Mean ±SE	Reduction%	Mean ±SE	Reduction%	Mean ±SE	Reduction%	
Abhold	**3.75±0.95**			**3.5±1.29**	**30.00 %**	**3.25±1.5**	**43.48^{ab}%**	**3.5±0.57**	**48.15^{ab}%**	**36.74^{ab}%**
Robic	**4.25±0.5**	**0**	**100%**			**0.25±0.5**	**96.16^{a}%**	**0**	**100^{a}%**	**98.72^{a}%**
Pleo	**4.5±1.29**	**0**	**100%**			**0**	**100^{b}%**	**0**	**100^{b}%**	**100^{b}%**
Untreated area	**3.75±0.5**	**4.50±0.57**		**5±1.15**		**5.75±0.95**		**6.75±0.5**		

In a column, means followed by the same letters are non-significantly different, P≥0.05.

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