COULD CHITIN SYNTHESIS INHIBITORS BE USED TO ENHANCE THE PERFORMANCE OF SYNTHETIC PYRETHROIDS AGAINST COTTON BOLLWORMS?

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Abstract

Pink and spiny bollworms are key pests of cotton in Egypt and are responsible about the greatest loss in cotton yield. In this study, alpha-cypermethrin (25gm AI/Feddan (4200m^2) and its mixture with flufenoxuron (17.5 + 7.5 gm AI/Feddan) were evaluated against the spiny bollworm, *Earias insulana* (Boisduval). Pink bollworm, *Pectinophora gossypiella* (Saunders) was not observed in cotton field during the period of this study. Tested chemicals were sprayed on July, 9 and repeated twice (July, 30 and August, 20). Three weeks after the third spray (September, 10), spiny bollworm infestation significantly reduced in the treatments of alpha-cypermethrin (0.0%) and its combination with flufenoxuron (2.0%) compared to the untreated check (33.0%). Combining flufenoxuron with alpha-cypermethrin in one mixture did not significantly affect the pyrethroid performance against pest population. Mean number of larvae counted in 100 green bolls on September, 10 were 1.0% or less in chemical treatments compared to 18.0% in the control. On September, 10; predator's population decreased in all treatments including the control. However, the peak of predator's activity in the control treatment was in the first half of August (350-363 individuals/25 plant). During this period, number of predators in alpha-cypermethrin treatment averaged 206-319 individuals/25 plant compared to 106-188 when alpha-cypermethrin was combined with flufenoxuron. Percentages of reduction in the general average of spiny bollworm and associated predator populations confirmed that alpha-cypermethrin alone or in its combination with flufenoxuron offered similar performance against the larval population of spiny bollworm. However, its negative impact on the predators was significantly increased when it was combined with flufenoxuron. Seed cotton yield averaged 7.65kentar/Feddan (157kg/4200m^2) for alpha-cypermethrin alone, 7.187 for alpha-cypermethrin/flufenoxuron mixture compared to 3.427 for the untreated check. The yield of seed cotton was insignificantly increased in the treatments of alpha-cypermethrin compared to that in the mixture treatment. Our study revealed that using the mixtures of IGR's and synthetic pyrethroids for the purpose of controlling bollworms is not valuable. The mixtures could be used when infestation of both cotton leaf worm and cotton bollworms are recorded.

Introduction

Cotton as "King of fibers" considered the leading commercial crop in many countries. Production of seed cotton is inflicted by insect pests (Anon, 1981 in India) particularly the last season infestation with bollworms (Nimbalkar, 2009 in India). Pink bollworm, *Pectinophora gossypiella* (Saunders) and spiny bollworm, *Earias insulana* (Boisduval) are major pests of cotton in Arab Republic of Egypt and both are responsible about the greatest loss in seed cotton yield. Egyptian farmers was named cotton "White gold"; however, cultivated areas with cotton are dramatically decreased, probably because of the high cost of pest control and harvest (hand picking); in addition, the low price of seed cotton yield that offered by the Egyptian Ministry of Agriculture. In 2009 cotton growing season, the price of seed cotton yield goes down to be 535 LE /Kentar (~$100/157kg seed cotton). In addition, Egyptian Ministry of agriculture encourages farmers to cultivate wheat; and the harvest of wheat is during the period from May to June, which does not match the optimum time for cultivating cotton.

The economic production of cotton is almost impossible to achieve without the chemical control of cotton pests particularly bollworms; in addition, cost of organic production of cotton is ~ 37% higher than that of conventional production using insecticides (Swezey *et al*., 2007). Egypt does not cultivate BT cotton because cotton plants are attacked not only by lepidopterous pests, but also by many other pests from different orders; in addition, it is possible for lepidopterous pests to develop resistance to the protein complex of BT (Liu *et al*., 1999; Bates *et al*., 2005 and Héma *et al.,* 2009).

For spiny and pink bollworms, larvae develop inside the green bolls and pesticides with contact properties must be carefully selected to affect egg and adult stages with minimum side effect on beneficial arthropods. Predators are more important than parasites for managing the population of bollworms (Stam and Elmosa, 1990, Syrian). In

Egypt, synthetic pyrethroids are commonly used against those two species and other key pests from the point view of their strong lipophilicity that makes them more effective as ovicides and adulticides (Ibrahim and Younis, 1990, Egypt); Khanzada, 2002, Pakistan; Younis, 2007, Egypt). However, because of the strong contact action, they may adversely affect natural enemies (Younis, 2007).

Insect growth regulators (IGR's) have received a great deal of attention because of their qualitative selectivity targeting biochemical and physiological systems are not available in vertebrates, as so-called "Third-generation insecticides" (Williams, 1968 & 1976). The IGR's include a group of insecticides named "Benzoyl Phenyl Urea's" (BPU's) or Antimoulting compounds which disrupt the development of insects via the inhibition of chitin biosynthesis in the new cuticle. Chitin synthesis inhibitors are known to be highly effective against many agricultural pests with a relatively low toxicity to mammals and natural enemies (Ishaaya, 1990).

Combinations of chitin inhibitors with conventional insecticides are typically used to broaden the spectrum of insect control or enhance the level of control of any given species through additive effect. Certain rare combinations surprisingly give a greater-than-additive or synergistic effect (Claus *et al.*, 2008). The mixtures of conventional insecticides and chitin synthesis inhibitors are recommended to use in the first spray of cotton bollworms control program to kill the larvae of cotton leaf worm. In 2009 cotton season, the experimental program by the Egyptian Ministry of Agriculture included a mixture of alpha-cypermethrin with flufenoxuron to be evaluated against cotton bollworms. In this study we try to clarify if IGR's when admixed with synthetic pyrethroids could have any additive effect against bollworms?

Materials and Methods

Chemicals tested:
A synthetic pyrethroid, alpha-cypermethrin and its mixture with a benzoyl phenyl urea (BPU), flufenoxuron were used in this study (see Table 1).

Pests and predators:
Tested chemicals were evaluated against the spiny bollworm, *Earias insulana*. Pink bollworm, *Pectinophora gossypiella* was not included in this study because it was not observed in cotton field during the period of bolls inspection (From July, 9 to September, 10). The natural enemies that recorded in cotton field are common green lacewing, *Chrysoperla carnea* Stephens; paper wasp, *Polistes gallicus* L; oriental hornet, *Vespa orientalis* L; the potter wasp, *Eumenus maxillosa* (De Geer); predatory bug, *Orius albidipennis* Reuter; eleven spot ladybird, *Coccinella undecimpunctata* Linnaeus and lady beetle, *Scymnus syriacus* Marseul.

Testing procedure:
The experiment was conducted during 2009 cotton growing season at the experimental Farm of the Faculty of Agriculture, Minia University-Egypt. An area of about 1.0 Feddan ($4200m^2$) was cultivated with the popular cotton hybrid, *Gossypium barbadens* (Giza 80) in April, 1. Some missing plants were re-sown on April, 20. All recommended agronomic practices were followed during the experimentation for proper crop management. The design of the experiment was laid out in Randomized Block Design (RBD) replicated four times with plot size of 25 x 14 square meters. Cotton cultivated area was divided to 12 plots of $350m^2$ each and the four replicates of the two chemical treatments and the control were arranged in completely randomized block design. Starting from June 18, samples of 100 green bolls were randomly collected from each plot, externally examined to determine the bollworms infestation and dissected to count the number of larvae. On July 9, the infestation with bollworms in collected samples reached ≥ 5.

The recommended rates of alpha-cypermethrin (25gm AI/Feddan) and its mixture with flufenoxuron (17.5 + 7.5gm AI/Feddan) as suggested by the Egyptian Ministry of Agriculture (see Table 1) were sprayed using a knapsack sprayer equipped with one nozzle, using 200 liters of water per Feddan. Total of 3 sprays were given at 21 day interval between each two successive sprays. The first spray started based on the economic threshold level of 5-10% infestation. EL-Shaarawy *et al.* (2009) determined the economic damage threshold for the spiny bollworm (*Earias insulana*) being 10 larvae and 8% infestation/100 green bolls. However, the Egyptian Ministry of Agriculture recommends starting the spraying program when %infestation reaches 5% or less. The spray program was started on July 9 and repeated twice, on July 30 and on August 20. After spray, samples of 100 green bolls were weekly collected (from July, 16 to September, 10), externally inspected and dissected as previously conducted in the pre-spray inspection. At the same intervals of counting bollworm; number of predators in each plot was monitored on 25 plants using the direct count technique.

For each treatment, reduction percentages in bollworm infestation, bollworm larval content and number of predators were calculated using Henderson and Tilton equation (Henderson and Tilton, 1955) as follows:

% Reduction = [1-{(Control before* Treatment after)/ (Control after * treatment before)}]* 100. On September 10 and September 30, seed cotton yield for each plot was harvested and weighed. Cotton sown on April, 1; however, because of the undesirable weather (cold and wind), some seeds did not germinate and the re-sown plants on April, 20 delayed in flowering and fruiting that requested twice picking of cotton on September, 10 and 30.

Data analysis:

The average values of observations (%infestation, mean number of larvae, mean number of predators and seed cotton yield) have been subjected for statistical analysis to assess the overall impact on pest suppression and seed cotton yield. Data were analyzed using analysis of variance followed by the least significant range at 5% level of probability (Gomez and Gomez, 1984).

Results and Discussions

Spiny bollworm data were collected during the period from July 9th till the first picking of seed cotton on September 10th. During this period, pink bollworm was not recorded at Minia University Farm. As reported in Table 2 and graphed in Figure 1, mean percentages of spiny bollworm infestation in the untreated check was 10% in July 9th and increased gradually to reach 33.0% in September 10th. In the treatments of alpha-cypermethrin and its combination with flufenoxuron, these percentages ranged from 6-7% in the pre-spray counts and significantly decreased to reach 0.0-2.0% in September 10th. However, Admixing flufenoxuron with alpha-cypermethrin did not enhance its performance since the difference between the level of spiny bollworm infestation in the treatments of the pyrethroid alone and its combination with the chitin synthesis inhibitor was not significant (Table 2).

Number of larvae counted inside the infested bolls was compared among control and the two chemical treatments (Table 2 and Figure 2). With the exception of the counts in July 16th (the first week after the first spray), mean numbers of larvae counted in 100 green bolls in both alpha-cypermethrin and its mixture with flufenoxuron was not significantly different, however was significantly less compared to the control. After one week from the first spray (in July 16th), mean number of counted larvae did not significantly differ among control and the mixture treatment, however were significantly greater than those in alpha-cypermethrin treatment.

Adverse effect of tested chemicals on predator population was also evaluated (Table 2 and Figure 3). Mean number of predators counted on 25 plants averaged 31 individuals in July 9th (pre-spray count) and gradually increased to reach 350-363 individuals/25 plants in the first two weeks of August, then gradually decreased to reach 69 individuals in September 10th. After three weeks of the third spray, Predators population decreased in all treatments including the control. The peak of predator's activity in the control treatment was in the first half of August (350-363 individuals/25 plants). At the same interval, number of predators in alpha-cypermethrin treatment averaged 206-319 individuals/25 plants compared to 106-188 when alpha-cypermethrin was combined with flufenoxuron.

Percentages of reduction in spiny bollworm population and the associated predators were calculated (Tables 3-4 and Figures 4-7). Data for the efficiency of alpha-cypermethrin when it was sprayed alone or in combination with flufenoxuron are recorded in Table (3) and Figures (4-6). Statistically, alpha-cypermethrin activity against the spiny bollworm did not significantly enhance by combining flufenoxuron except in the first week after the first spray and the second and third weeks after the second spray. During these counts (July 16; August 13 and August 20), alpha-cypermethrin alone was much more efficient than its mixture with chitin synthesis inhibitor, flufenoxuron.

The combined data regarding percentages of reduction in spiny bollworm and associated predators population are reported in Table 4 and Figure 7. These data confirmed that alpha-cypermethrin alone or in its combination with

flufenoxuron offered similar performance against the larval population of spiny bollworm. However, its negative impact on the predators was significantly increased in the mixture.
Seed cotton yield in the control treatments that did not receive any insecticidal treatments during the whole season was highly significantly reduced compared to insecticidal treatments (seed cotton yield data not shown). However, cotton yield in the treatments of alpha-cypermethrin alone or its combination with flufenoxuron did not significantly differ. Mean yield of seed cotton averaged 7.65Kentar/Feddan (for alpha-cypermethrin alone), 7.18Kentar/Feddan (for alpha-cypermethrin/flufenoxuron mixture) and 3.42Kentar/Feddan (for untreated check).

Discussion

As reported in the current study and the previous studies (El-Saadany *et al.*, 1985) and Stam and Elmosa, 2006), Spiny bollworm was the key pest of cotton during the period from July until the end of cotton season and is responsible about the greatest loss in seed cotton yield**.** A unit infestation degree by spiny bollworm causes a loss varied from 6–9% in seed cotton yield (El-Saadany *et al.,* 1985). In 2009 cotton season, pink bollworm did not record in cotton field during the period from July to September; however, appeared late in October with less abundant (<10% infestation) than the spiny bollworm (36% infestation). The short duration of pink bollworm in cotton fields under Egyptian conditions (almost one month) was confirmed in Pakistan by the finding of Bhatti *et al*., (2007) who reported that *Earias* spp dominated from July to September, whereas, *P. gossypiella* dominated from mid-September to harvest. Low population (less than 10%) of pink bollworm in Egyptian cotton fields is probably because of the limited cultivated areas with cotton and the burning of cotton plants having diapausing larvae eliminate the next year infestation.

In the present study, it was obvious that flufenoxuron in its mixture with alpha-cypermethrin did not significantly affect the performance of the pyrethroid insecticide against spiny bollworm; however, significantly increased the negative impact on beneficial predators. Previous studies and current study confirmed the importance of beneficial arthropods for managing the populations of bollworms in cotton fields. Stam and Elmosa (2006) found among the entomophagous insects, coccinellids were more numerous during the months June and July and the hemipterous predators were more abundant during August and September. In the current study, the predator *Orius albidipennis* was recorded in cotton fields that was confirmed by the finding of Abdel-Razak *et al*., (2006), indicating that predator, *Orius albidipennis* (Hemiptera: Anthocoridae) was the most dominated predator on cotton plants and the predator peak was synchronous with the peak of spiny boll worm *Earias insulana* eggs.

Seed cotton yield in the treatments of alpha-cypermethrin alone or its combination with flufenoxuron did not significantly differ. Cotton production was insignificantly greater in the treatment of alpha-cypermethrin alone probably because a part (7.5gmAI/Feddan) of its concentration alone (25gmAI/Feddan) was replaced by the same amount of flufenoxuron in the mixture (17.5 + 7.5gmAI/Feddan (for explanation, see Table 1). In the control treatment, seed cotton yield was highly significantly reduced compared to insecticidal treatments. Flufenoxuron alone at 7.5gmAI/Feddan have no significant impact (compared to the untreated check) against either spiny bollworm or beneficial arthropods (data not shown).

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Table 1. Chemicals

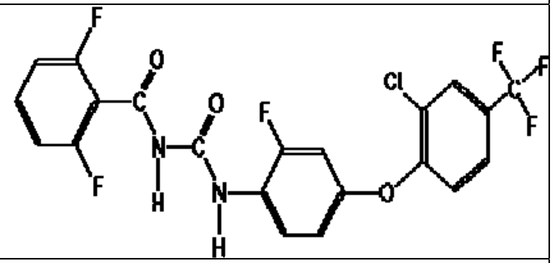
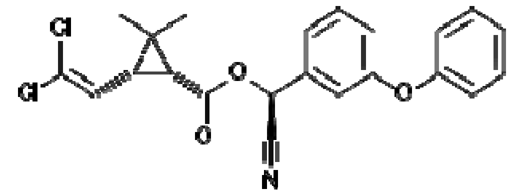
Chemicals	Trade name and formulation	Rate of application	Chemical name	Chemical structure
Flufenoxuron	Cascade	7.5gm AI/Feddan	CAS Name: N-[[[4-[2-chloro-4-(trifluoromethyl)phenoxy]-2-fluorophenyl]amino]carbonyl]-2,6-difluorobenzamide	
Alpha-cypermethrin	Bestox, 15%EC	165ml/ Feddan (25gm AI/Feddan)	R)-Cyano-3-phenoxybenzyl (1S,3S)-rel-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylate	
Mixture	Cegeron, 10%EC (3% + 7%)	17.5 +7.5gmAI		

Table 2. Spiny bollworm infestation (%), larval content (%) and mean number of predators counting in cotton field at weekly intervals during the period from July, 9 to September, 10 (2009-cotton season, Minia region, Egypt).

Measured parameter	Treatment	July 9	First spray			Second spray			Third spray		
			July, 16	July, 23	July, 30	Aug., 6	Aug., 13	Aug., 20	Aug., 27	Sept., 3	Sept, 10
%Infest.	Alpha-cypermethrin	6	6B	5B	4B	5B	1.0B	2.0B	0.0B	0.0B	0.0B
	Mixture	7	19A	7B	4B	7B	4B	6B	2B	0.0B	2.0B
	Check	10	21A	34A	23A	30A	28A	27A	36A	30A	33A
%Larval content	Alpha-cypermethrin	6	3B	1B	1B	6B	1B	0.0B	0.0B	0.0B	0.0B
	Mixture	7	13A	3B	2B	3B	0.0B	2B	1.0B	0.0B	1.0B
	Check	4	11A	12A	16A	15A	14A	12A	16A	11A	18A
No. of predators/ 25plant	Alpha-cypermethrin	38	38C	119A	44B	319A	206B	131B	69B	44C	44
	Mixture	38	63B	63B	69B	188B	106C	81C	50B	106B	38
	Check	31	169A	163A	113A	363A	350A	225A	231A	150A	69

For each measured parameter at each date of inspection, means followed by different letters are significantly different ($LSD_{0.05}$).

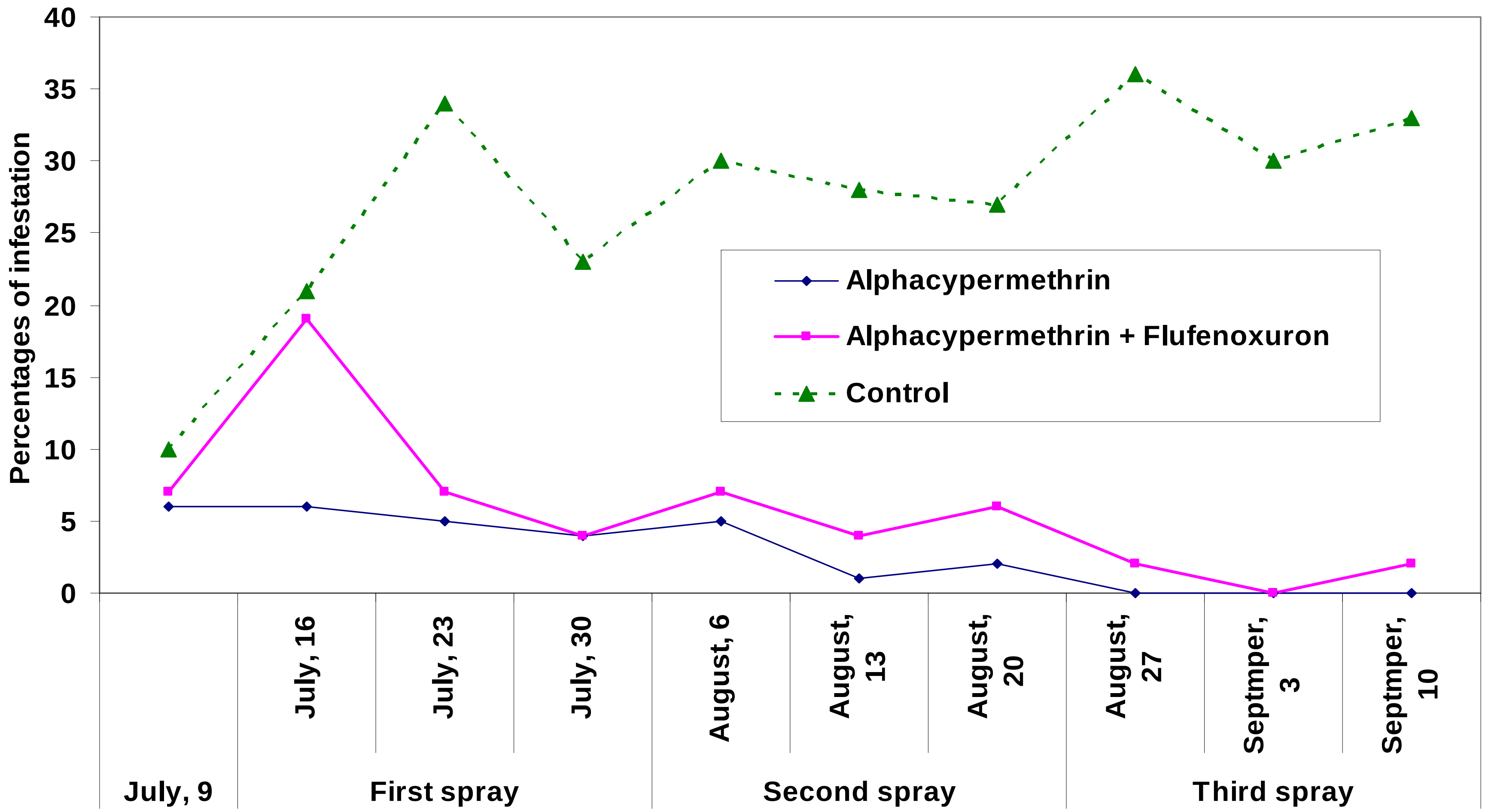


Figure 1. Percentages of spiny bollworm infestations at different dates post spraying alpha-cypermethrin alone or in combination with chitin synthesis inhibitor, flufenoxuron (2009-cotton growing season, Minia region, Egypt).

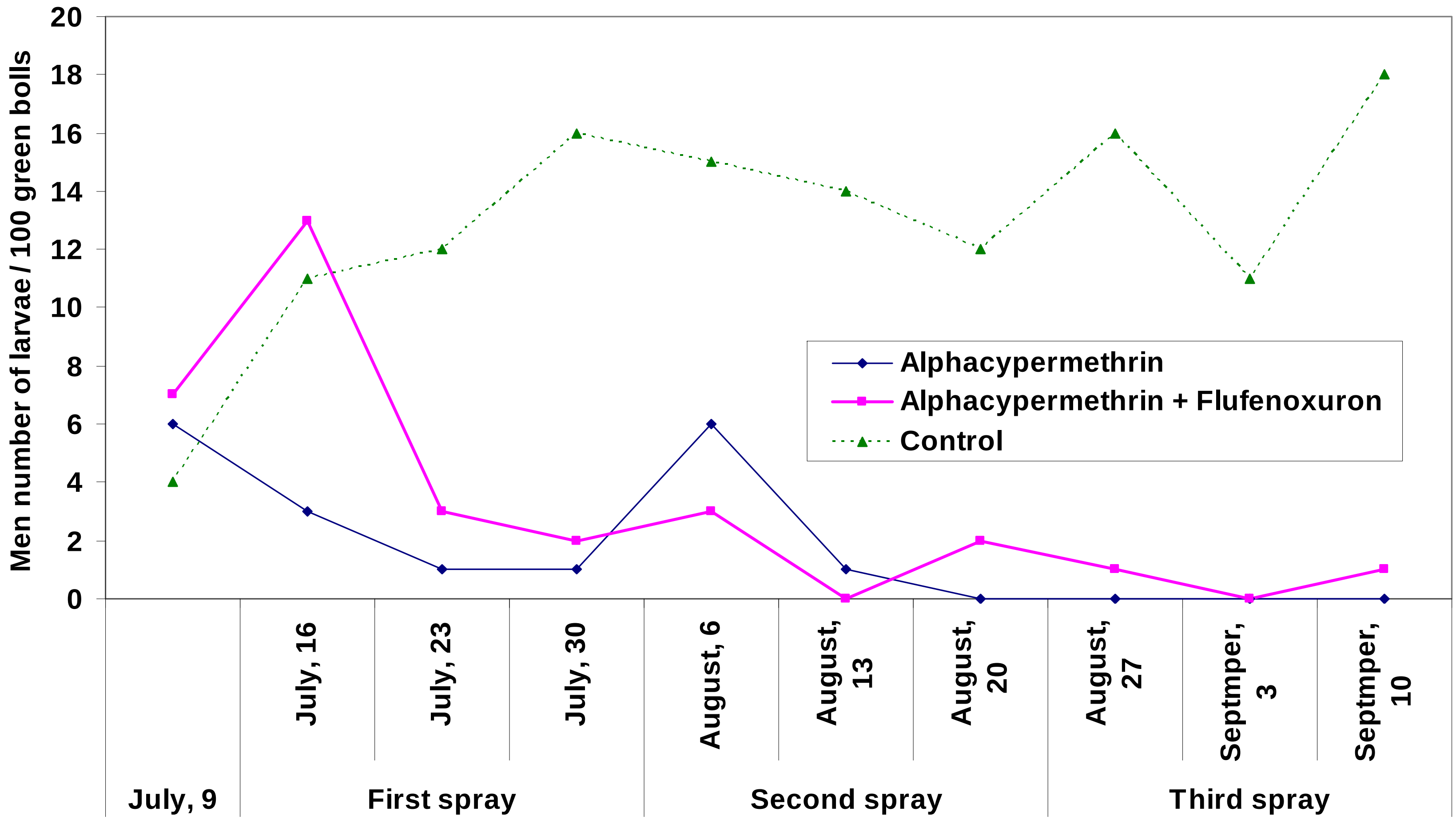


Figure 2. Mean number of spiny bollworm larvae counted in 100 green bolls at different intervals pre- and post-spraying alpha-cypermethrin and its combination with flufenoxuron (2009-cotton growing season, Minia region, Egypt).

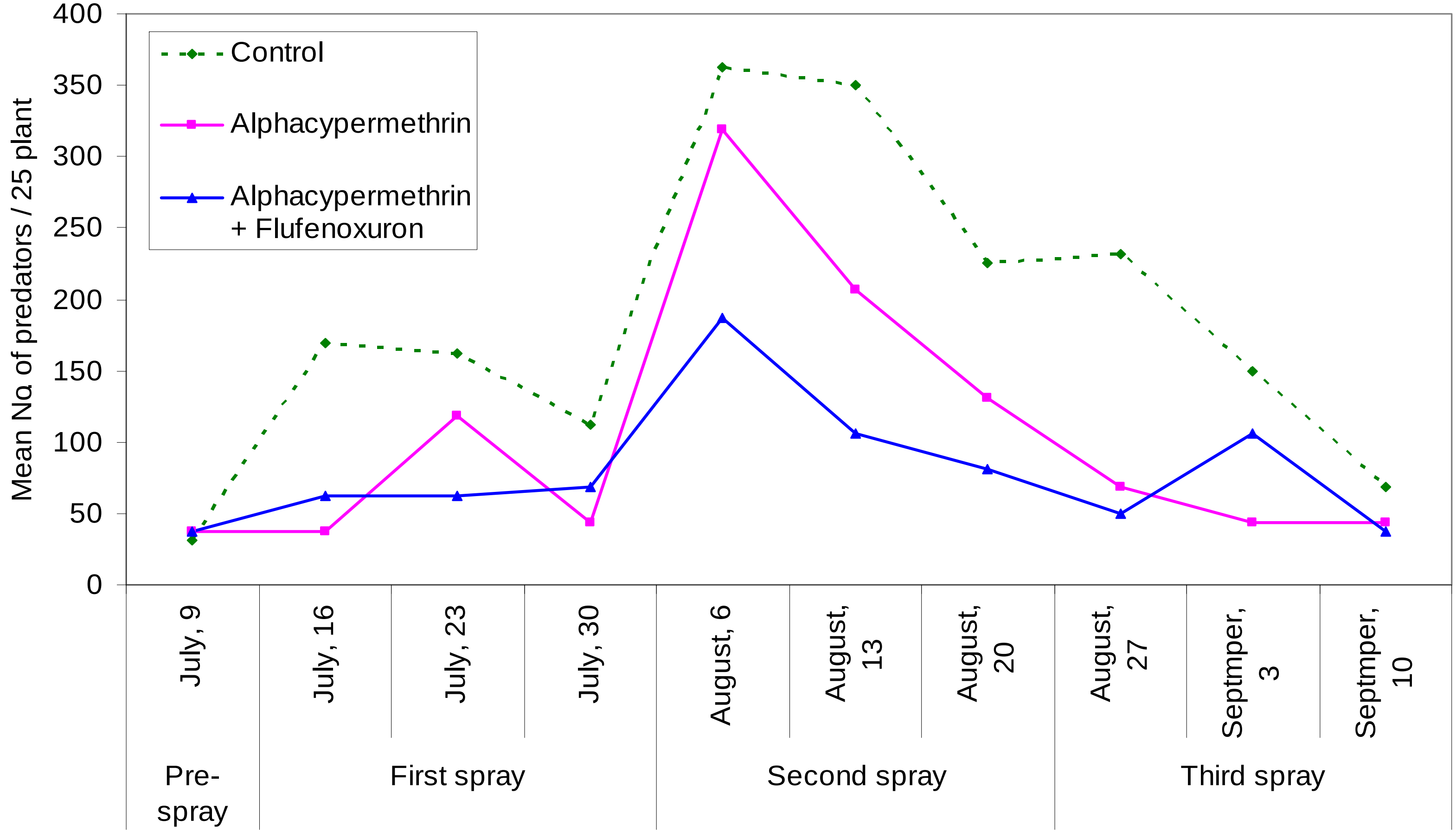


Figure 3. Mean number of predators at weekly intervals post applying tested chemicals in three successive sprays of 3 weeks intervals (2009-cotton growing season, Minia region, Egypt).

Table 3. Comparative effectiveness of alpha-cypermethrin and its mixture with flufenoxuron against the spiny bollworm and associated predators (2009-cotton growing season, Minia region, Egypt).

Measured parameter	Treatment	First spray			Second spray			Third spray		
		July 16	July 23	July, 30	Aug. 6	Aug. 13	Aug. 20	Aug. 27	Sept. 3	Sept. 10
%Reduction (infestation)	Alpha-cypermethrin	52.38A	75.49A	71.02A	72.22A	94.05A	87.65A	100.0A	100.0A	100.0A
	Mixture	-29.25B	70.59A	75.16A	66.67A	79.59B	68.25B	92.06A	100.0A	91.34A
%Reduction (Larval content.)	Alpha-cypermethrin	81.82A	94.44A	95.83A	73.33A	95.24A	100.0A	100.0A	100.0A	100.0A
	Mixture	32.47B	85.71A	92.86A	88.57A	100.0A	90.48A	96.43A	100.0A	96.83A
%Reduction (No. of predators)	Alpha-cypermethrin	81.51A	39.08B	67.56A	26.71B	50.88B	51.37A	75.23A	75.69A	46.97A
	Mixture	69.18B	67.95A	49.07B	56.90A	74.69A	69.91A	81.94	40.97B	54.55A

For each measured parameter at each date of inspection, percentages followed by different letters are significantly different (Paired *t* test).

Table 4. Reduction percentages in the spiny bollworm and associated predators during the three successive sprays and the general average of reduction (G. A.) during the period of insect activity.

Treatment	Reduction percentages in bollworm infestation				Reduction percentages in bollworm larval content				Reduction percentages in mean number of predators			
	1st spray	2nd spray	3rd spray	G. A.	1st spray	2nd spray	3rd spray	G. A.	1st spray	2nd spray	3rd spray	G. A.
Alpha-cypermethrin	66.3A	84.6A	100.0A	83.7A	90.7A	89.5A	100A	93.4A	62.4A	41.7B	71.0A	53.7B
Mixture	38.8B	71.5A	94.5A	68.3B	70.3B	93.0A	97.8A	87.04A	63.6A	66.7A	64.1A	65.3A

For each measured parameter at each date of inspection, percentages followed by different letters are significantly different (Paired *t* test).

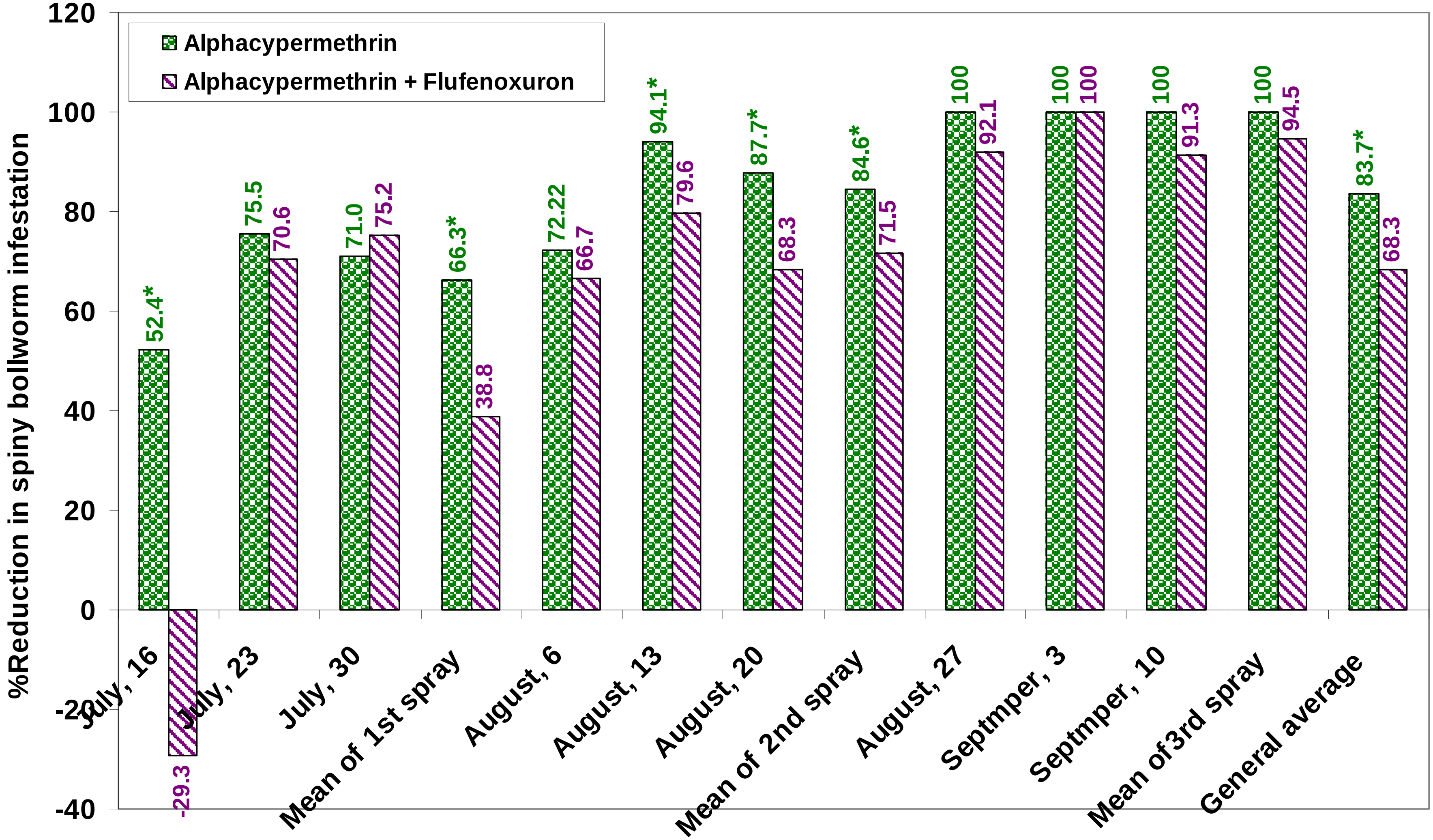


Figure 4. Percentages of reduction in spiny bollworm infestation at weekly intervals post three successive sprays of three weeks interval (2009-cotton growing season, Minia, Egypt).

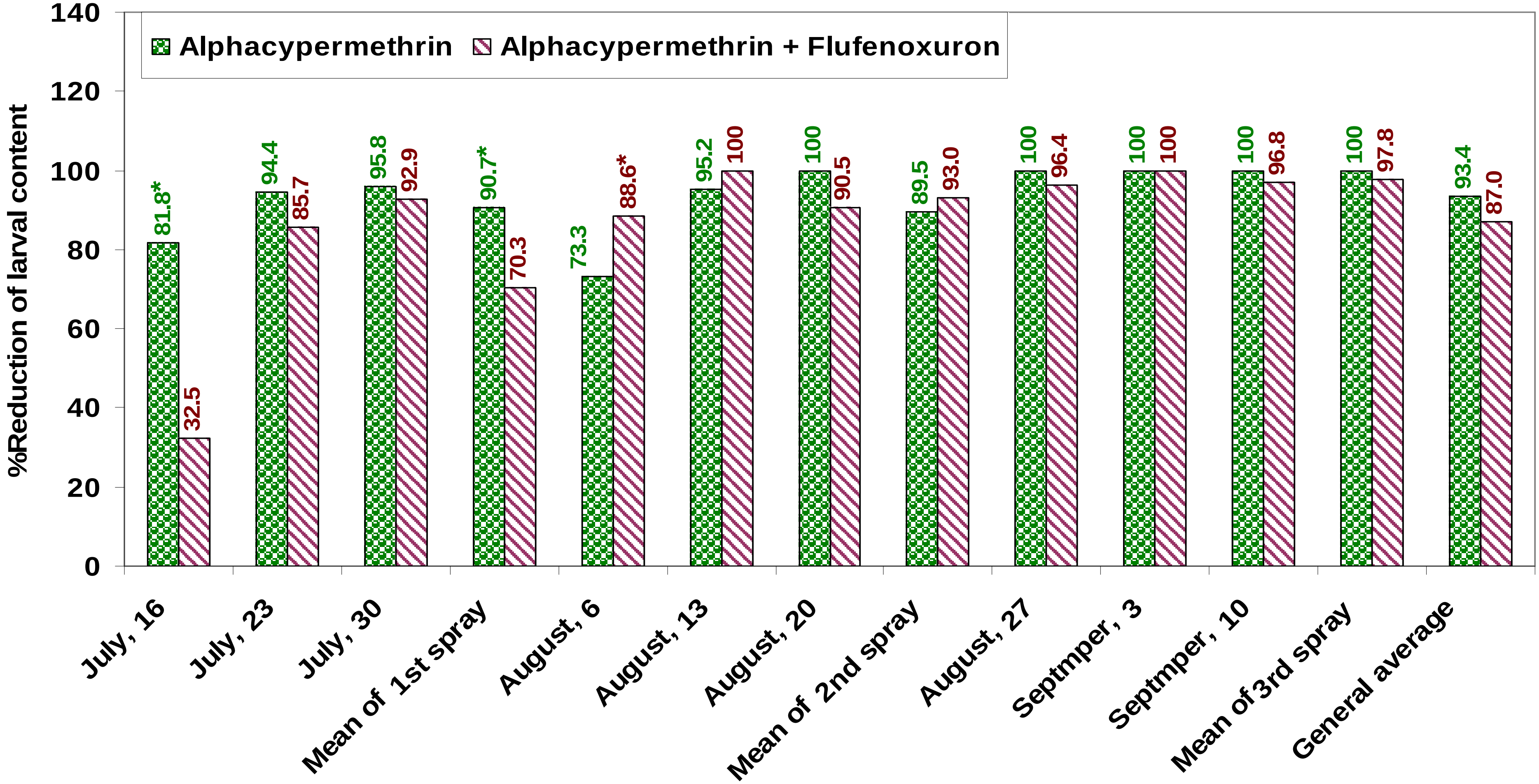


Figure 5. Reduction percentages of spiny bollworm larvae at weekly intervals post three successive sprays of three weeks interval (2009-cotton growing season, Minia, Egypt).

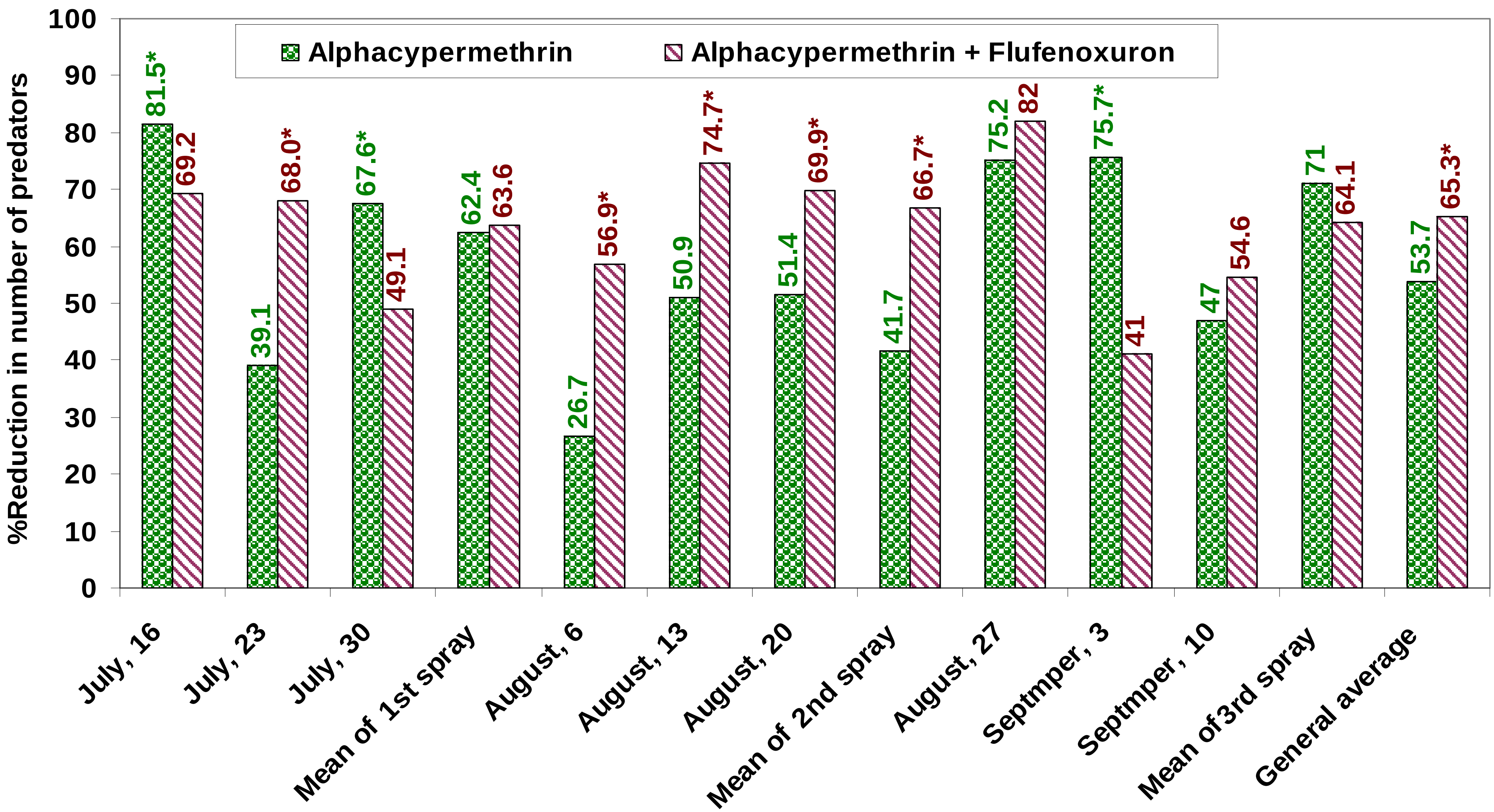


Figure 6. Reduction percentages in mean number of predators when alpha-cypermethrin was sprayed alone or in combination with flufenoxuron (2009-cotton growing season, Minia, Egypt).

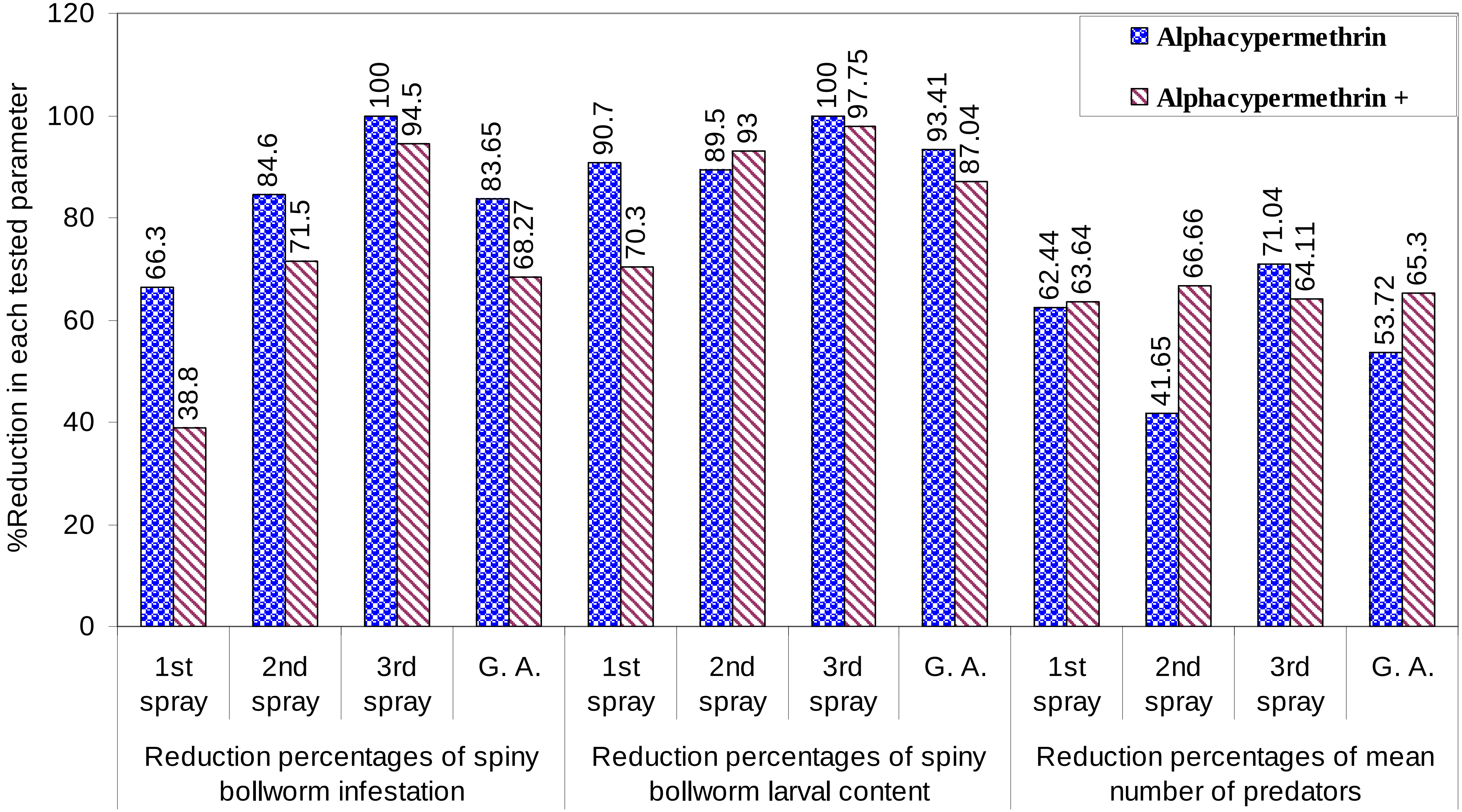


Figure 7. Efficacy of alpha-cypermethrin against spiny bollworm and its negative impact on the associated predators when it used alone or in combination with flufenoxuron 2009-cotton growing season, Minia, Egypt).