

## Integration of Selected Novel Pesticides with *Trichogramma chilonis* (Hymenoptera: Trichogrammatidae) for Management of Pests in Cotton

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### ABSTRACT

Selected pesticides widely used worldwide to control key pests on cotton plants were evaluated for integration with the *Trichogramma chilonis* (Ishii). Chlorantraniliprole showed a mean emergence of approximately 79–82 and 87–91% at both 6.25x and 9.4x doses when host eggs (*Sitotroga cerealella* Olivier) were treated at larval and pupal stage of parasitoids, respectively. *Helicoverpa armigera* NucleoPolyhedroses Virus (HaNPV) revealed mean emergence ranging from 80 to 84% at both 12.5x and 6.25x doses in the pupal stage treatment. Spiromesifen demonstrated mean emergence approximately ranging from 82.35 to 88.55% at 5x, x, and 0.5x doses in both the egg and pupal stage treatments, and in addition at 8.3X dose in the pupal stage treatment. Spiromesifen led to mean emergence of 88.20 and 79.52% at 2x and 5x doses, respectively, when parasitoid was treated at larval stage. When individual *T. chilonis* females were exposed to the previously treated host eggs (*S. cerealella*), chlorantraniliprole resulted in mean parasitism ranging from 17.88 to 20.88 parasitized host eggs at both 5x and 3.13x doses, while spiromesifen led to mean parasitism of 7.20 parasitized host eggs at 10.4x dose and showed significant effect on parasitism by parasitoid. The results indicated that the pesticides tested against emergence exhibited no significant toxic effects on the parasitoid.

**Keywords:** Chlorantraniliprole, Emergence, Integration, Parasitism, *Trichogramma chilonis*.

### INTRODUCTION

Cotton (*Gossypium hirsutum* L) is an important cash crop found worldwide including in Pakistan. Variety of pest species in cotton agro-ecosystem exhibit different feeding habits, behavior, and ecology and provide a challenge to implement integrated pest management or IPM (Barros *et al.*, 2018). Furthermore, the long phenology of cotton allow for colonization and multiple generations of pest species. Thus, integration of pesticides with the biological control may be needed to manage the multiple pest populations in cotton.

The use of agrochemicals, particularly pesticides, can adversely affect the efficacy of

natural enemies, and lead to disruption of their ecosystem services (Desneux *et al.*, 2007;

Stark *et al.*, 2007; Stavrinides and Mills, 2009; Dhawan *et al.*, 2009; Martinou *et al.*, 2014; Khan *et al.*, 2015; Martinez *et al.*, 2018). Assessment of acute mortality by the pesticides provides important information on the risk they pose to natural enemies (Candolfi *et al.*, 2001; Martinou *et al.*, 2014). Furthermore, use of such chemicals also leads to sub-lethal effects on development and reproduction of predators and parasitoids (Croft, 1990; Desneux *et al.*, 2007; Biondi *et al.*, 2012; Pekár, 2012; Martinou *et al.*, 2014).

The adverse impacts of pesticides on beneficial insects can be minimized by evaluation of pesticide effects on natural enemies, and subsequent adoption of strategies

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such as use of selective compounds, and altered dosage or schedule of pesticide application (Way, 1986; Hassan *et al.*, 1994; Martinson *et al.*, 2001; Khan *et al.*, 2014; Ghorbani *et al.*, 2016). Therefore, successful integration of chemical and biological control requires evaluation of both lethal and sub-lethal effects of pesticides on natural enemies (Croft, 1990; Ruberson *et al.*, 1998; Stark *et al.*, 2007; Jiang *et al.*, 2018). Hymenopteran parasitoids including *Trichogramma* have been shown to be highly effective in suppressing crop pest populations (Willow *et al.*, 2019).

*Trichogramma* are used worldwide in biological control (Khan *et al.*, 2015b), and are among the most widely used parasitoid species worldwide (Parreira *et al.*, 2019). They control over 400 host species belonging to a variety of insect orders, particularly Lepidoptera (Lingathurai *et al.*, 2015). The parasitic wasps have been reportedly released in 30 countries on an estimated area of 80 million acres including agricultural land and forests annually to cope with important insect pests (Li, 1994; Khan *et al.*, 2015b) in corn, rice, cotton, sugar-beet, tomatoes, vegetables, and orchards (Hassan, 1993; Smith, 1996; Khan *et al.*, 2014).

*Trichogramma chilonis* (Ishii) successfully controls more than 400 pest species, mostly

lepidopteran pests, and has been successfully used in augmentation worldwide (Lingathurai *et al.*, 2015; Pinto and Stouthamer, 1994). They are widely distributed throughout the Indian subcontinent (Manjunath *et al.*, 1985; Ananthakrishnan *et al.*, 1991; Khan *et al.*, 2014). They control pests including *Chilo* spp. in sugarcane, maize, and *Helicoverpa armigera* Hübner in cotton, tomato, and Lady's finger in India (Singh, 2001). In Pakistan, it is an important egg parasitoid of lepidopteran pests (Sattar *et al.*, 2011; Khan *et al.*, 2014). It successfully manages some of the common hosts i.e., sugarcane borer (*Chilo sacchariphagus indicus*) in sugar cane, diamondback moth (*Plutella xylostella* (Linnaeus)) in cabbage and other vegetables, and cotton bollworms (*Helicoverpa armigera* (Hübner)) in cotton and corn (Rasool *et al.*,

2002). *T. chilonis* is an effective parasitoid of rice leaf folder *Cnaphalocrocis medinalis* (Guenée) in Pakistan (Sagheer *et al.*, 2008).

The aim of the current research was to evaluate the effects of selected pesticides commonly used on the cotton plants on the immature mortality and parasitism of *T. chilonis*. The pesticides selected were chlorantraniliprole, HaNPV, and spiromesifen, applied at very high doses ( $\geq 5X$  dose of field dose: X) in order to integrate the experimental pesticides with *T. chilonis* for more successful control of important pests on cotton field.

## MATERIALS AND METHODS

### Rearing of *Sitotroga cerealella* Olivier

The young larvae of grain moth *Sitotroga cerealella* Olivier hatched and infested the sterilized wheat grain within a week in a plastic tray (30×18 cm) in the laboratory of Entomology Division, Nuclear Institute of Food and Agriculture (NIFA), Peshawar, (Pakistan). The infested wheat was maintained in plastic rearing jars (15×20 cm) in the laboratory at average conditions of 24±6°C, 65±10% Relative Humidity (RH) and 16:8 (L:D) until adults emerged after 20-25 days. The emerged adult moths were regularly collected every 24 hours by an electric suction apparatus in the oviposition jar (10×15 cm) covered at bottom by mesh (mesh no. 30 to 40 pore size). The jar was placed on corn flour in a plastic tray until next day (24 hours) to lay eggs in the flour, and eggs were subsequently collected by sieving the flour, and were used in the laboratory.

### Rearing of *Trichogramma chilonis* (Ishii)

Approximately 1,000-1,300 ultraviolet untreated fresh eggs of *S. cerealella* were sprinkled on glued paper card (4×7 cm) followed by glue drying for 1-2 hours, and card was exposed to parasitoids in glass jars

(5×12 cm) containing approximately 20-30 pairs of adult of *T. chilonis* in the laboratory at conditions described earlier. The parasitized card was removed and was transferred to another glass jar of the same size, and the jar was incubated at the 23±3°C, 70±10% RH and 14:10 (L:D) conditions until adult emergence. The stock culture of *T. chilonis* was maintained for use in the experimental work.

#### Preparation for Testing of Pesticides against Pre-Imaginal Stages of *T. chilonis*

Approximately 200-300 fresh *S. cerealella* eggs were glued to the hard paper card (5×8 cm), and allowed to dry 1-2 hours before offering to parasitoids in glass jars (5×12 cm) containing approximately 14 to 20 mixed-gender adults of *T. chilonis* depending on the number of host eggs on the card in the laboratory at average conditions of 24±6°C, 65±10% RH and 16:8 (L:D). After 24 hours of exposure, the parasitized card was subsequently removed and was cut to small card strips (0.8×8 cm). The trial involved 10 replicated card strips, each containing 20-40 host eggs, prepared separately for field dose (x), and 0.5x, 2x, 5x, 6.25x, 8.3x, 9.4x, and 12.5x doses. The different doses were prepared based on the reason: (1) To integrate several times higher doses than field dose with the parasitoids, (2) to achieve more successful control of pests. The above mentioned card strips were treated at appropriate time post-parasitization for the different immature stages that were less than 1 day (< 24 hours old) for eggs stage, 3 days (72 hours old) for

larvae, and 6 days (144 hours old) for pupae.

#### Preparation of Pesticide Solutions

Commercial products of formulated pesticides (Table 1) were diluted with tap water to prepare two to four different concentrations of pesticides for use in the experiments by the following formula:

$$C_1V_1=C_2V_2$$

Where,  $C_1$  and  $V_1$  are the Concentration and Volume of commercial pesticides/stock solution, respectively, and  $C_2$  and  $V_2$  are the Concentration and Volume of the required pesticide solutions (diluted), respectively.

#### Testing against Immature Stages of *T. chilonis*

Chlorantraniliprole was tested against both larval and pupal stages of parasitoid at both 6.25X and 9.4X doses, while spiromesifen was tested against both egg and pupal stages at X, 0.5X and 5X doses and, in addition, 8.3X dose was tested only against pupae. However, spiromesifen was tested at both 5X and 2X doses against the larval stage of parasitoids, and HaNPV was tested against the pupal stage of *T. chilonis* at both 6.25X and 12.5X doses. The pesticides were tested by dipping paper card strips (0.8×8 cm) each containing approximately 10-15 parasitized host eggs (< 24 hours) for 1-2 seconds in the pesticide solution or in water (untreated control). The card strip was then removed; the excess of pesticide solution or water was removed with filter paper, and card was subsequently air dried at room temperature

**Table 1.** Label descriptions of the pesticides used in the experiments.

Trade name and formulation <sup>a</sup>	Type	Active ingredient	Chemical class	Field rate/Acre or per liter solution
Coragen 200 SC	Insecticide	Chlorantraniliprole	Anthranilicdiamide	80 ml acre <sup>-1</sup> or 0.8 ml 1 L <sup>-1</sup>
HaNPV	Insecticide	HaNPV (7.5×10 <sup>12</sup> OB/L)	Biopesticides	80 ml acre <sup>-1</sup> or 0.8 ml 1 L <sup>-1</sup>
Oberon 240 SC	Miticide	Spiromesifen	Tetronic acids	250 ml acre <sup>-1</sup> or 2.5 ml 1 L <sup>-1</sup>

<sup>a</sup> SC (Soluble Concentrate). HaNPV (*Helicoverpa armigera* NucleoPolyhedroses Virus).

for 1 h. Each dried card strip was transferred into a vial (1×10 cm), and was incubated at controlled conditions: 23±3°C, 70±10% (RH) and 14:10 (L:D) until adult emergence. The number of pupae and the number of specimens emerging from the pupae on the pesticides dipped card were determined and recorded separately for each concentration and stage treated.

#### Assessing Pesticides Effects on Parasitism of Previously Treated Host Eggs (No-Choice Test)

Approximately 100 to 175 fresh *S. cerealella* eggs were glued on hard paper card (5×8 cm). The card was dried for 1-2 hours and was subsequently cut into several strips (0.9×8 cm each) in such a manner that each card strip contained approximately 30-35 host eggs. Card strips were treated by dipping for 1-2 seconds for each pesticide including chlorantraniliprole (Doses: 5X and 3X), and spiromesifen (dose: 10.4X) in their respective solution, in addition to water. Each card was dried at the aforementioned laboratory conditions and was subsequently transferred to a glass vial (1×10 cm) containing one pair of *T. chilonis* (< 24 hours old). The vial was exposed to light for 24 hours for completion of parasitisation. The trial was replicated 10 times for each concentration. The exposed parasitizing female was removed after 24 hours from each vial, and all the vials were incubated at aforementioned conditions until pupae formation. The data were recorded separately for all doses of pesticides by counting darkened eggs (pupae) 7 days after exposure to parasitoids.

#### Statistical Analysis

The data were analyzed using a general linear model through factorial analysis of variance for each of the response variables, and the differences between treatments were analyzed statistically by Tukey's HSD all-

pairwise multiple comparison tests ( $P= 0.05$  or 95% CI, or  $P= 0.001$  or 99.9% CI) by using statistical software "Statistix" (version 9).

The assessment of reduction in emergence (%) or reduction in parasitism (%) over the controls was carried out by toxicity categories (laboratory and field scales) of International Organization for Biological Control (IOBC)/West Palaearctic Regional Section (WPRS) (Hassan, 1994; Sterk *et al.*, 1999): 1= harmless ( $E < 30\%$ ); 2= slightly harmful ( $30 \leq E \leq 79\%$ ); 3= moderately harmful ( $79 < E \leq 99\%$ ); 4= harmful ( $> 99\%$ ), where "E" is the Effect of the pesticide on the biological control agent measured as the reduction in percentage of emergence or parasitism over the control.

## RESULTS AND DISCUSSION

### Pesticides Effects on Emergence

All the three pesticides and their doses tested against the immature stages of *T. chilonis* were found harmless ( $E < 30\%$ ) for emergence of parasitoid. Chlorantraniliprole demonstrated mean emergence (Table 2) ranging approximately from 80 to 91% at both 6.25X and 9.4X doses in both larval and pupal stage treatments. Similarly, HaNPV showed (Table 3) mean emergence ranging approximately from 80 to 84% at both 6.25X and 12.5X doses when host eggs were treated at pupal stage of parasitoid. All the doses of chlorantraniliprole and HaNPV showed no significance difference with respective controls ( $P > 0.05$ ). Spiromesifen revealed (Table 4) mean emergence ranging approximately from 82 to 88% at 5X, X and 0.5X doses in the egg stage treatment, while in the pupal treatment, emergence ranged approximately from 83 to 87% at aforementioned doses plus 8.3X dose. All doses of spiromesifen used both in egg and pupal stage treatments were statistically at par with the respective controls ( $P > 0.05$ ). In the larval stage treatment (Table 4), spiromesifen led to 88% mean emergence at

**Table 2.** Percentage emergence (mean±SE) of *T. chilonis* adults from the host eggs (*S. cerealella*) treated with chlorantraniliprole at larval and pupal stages of parasitoid, means comparison (Tukey's HSD, P= 0.05 or 5%), and toxicity ranking.<sup>a</sup>

Stage	Doses (means±SE) and toxicity ranking				
	9.4X	C	6.25X	C	Control
Larval	81.61±0.75 bc	1	79.83±0.73 c	1	80.15±0.87 c
Pupal	90.98±3.63 a	1	87.58±1.33 abc	1	88.78±1.60 ab

<sup>a</sup> Means followed by the same letter within or among columns are not significantly different (Tukey's HSD, P> 0.05). "C" indicate toxicity Class (International Organization of Biological Control: IOBC).

**Table 3.** Percentage emergence (mean±SE) of *T. chilonis* adults from the host eggs (*S. cerealella*) treated with HaNPV at pupal stage of parasitoid, means comparison (Tukey's HSD, P= 0.05 or 5%), and toxicity ranking.<sup>a</sup>

Doses (means±SE) and toxicity ranking				
12.5X	C	6.25X	C	control
80.15 ± 0.84 a	1	84.07 ± 2.10 a	1	84.67 ± 1.46 a

<sup>a</sup> Means followed by the same letter among columns are not significantly different (Tukey's HSD, P> 0.05).

**Table 4.** Percentage emergence (mean±SE) of *T. chilonis* adults from the host eggs (*S. cerealella*) treated with spiromesifen at immature stages of parasitoid, means comparison (Tukey's HSD, P= 0.05 or 5%) and toxicity ranking.<sup>a</sup>

Stage	Doses (means ± SE) and toxicity ranking										
	8.3X	C	5X	C	2X	C	X	C	0.5X	C	Control
Egg	-	-	82.35±0.79 a	1	-	-	83.94±0.82 a	1	88.55±1.09 a	1	88.59±1.65 a
Larval	-	-	79.52±2.74 b	1	88.20 ±1.76 a	1	-	-	-	-	88.21±1.33 a
Pupal	83.11±1.69 a	1	87.34±1.15 a	1	-	-	83.44±1.69 a	1	86.36±2.96 a	1	80.84±1.87 a

<sup>a</sup> Means followed by the same letter in the row are not significantly different (Tukey's HSD, P> 0.05). "C" indicates toxicity Class (IOBC).

2X dose, while 79.52% at 5X dose, which was significantly different from the control (P< 0.05). The results demonstrated that neither chlorantraniliprole nor HaNPV and spiromesifen showed adverse impacts on emergence at all used doses and all stages treated.

### Pesticides Effects on Parasitism

Exposure of females *T. chilonis* to the previously treated host eggs of *S. cerealella*, chlorantraniliprole led to reduced mean parasitism (Table 5): 17.88 at 5X dose, and 20.88 at 3.13X dose, and both doses were statistically at par with the control (P>

0.001) and were ranked as harmless (P< 30%) for parasitism. However, spiromesifen demonstrated (Table 6) significantly reduced mean parasitism i.e., 7.20 at 10.38X, which was significantly different from the control (P<0.05) and was categorized as slightly harmful (30≤ E≤ 79%) for parasitism.

The three pesticides used in the current study, including chlorantraniliprole, HaNPV and spiromesifen, are widely used worldwide for the control of key pests on the cotton plants as well as broadly in the agroecosystem.

The insecticide chlorantraniliprole belongs to a novel chemical class, anthranilic diamides and is selective to beneficial arthropods. It activates insect ryanodine



**Table 5.** The number of successfully parasitised *S. cerealella* eggs (mean±SE) previously treated with chlorantraniliprole by single female *T. chilonis*, means comparison (Tukey's HSD, P= 0.001 or 0.1%), and toxicity ranking.<sup>a</sup>

Doses (means±SE) and toxicity ranking				
5X	C	3.13X	C	control
17.88±1.92 a	1	20.88±2.15 a	1	23.00±2.02 a

<sup>a</sup> Means followed by the same letter within a column/among columns are not significantly different (Tukey's HSD, P> 0.001). "C" indicates toxicity Class (IOBC).

**Table 6.** The number of successfully parasitised *S. cerealella* eggs (mean±SE) previously treated with spiromesifen by single female *T. chilonis*, means comparison (Tukey's HSD, P= 0.05 or 5%), and toxicity ranking.<sup>a</sup>

Doses (means±SE) and toxicity ranking		control
10.38X	C	
7.20±1.70 b	2	18.7±2.93 a

<sup>a</sup> Means followed by the same letter within a column/among columns are not significantly different (Tukey's HSD, P> 0.05). "C" indicates toxicity Class (IOBC).

receptors, which stimulates release of calcium from the internal store of smooth and striated muscle, and results in impaired muscle regulation, paralysis, and insect death, due to abnormalities in the normal contraction of muscles. Chlorantraniliprole acts mainly by ingestion and has little contact activity. In Pakistan, it is used to control mainly lepidopterous insects, such as American bollworms, boll-worms and armyworms in sugarcane, corn, rice, cotton, tomato, lady finger, cabbage, mustard, spinach, apple and peach, etc.

The *Nuclear PolyhedroViruses* (NPV) are microbial insecticides belonging to the sub group Baculoviruses, and have been used to manage pest insects, predominantly moths and butterflies in cereal crops and vegetables. The polygonal structure of the virus known as the capsid protects and facilitates the virus's infestation of host cells and helps in virus reproduction. The virus strains are released and start reproduction in the host by rupturing the capsid. The NPV *Baculovirus heliothis* control *Sheliothines* spp, an important pest group in at least 30 crops worldwide. The *Helicoverpa armigera* *Nuclear PolyhedroVirus* (HaNPV) is an important

virus strain for controlling the important cotton pest *H. armigera*.

Spiromesifen is a novel acaricide, belonging to the insecticide class ketoenols, acts as a lipid biosynthesis inhibitor. Spiromesifen disrupts lipogenesis by preventing formation of fatty acid and their chemical derivatives. The acaricide is used against whiteflies and all major mites including red mites, two-spotted spider mites and bud mites at all stages on the cotton, maize, brinjal, cabbage, cauliflower, chilli, cucurbit, potato, lettuce, spinach, tomato and strawberry, etc., in Pakistan.

Repeated use of many pesticides in the agro-ecosystem can result in pest resistance to pesticides and appearance of secondary pests. Therefore, the present study was an endeavour to determine the effects of pesticides on *T. chilonis* at the rates several times high than field dose, in order to stop or reduce the development of resistance in pest insects found in cotton in response to the field dose, and for more effective control of pests in the future.

Sufficient literature is not available on the novel pesticides effects on *Trichogramma* (Khan *et al.*, 2015b). Furthermore, the pesticides tested against the parasitoids in the present studies were not evaluated previously

at the same doses against the *Trichogramma* or any other natural enemies to assess their emergence or parasitism. However, there are few previous studies on the pesticides effects on the tiny parasitoids.

HaNPV was found to be harmless for emergence of *T. chilonis* in the current study as well as by Khan *et al.* (2014) who found the same insecticide as harmless for emergence of adults *T. chilonis* from the host eggs (*S. cerealella*) treated with the same insecticide at field dose (x), 2x, and 0.5x doses, when parasitoids were at egg, larval, and pupal stages. The percent reduction in mean emergence relative to the controls of *T. chilonis* was less than 10% for all doses and life stages treated. Moreover, the previous studies with other microbial insecticides also supported the fact that microbial insecticides are harmless to *Trichogramma* such as Gandhi *et al.* (2005), who described that emergence success of the *T. chilonis* was not influenced by the treatment of bacterium *Pseudomonas fluorescens*. Furthermore, Nasreen *et al.* (2004) also found *Bacillus thuringiensis* (microbial insecticide) as relatively safe for emergence of the *T. chilonis*, when host eggs of *S. cerealella* were treated at late pupal stage of parasitoid under semi-field conditions.

Both chlorantraniliprole and spiromesifen were found harmless for emergence of *T. chilonis* in the present study. This is supported by Hussain *et al.* (2012), who evaluated toxicity of chlorantraniliprole under laboratory conditions and demonstrated that the same insecticide resulted in maximum emergence of *T. chilonis* from the host eggs (*S. cerealella*) treated after 8 days of parasitism (treatment of pupal stage) and showed minimum adverse effect on emergence of the tiny wasp from 1, 3, 5 and 7 days old parasitized treated eggs. Similarly, Khan and Ruberson (2017) observed that both chlorantraniliprole and spiromesifen caused no significant mortality to immature stages of *T. pretiosum*.

Literature is not available on the effects of chlorantraniliprole and spiromesifen on parasitism by females of *Trichogramma* emerged from the host eggs treated at the immature stages. Although Khan *et al.* (2015a)

observed that chlorantraniliprole and spiromesifen were harmless and slightly harmful for parasitism by the female *T. chilonis*, respectively, exposed to the previously treated host eggs of *S. cerealella*. Similarly, Khan and Ruberson (2017) observed that both chlorantraniliprole and spiromesifen caused no significant effect on foraging behaviour of female *T. pretiosum* exposed to previously treated host eggs of *Helicoverpa zea* (Boddie).

## CONCLUSIONS

The experimental pesticides were evaluated as harmless for emergence of, as well as parasitism by, *T. chilonis* at all used doses. Moreover, chlorantraniliprole demonstrated as harmless for parasitism at both 3.13x and 5x doses, while spiromesifen showed slightly harmful for parasitism by parasitoid at 10.38x doses. The selected pesticides at harmless doses and can be integrated with the parasitoids at the same doses provided that the doses will not adversely affect other natural enemies in the agro-ecosystem. The current study deals with research under the laboratory conditions, however, further study under field conditions are required for more effective integration of parasitoids wasps with the pesticides under natural conditions. Moreover, further studies are needed to determine the adverse effects of the used pesticides on other natural enemies in agro-ecosystem for comprehensive integration of biological and chemical controls to effectively control the different pests in the agroecosystem.

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### مدیریت آفت های پنبه با تلفیق آفتکش های جدید با *Trichogramma chilonis* (Hymenoptera: Trichogrammatidae)

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چکیده

در این پژوهش، چند آفتکش منتخب که برای کنترل آفت های عمده پنبه در جهان رایج است از نظر تلفیق با *Trichogramma chilonis* (Ishii) ارزیابی شد. بر اساس نتایج، میانگین ظهور (mean emergence) در مورد Chlorantraniliprole، در هر دو دُز 6.25x و 9.4x و هنگامی که تخم های میزبان (*Sitotroga cerealella* Olivier) در مرحله لارو و شفیره پارازیتوئید تیمار شده بود به ترتیب نزدیک به ۸۲-۷۹٪ و ۹۱-۸۷٪ بود. همچنین، ویروس (HaNPV) میانگین ظهور را در تیمار مرحله شفیره در هر دو دُز 12.5x و 6.25x بین ۸۰٪ تا ۸۴٪ نشان داد. نیز، Spiromesifen میانگین ظهور را بین 82.35% تا 88.55% در دُز های 5x، 0.5x، مراحل تخم و شفیره و در دُز 8.3x در مرحله شفیره نشان داد. در هنگامی که پارازیتوئید در مرحله لاروی تیمار شد، Spiromesifen منجر به میانگین ظهور 88.20% و 79.52% به ترتیب در دُز های 2x و 5x شد. هنگامی که هر *T. chilonis* ماده در معرض تخم قبلا تیمار شده میزبان (*S. cerealella*) قرار میگرفت، chlorantraniliprole در دُز های 5x و 3.13x منجر به میانگین پارازیتیزم در تعداد ۱۷/۸۸ تا ۲۰/۸۸ عدد از تخم پارازیت شده میزبان شد، در حالیکه spiromesifen در دُز 10.4x منجر به میانگین پارازیتیزم در تعداد ۷/۲۰ تخم میزبان شد و تاثیر معناداری روی پارازیتیزم پارازیتوئید نشان داد. نتایج حاکی از آن بود که آفتکش های آزمون شده بر علیه ظهور، هیچ اثر سمی عمده ای برای پارازیتوئید نداشتند.