

## Biology and fertility life table of *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) exposed to neem-based formulations

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**ABSTRACT:** We evaluate the effect of neem-based formulations on biological and population parameters of *Helicoverpa armigera*. Newly hatched larvae were fed for 72 h on diet containing Neenmax<sup>®</sup>, Nim-I-go<sup>®</sup>, Emulzinim<sup>®</sup> (neem-based products); Pirate<sup>®</sup> (positive control) and distilled water (negative control). Biological parameters were evaluated on a daily basis, and a fertility life table was used to assess populational growth. The experimental design was completely randomized, with five treatments (products and controls) and 10 replications. Means were submitted to analysis of variance and compared by Tukey test (5% probability). Neenmax<sup>®</sup> did not differ from Pirate<sup>®</sup> for larval survival. Duration and viability of larval and pupal periods, pupal weight at 24 h, oviposition period, adult longevity and female fertility were significantly affected by Emulzinim<sup>®</sup> and Nim-I-go<sup>®</sup>. Sex ratio and pre-oviposition period were not affected. Net reproductive rate (Ro), population doubling time (DT), intrinsic rate of increase (Rm) and finite rate of increase ( $\lambda$ ) differed statistically for neem formulations when compared to the negative control (distilled water). Based on our results, we conclude that the neem-based formulations evaluated in this study represent a potentially viable alternative for chemical control in the management of *H. armigera*.

**Key words:** artificial diet; *Azadirachta indica*; botanical insecticides; cotton bollworm; population parameters

## Biologia e tabela de vida de fertilidade de *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) exposta a formulações à base de nim

**RESUMO:** O objetivo deste estudo foi avaliar o efeito de formulações à base de nim sobre parâmetros biológicos e populacionais de *Helicoverpa armigera*. Lagartas recém-eclodidas foram alimentadas por 72 h com dieta contendo Neenmax<sup>®</sup>, Nim-I-go<sup>®</sup>, Emulzinim<sup>®</sup> (produtos à base de nim); Pirate<sup>®</sup> (controle positivo) e água destilada (controle negativo). Os parâmetros biológicos foram avaliados diariamente, e a tabela de vida de fertilidade foi usada para estimar o crescimento populacional. O delineamento experimental foi inteiramente casualizado, com cinco tratamentos (produtos e controles) e 10 repetições. As médias foram submetidas à análise de variância e comparadas pelo teste de Tukey (5% de probabilidade). Neenmax<sup>®</sup> não diferiu de Pirate<sup>®</sup> quanto à sobrevivência larval. A duração e viabilidade dos períodos larval e pupal, peso das pupas após 24 h, período de oviposição, longevidade dos adultos e fertilidade das fêmeas foram significativamente afetados por Emulzinim<sup>®</sup> e Nim-I-go<sup>®</sup>. A razão sexual e o período de pré-oviposição não foram afetados. Com relação aos parâmetros populacionais, a razão líquida de reprodução (Ro), tempo para duplicação da população (DT), razão intrínseca de aumento (Rm) e razão finita de aumento ( $\lambda$ ) diferiram estatisticamente para as formulações à base de nim, quando comparadas ao controle negativo (água destilada). De acordo com os resultados, as formulações à base de nim avaliadas neste estudo se apresentam como alternativas interessantes para uso da técnica de controle químico no manejo de *H. armigera*.

**Palavras-chave:** dieta artificial; *Azadirachta indica*; inseticidas botânicos; lagarta-do-velho-mundo; parâmetros populacionais

## Introduction

The cotton bollworm, *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) was responsible for devastating attacks on soybean crops in the Central-South region of Brazil during the 2012/2013 growing season (Czepak et al., 2013), and was subsequently observed in several other areas of production in the country (Specht et al., 2013). This pest, hitherto quarantined for Brazil (Hirose & Moscardi, 2012; Ávila et al., 2013), uses a wide range of cultivated plants as hosts (including important agricultural commodities such as soybean, maize and cotton) and causes considerable damage throughout the entire phenology of the crop (Czepak et al., 2013; Specht et al., 2013).

*H. armigera* is typically controlled with synthetic chemical insecticides. In addition to the environmental damage they cause, these chemicals are costly and often inefficient due to the bollworm's impermeable tegument and its ability to rapidly evolve pesticide resistance (Alvi et al., 2012); there are currently 856 reported cases of *H. armigera* resistance to 48 pesticides, most of them synthetic (Arthropod Pesticide Resistance Database, 2018).

In contrast to synthetic pesticides, botanical insecticides have a lower environmental impact and frequently contain a mixture of several active principles making it harder for target species to evolve resistance (Ahmad et al., 2015). For these reasons, the use of botanical insecticides is increasing in Integrated Pest Management (IPM) programs around the world (Aoyama & Labinas, 2012). One of the most effective sources of botanical pesticides is the neem tree (*Azadirachta indica* A. Juss.), the extracts of which combine low toxicity to mammals with reported effects on more than 400 species of insects and mites of agricultural importance (Ahmad et al., 2013a).

One of the guiding principles of IPM is that the higher the dose of pesticide applied, the greater the selection pressure and the chance of resistance development (Norris et al., 2003). Thus, effective dosage is a critical factor in choice of chemical control, and has not yet been evaluated for botanical insecticides against *H. armigera*. IPM also advocates continuous management of pests, with the aim of preventing insects from reaching a population density capable of causing economic damage.

To verify the efficacy of insecticides, it is important to assess both lethal (mortality) and sublethal effects. The

parameters of the fertility life table, especially the intrinsic rate of increase ( $R_m$ ) and the net reproductive rate ( $R_o$ ) of the population, are key indicators for determining the toxic effects of any product on pest population dynamics (Stark et al., 2007). Fertility life tables can be used to estimate the total effect of insecticides, including survival and the development of future generations through their reproductive potential (Ahmad et al., 2015). Such a full evaluation is ideal for identifying the appropriateness of an insecticide for use in an IPM program. Thus, the aim of this study is to use fertility life tables to evaluate the effect of neem-based formulations on the biology and population parameters of *H. armigera*.

## Materials and Methods

Bioassays were carried out in the Laboratory of Entomology of Embrapa Tabuleiros Costeiros / Unidade de Execução de Pesquisa e Desenvolvimento de Rio Largo (at  $25 \pm 1$  °C, relative humidity of  $80 \pm 10\%$  and photophase of 12 h), in Rio Largo, AL, Brazil, using the artificial diet of *Anticarsia gemmatalis* Hübner (Lepidoptera: Noctuidae), modified for *H. armigera* by Ribeiro (2017).

The neem-based formulations Neenmax<sup>®</sup>, Emulzinim<sup>®</sup> and Nim-I-go<sup>®</sup> and the synthetic insecticide Pirate<sup>®</sup> (positive control) were evaluated in concentrations of 2.0, 2.0, 1.0 and 0.16 mL, respectively, for 200 mL of artificial diet, in order to obtain the concentrations recommended by the manufacturers (Table 1). After the artificial diet preparation, the products were separately added and homogenized for approximately two minutes, prior to solidification.

Eggs of *H. armigera* were obtained from BUG Biological Agents (Piracicaba, SP, Brazil). Neonate larvae were individually placed in six-cell tissue culture plates. A cube of approximately  $1.0 \times 3.0 \times 1.0$  cm of artificial diet treated or not (negative control) with neem-based formulations (treatments) and Pirate<sup>®</sup> (positive control) were offered to each larva. After 72 h, larvae were placed in new containers and provided with an artificial diet free of insecticides until their death or pupation. The plates were daily cleaned, and new diet cubes were daily offered to the remained larvae. The 24 h-old pupae were weighed on analytical balance, sexed and kept in plastic pots containing a filter paper disc moistened with distilled water and closed with voile tissue. For each treatment the duration and viability of the larval and pupal periods, the pupal weight with 24 h and the sex ratio were evaluated. The sex ratio was

**Table 1.** Commercial neem-based formulations tested on *H. armigera* in artificial diet and their composition, recommended dose and registrant/manufacturer.

Commercial name	Composition	Recommended dose	Registrant/Manufacturer
Neenmax <sup>®</sup>	Azadirachtin: 2,389 ppm (neem oil); organic emulsifier	2 L ha <sup>-1</sup> 200 mL 20 L <sup>-1</sup>	Amigos do Nim
Emulzinim <sup>®</sup>	Azadirachtin: 0.12% (neem oil); vehicle: 99.88%; natural emulsifier.	1 L 100 L <sup>-1</sup>	Insetimax
Nim-I-go <sup>®</sup>	Azadirachtin: 2,000 ppm; natural emulsifier.	5 mL L <sup>-1</sup>	Agrobiológica Soluções Naturais
Pirate <sup>®</sup>	Chlorfenapyr: 240 g L <sup>-1</sup> ; other ingredients: 860 g L <sup>-1</sup>	0.8 - 1.2 g L <sup>-1</sup>	Basf Corporation

obtained by the formula  $SR = NF / (NF + NM)$ , where SR: sexual ratio; NF: number of females; and NM: number of males.

After adult emergence, pairs of the same age were formed for each treatment, confined in PVC (polyvinyl chloride) cages (200 mm in diameter × 35 cm high, internally lined with sulphite paper). The adults were fed with 10% honey solution and each cage contained soybean (*Glycine max* (L.) Merrill.) leaves (substrate and stimulant for oviposition). The number of eggs laid was recorded on a daily basis until the insect's death. Pre-oviposition and oviposition periods, longevity and female fecundity were also evaluated. Hatching success of eggs and fertility were also determined.

The experimental design was completely randomized, with five treatments and 10 replications. Each replication was composed by a group of five larvae for the assessment of larval development, or by a couple of adult insects for the assessment of adult parameters. The means were analyzed through ANOVA, performed with the GENES program (Cruz, 2013; 2016), and compared by the Tukey test, at a 5% probability level.

Based on the biological data obtained for *H. armigera*, fertility life table parameters were calculated and compared by Tukey's test at 5% probability, using the SAS statistical program (SAS Institute, 2001). Proportion of individuals alive at age  $x$  ( $l_x$ ) and the number of female progeny produced per female during age interval  $x$  ( $m_x$ ) were determined from daily observation data. The following demographic parameters were calculated: 1) net reproductive rate,  $R_0 = \sum l_x m_x$ ; 2) mean generation time,  $T = \sum (l_x m_x) x / \sum (l_x m_x)$ ; 3) intrinsic rate of increase,  $R_m = (\ln R_0) / T$ ; 4) population doubling time,  $TD = \ln(2) / r_m$ ; and 5) finite rate of increase,  $\lambda = \exp(r_m)$ .

## Results and Discussion

The neem-based formulations caused significant larval mortality ( $p < 0.05$ ). Emulzinim® and Nim-I-go® were lethal to 36 and 46% of larvae, respectively. Neenmax® caused 94% mortality, not differing from the synthetic insecticide Pirate®, which caused 100% of larval mortality (Table 2). Although the three formulations evaluated here are neem-based, Neenmax presents the highest azadirachtin concentration, which may explain the higher mortality observed in this treatment.

In general, larval survival of *H. armigera* was strongly affected by Neenmax®, corroborating previous studies. For example, Ma et al. (2000) recorded mortality in the first

instars larvae of *H. armigera* in cotton plants sprayed with the emulsified oil of neem with 3% azadirachtin. A similar study (Viana & Prates 2003) offered leaves of corn submerged and sprayed with neem extract (10 mg mL<sup>-1</sup>) to newborn larvae of *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae), and observed 100% mortality. Kumar (2014) also verified the high effectiveness of neem oil (4 mL L<sup>-1</sup>) on *H. armigera* in comparison with four synthetic insecticides.

In the present study, the synthetic insecticide Pirate® had a knock-down effect with 100% of mortality within 48 h of exposure. In contrast, *H. armigera* larvae submitted to Neenmax® did not die during dietary exposure (72 h), but rather during the larval development period. However, by the end of the bioassay both insecticides had reached approximately the same level of cumulative mortality. These results suggest that Neenmax® could be equally effective as a synthetic insecticide when used on *H. armigera* and confirms the growth regulator action of neem-based formulations.

Neenmax® caused 100% pupal mortality, differing statistically from Emulzinim® and Nim-I-go® (14 and 12%, respectively) ( $p < 0.05$ ). The use of neem-based formulations may control insects not only by lethal effects, but also by long-term toxic effects on subsequent stages of development (Ahmad et al., 2015). Such formulations may also affect biological parameters such as fecundity and fertility through sublethal effects, thereby reducing the population of future generations (Stark & Banks, 2003).

Perhaps the most studied effects of sublethal doses are those related to insect feeding inhibitory activity (deterrent effect) and those that influence insect development, especially ecdysis (Silva et al., 2013). These could explain the observed pupal mortality in larvae fed with neem-based products. Inhibition of larval feeding can result in fewer energy reserves at the pupal stage, thereby decreasing the probability of surviving the period of starvation preceding the emergence of the adult. In the present study, all pupae of larvae treated with the Neenmax® diet were characterized by deformities and none reached adulthood. Some larvae fed with Emulzinim® and Nim-I-go® treated diet failed to pupate or produced malformed pupae (e.g. pupae with larval features, such as legs and cephalic capsule).

An increase in the period of larval development was observed for Nim-I-go® (18.7 days) when compared to Emulzinim® and the negative control (15.43 and 15.78 days, respectively) ( $p < 0.05$ ) (Table 2). Ahmad et al.

**Table 2.** Viability and duration of larval and pupal periods, sex ratio and pupal weight (mean ± SE) of *Helicoverpa armigera* fed on an artificial diet treated with neem-based formulations (72 h) and controls (temperature: 25.0 ± 1 °C; RH: 80 ± 10%; photophase: 12 h)<sup>1</sup>.

Products	Viability (%)		Duration (days)		Sex ratio	Pupal weight (g)
	Larval	Pupal	Larval	Pupal		
Emulzinim®	64 ± 8.33 b	86 ± 5.21 b	15.43 ± 0.42 b	11.85 ± 0.35 a	0.58 ± 0.08 a	0.30 ± 0.00 ab
Neenmax®	6 ± 4.27 c	0 ± 0.00 c	-	-	-	-
Nim-I-go®	54 ± 5.21 b	88 ± 3.27 b	18.69 ± 0.77 a	11.48 ± 0.32 a	0.52 ± 0.02 a	0.28 ± 0.02 b
Pirate®	0 ± 0.00 c	-	-	-	-	-
Control	100 ± 0.00 a	100 ± 0.00 a	15.78 ± 0.34 b	11.82 ± 0.26 a	0.64 ± 0.04 a	0.33 ± 0.01 a

<sup>1</sup> Means (± SE) followed by the same letter in the column did not differ by Tukey's test ( $p \geq 0.05$ ).

(2013b) observed a similar extension of *H. armigera* larval period when exposed to 15 and 20 mg L<sup>-1</sup> Neemazal® (1% azadirachtin). Such prolongation is probably due to the effect of neem on larval feeding, reducing the rate of accumulation of nutrients for ecdysis. An extended larval period also results in increased exposure of the insects to the action of natural enemies (Wondafrash et al., 2012). Finally, decreasing the rate of development could reduce the number of generations within a growing season and reduce population growth, which is stepped over generations.

Previous studies have shown that ingestion of neem-based formulations by newly hatched *S. frugiperda* larvae hinders their normal development, even if they were later fed with a nutritional source without insecticides (Viana & Prates, 2003). The role of azadirachtin in blocking the synthesis and release of ecdysteroids, causing incomplete ecdysis in immature insects, is well known (Isman, 2006). However, despite interference during phase changes, especially from larval to pupal, and from pupa to adult, in the present study none of the experimental products affected the duration of the pupal period or the sex ratio of the insects (Table 2).

After 24 hours, negative control pupae had a mean weight of 0.33 g; higher than Nim-I-go® treatment (0.28 g) ( $p < 0.05$ ) (Table 2). Ma et al. (2000) did not observe a reduction in the weight of *H. armigera* pupae when the larvae were fed for 96 h with cotton leaves sprayed with the emulsified oil of neem at 3% concentration. However, the product was subsequently observed to reduce fecundity and fertility of adult females.

Emulzinim® and Nim-I-go® treated individuals had lower longevity when compared to the negative control, for both males and females ( $p < 0.05$ ) (Table 3). The males had a mean longevity of 8.3 and 8.2 days, while the females survived a little longer, reaching a mean of 9.5 and 9.1 days, respectively, for Emulzinim® and Nim-I-go®. In the negative control, a respective longevity of 10.2 and 11.2 days for males and females was observed. For the pre-oviposition period, there

was no statistical difference between the treatments and the negative control, with an average period of about three days before oviposition ( $p < 0.05$ ). Ahmad et al. (2015) also found no adverse effect of neem-based products on the pre-oviposition period of *H. armigera*. However, in the present study, neem-based formulations were associated with a shorter oviposition period, statistically differing from the negative control (Table 3).

Neem has been recorded to sterilize adult female insects (Isman 2006; Abedi et al. 2014). In the present study, we observed a drastic reduction in the fecundity of females exposed to neem-based formulations. For total fecundity (the end of the oviposition period), all treatments differed statistically, with an average of 527.5 eggs for Emulzinim®, 970.1 eggs for Nim-I-go® and 2,116.8 eggs for the negative control ( $p < 0.05$ ) (Table 4). A reduction in *H. armigera* fecundity exposed to azadirachtin has previously been observed (Ahmad et al. 2013a). Decreased fecundity may be due to a reduction in ovarian protein and disruption of the synthesis and absorption of vitellogenin in developing oocytes, preventing egg production (Pineda et al., 2009; Ahmad et al., 2012). The number of viable eggs was higher in the control, with a mean of 1,117.1 fertile eggs, differing from Emulzinim® and Nim-I-go® ( $p < 0.05$ ) (Table 4).

The fertility life table indicates that all treatments differed from the negative control for net reproductive rate ( $R_0$ ), intrinsic rate of increase ( $R_m$ ), population doubling time (TD) and finite rate of increase ( $\lambda$ ) (Table 5). Moreover, the intrinsic rate of increase ( $R_0$ ) - the capacity of the population to increase in number to each generation - was strongly affected by the action of neem-based formulations. Thus, for Emulzinim® and Nim-I-go®, *H. armigera* population may increase 195.77 and 273.25 times, respectively, for each generation. Such values are low when compared to the negative control, where the population would increase 1,354.75 times. The mean generation time (T) of 23.3 (Emulzinim®) and 25.7 days (Nim-I-go®) were not different from the negative control (23.37 days).

**Table 3.** Adults longevity, pre-oviposition and oviposition periods (mean  $\pm$  SE) of *H. armigera* fed on artificial diet treated with neem-based formulations (72 h) and control (temperature: 25.0  $\pm$  1 °C; RH: 80  $\pm$  10%; photophase: 12 h)<sup>1</sup>.

Products	Longevity (days)		Mean longevity (days)	Pre-oviposition period (days)	Oviposition period (days)
	Male	Female			
Emulzinim®	8.3 $\pm$ 0.50 b	9.5 $\pm$ 0.31 b	8.9 $\pm$ 0.30 b	3.4 $\pm$ 0.16 a	3.9 $\pm$ 0.18 b
Nim-I-go®	8.2 $\pm$ 0.33 b	9.1 $\pm$ 0.28 b	8.7 $\pm$ 0.24 b	3.0 $\pm$ 0.21 a	4.6 $\pm$ 0.27 b
Control	10.2 $\pm$ 0.42 a	11.2 $\pm$ 0.29 a	10.7 $\pm$ 0.25 a	2.9 $\pm$ 0.18 a	5.5 $\pm$ 0.17 a

<sup>1</sup> Means ( $\pm$  SE) followed by the same letter in the column did not differ by Tukey's test ( $p \geq 0.05$ ).

**Table 4.** Fecundity (daily and total) and fertility (mean  $\pm$  SE) of *H. armigera* fed on artificial diet treated with neem-based formulations (72 h) and control (temperature: 25.0  $\pm$  1 °C; RH: 80  $\pm$  10%; photophase: 12 h)<sup>1</sup>.

Product	Daily fecundity						Total fecundity	Fertility
	1 <sup>st</sup> day	2 <sup>nd</sup> day	3 <sup>rd</sup> day	4 <sup>th</sup> day	5 <sup>th</sup> day	6 <sup>th</sup> day		
Emulzinim®	97.9 $\pm$ 8.27 b	183.7 $\pm$ 29.92 c	149.3 $\pm$ 21.1 b	87.2 $\pm$ 19.77 b	9.4 $\pm$ 9.40 c	0 $\pm$ 0.00 b	527.5 $\pm$ 42.27 c	211.6 $\pm$ 28.15 b
Nim-I-go®	76.4 $\pm$ 11.8 b	305.9 $\pm$ 26.11 b	260.7 $\pm$ 20.48 b	189.2 $\pm$ 33.29 b	126.1 $\pm$ 42.27 b	11.8 $\pm$ 11.8 b	970.1 $\pm$ 104.36 b	216.1 $\pm$ 24.31 b
Control	259.2 $\pm$ 37.64 a	443 $\pm$ 36.06 a	562 $\pm$ 54.22 a	431.4 $\pm$ 40.07 a	303.9 $\pm$ 31.33 a	117.3 $\pm$ 40.52 a	2,116.8 $\pm$ 129.08 a	1,117.1 $\pm$ 91.38 a

<sup>1</sup> Means ( $\pm$  SE) followed by the same letter in the column did not differ by Tukey's test ( $p \geq 0.05$ ).

**Table 5.** Net reproductive rate (Ro), mean generation time (T), population doubling time (TD), intrinsic rate of increase (Rm) and finite rate of increase ( $\lambda$ ) (mean  $\pm$  SE) of *H. armigera* fed on artificial diet treated with neem-based formulations (72 h) and control (temperature: 25.0  $\pm$  1 °C; RH: 80  $\pm$  10%; photophase: 12 h)<sup>1</sup>.

Product	Ro	T (days)	DT (days)	Rm	$\lambda$ (individuals/ <sup>♀</sup> /day)
Emulzinim®	195.77 $\pm$ 15.70 a	23.30 $\pm$ 0.38 a	3.06 $\pm$ 0.04 a	0.22 $\pm$ 0.003 a	1.25 $\pm$ 0.004 a
Nim-I-go®	273.25 $\pm$ 29.01 a	25.70 $\pm$ 0.98 a	3.17 $\pm$ 0.16 a	0.21 $\pm$ 0.01 a	1.24 $\pm$ 0.01 a
Control	1354.75 $\pm$ 82.61 b	23.37 $\pm$ 0.43 a	2.24 $\pm$ 0.05 b	0.31 $\pm$ 0.006 b	1.36 $\pm$ 0.008 b

<sup>1</sup> Means ( $\pm$  SE) followed by the same letter in the column did not differ by Tukey's test ( $p \geq 0.05$ ).

The finite rate of increase ( $\lambda$ ), which represents the number of offspring per female per day added to the population, was 1.25 and 1.24 for Emulzinim® and Nim-I-go®, respectively; this was significantly lower than the control (1.36). These results clearly demonstrate that neem-based formulations negatively affect the development of future generation of *H. armigera*.

More generally, the results of our study corroborate those of Ahmad et al. (2015) who reported the negative effects of neem-based formulations, Neemarin® (0.15% azadirachtin); Neemazal® (1% azadirachtin); Neemix® (0.25% azadirachtin) and neem oil (1% azadirachtin) on *H. armigera*. This study verified the negative effects of all the studied products on biological and demographic parameters, and highlighted the potential of Neemazal®, which mainly affects the intrinsic rate of increase (Rm) and the finite rate of increase ( $\lambda$ ) of *H. armigera* populations.

The use of neem is rapidly increasing in contemporary agriculture and is becoming an important tool within many IPM programs. Neem-based formulations may be particularly useful in this context due to their multiple modes of action (related to azadirachtin and other associated metabolites). The lethal and sublethal effects of neem formulations on the pest *H. armigera* demonstrated in the present study highlights their enormous potential as environment-friendly alternatives to synthetic pesticides, and indicates that they may be particularly well-suited for use in organic agriculture and family farming.

## Conclusions

The neem-based formulation Neenmax® (added to an artificial diet at the commercial dose recommended by the manufacturer) significantly reduced the viability of *H. armigera* larval and pupal stages, with an overall effect similar to that of the synthetic chemical insecticide Pirate®.

The Nim-I-go® and Emulzinim® formulations negatively affected several key biological parameters of *H. armigera*, including larval/pupal viability, adult longevity, oviposition period, fecundity and fertility.

Our data strongly supports the use of neem-based formulations as a tool for the management of *H. armigera*. Significantly, these formulations appear to be effective at the commercial doses recommended by the manufacturers, generating lethal and sublethal effects and affecting the insect's biology and population parameters.

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