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Effect of *Azadirachta indica* (Sapindales: Meliaceae) oil on *Spodoptera frugiperda* (Lepidoptera: Noctuidae) larvae and adults

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Abstract

Azadirachta indica A. Juss. (Meliaceae) is used to control insects due to its compounds that have insecticidal, repellent, and antifeedant properties. These effects also may cause sublethal impacts on insects that reduce populations of target species. Such species can have economic importance, like *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae), which feeds on various plant species. Thus, the objective of this study was to evaluate the larval mortality, lethal concentration, and sublethal effects on larvae, pupae, and adults of *S. frugiperda* after larvae were fed on an artificial diet containing *A. indica* oil at different concentrations. The oil was incorporated into the artificial diet at the concentrations of 5,000, 10,000, and 15,000 ppm, plus a control group. Four replicates of 35 second instar caterpillars were made for each treatment, and observed daily until pupation and emergence. The larval survival, duration of the larval and pupal periods, pupal weight and viability, wing length, and deformation of adults were evaluated. Longevity, fertility, and fecundity also were recorded for each pairing (mating arrangement) of males and females that developed on either a diet without oil and propanone, with propanone only, and with both oil and propanone. *Azadirachta indica* oil in the diet reduced survival and prolonged duration of the larval and pupal periods. Pupae in the groups that contained oil weighed less, and the adults had smaller wing length than in the control group. There was no influence on pupal viability, but the number of deformed adults increased. The oil reduced fecundity and longevity, but it did not alter fertility. These results suggest that the oil may reduce the probability of this insect remaining in the environment due to its ability to decrease the offspring and longevity of this species.

Key Words: development; fall armyworm; mortality; neem oil; reproduction

Resumo

Azadirachta indica A. Juss. (Sapindales: Meliaceae) é usada no controle de insetos devido a seus compostos que possuem atividade inseticida, de repelência e de deterrência alimentar. Esses efeitos também podem causar impactos subletais, reduzindo as populações de espécies-alvo. Espécies essas que podem ter importância econômica, como *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae) que se alimenta de várias espécies de plantas. Assim, esse estudo teve como objetivo avaliar a mortalidade larval, concentração letal e o efeito subletal em larvas, pupas e adultos de *S. frugiperda* cuja larva se alimentou de uma dieta contendo óleo de *A. indica* em diferentes concentrações. O óleo foi incorporado, com propanona, em dieta artificial em concentrações de 5.000, 10.000, e 15.000 ppm, e o grupo controle. Quatro réplicas de 35 lagartas de segundo ínstar foram feitas para cada tratamento e observadas diariamente até a pupação e a emergência. Foram avaliados a sobrevivência larval, a duração da fase larval e pupal, o peso e a viabilidade pupal, o comprimento da asa e a proporção de adultos deformados. A longevidade, fertilidade e fecundidade também foram registradas para cada combinação de machos e fêmeas que se desenvolveram em dieta sem óleo e propanona, com somente propanona ou com óleo e propanona. O óleo de *Azadirachta indica* na dieta reduziu a sobrevivência e retardou a fase larval e pupal. As pupas dos grupos que continham óleo pesaram menos e os adultos tiveram um menor comprimento de asa do que os do grupo controle. Não houve influência na viabilidade pupal, mas o número de adultos deformados foi aumentado. O óleo reduziu a fecundidade e a longevidade, mas não alterou a fertilidade. Esses resultados sugerem que o óleo pode reduzir a probabilidade do inseto permanecer no ambiente devido a sua habilidade de diminuir a prole e a longevidade dessa espécie.

Palavras Chave: desenvolvimento; lagarta-do-cartucho; mortalidade; óleo de nim; reprodução

Azadirachta indica A. Juss. (Meliaceae), a plant known as neem or Indian lilac, is originally from Southeast Asia and the Indian subcontinent (Brahmachari 2004; Campos et al. 2016). Every part of the plant, including fruits, seeds, twigs, flowers, stem, and root bark, produce active secondary compounds that have been used for centuries to control insects (Mordue (Luntz) 2004), with seed kernels the part that has the highest concentration of these compounds (Brahmachari 2004).

This plant has been used for insect control due to its more than 100 active biological compounds, principally terpenoids (Campos et al. 2016), which have insecticidal, repellent, and antifeedant properties. Azadi-

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rachtin is the principal compound responsible for antifeedant activity and toxic effects on insects (Mordue (Luntz) & Nisbet 2000) and because of this, it is the most-studied compound with respect to the mode of action. It acts on chemoreceptors by blocking receptors that respond to phagostimulants or stimulating "deterrent" cells (Mordue (Luntz) & Blackwell 1993). In addition, this compound inhibits the release of morphogenetic peptides from the brain, which results in a blockage of the synthesis of ecdysteroids, and a regulation of the release of juvenile hormone (Mordue (Luntz) & Nisbet 2000). Azadirachtin also may have other modes of action, such as inhibiting mitosis, necrotizing fat body, and acting on the muscles of flight, gut, and movement (Mordue (Luntz) & Blackwell 1993).

Azadirachtin has great importance for insect population management because it can regulate insect populations, either the current generation or the offspring in the next generation (França et al. 2017), and can even affect other organisms in the trophic chain, such as predators or parasitoids (Schmutterer 1997). Thus, knowing the effects of this oil on the biology of the target species is fundamental to developing strategies to control pest populations.

Among agricultural pests that often need to be controlled is the fall armyworm, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae). It mostly attacks plants in the family Poaceae (Capinera 2008), but also can feed on plants of other botanical families (Pogue 2002; Capinera 2008). Corn (Viana & Prates 2003) and rice (Busato et al. 2002) are the principal crops affected, but cotton, beans, tomatoes, sugar cane, peanuts, potatoes, and cabbage also may be damaged by this pest (Sarmento et al. 2002).

In addition to killing *S. frugiperda* (Lima et al. 2010; Roel et al. 2010; Campos & Boiça Jr 2012), neem oil also can increase the duration of larval and pupal periods, reduce pupal weights and viability (Lima et al. 2010; Roel et al. 2010), shorten adult longevity, increase the prevalence of morphogenetic deformities in pupae and adults (Lima et al. 2010), and cause histophysiological changes in the peritrophic membrane (Roel et al. 2010). Although several effects are known in the immature stages of this species, there is a lack of knowledge about the impact in adults that were exposed to neem oil during their larval period.

Thus, the purpose of this study was to evaluate larval mortality, lethal concentration, and sublethal effect on larvae, pupae, and adults of *S. frugiperda* after larvae were fed on an artificial diet containing different concentrations of *A. indica* oil.

Materials and Methods

REARING OF *SPODOPTERA FRUGIPERDA*

The *S. frugiperda* colony was started with pupae obtained from Promip® (Engenheiro Coelho, São Paulo, Brazil). The adults were kept in cages made of polyvinyl chloride (PVC) pipes (20 cm H × 15 cm D) with holes on the sides. They were fed with a solution of water and 20% honey, replaced every 2 d.

The caterpillars were kept individually in 50 mL plastic cups containing artificial diet adapted from Bowling (1967) (500 g cooked pinto beans, 75 g brewer's yeast, 15 g ascorbic acid, 20 g methylparaben, 2.5 g sodium benzoate, 45 g agar, and 200 mL distilled water). The insect colony and those used in the bioassays were kept in an air-conditioned room at 27 ± 2 °C, 70 ± 10% RH, and a 14:10 h (L:D) photoperiod.

APPLICATION OF *AZADIRACHTA INDICA* OIL

The *A. indica* oil (Naturalneem unemulsified pure oil, 2,000 ppm azadirachtin) was obtained from Dogneem® (Jaguariúna, São Paulo, Brazil). The oil was incorporated into the artificial diet at concentrations of 5,000, 10,000, and 15,000 ppm (1 ppm = 1 µg oil per g artificial diet). Along with the oil, 20 mL of propanone was added as an emulsifier for each 200 g of prepared diet. After homogenizing the mixture, the diet was left at room temperature for 24 h to let the propanone evaporate. To evaluate the immature stages, only propanone was added to the artificial diet of the control group. For evaluation of adults, 2 control groups were established: the larvae of 1 group consumed the same artificial diet as the original colony without additives, and the other group only propanone was added to the diet.

EFFECTS OF *AZADIRACHTA INDICA* OIL ON BIOLOGICAL PARAMETERS OF *SPODOPTERA FRUGIPERDA*

Four replicates of 35 second instar caterpillars (4 d after hatching) were used for each treatment. Each caterpillar, weighing between 2 and 3 mg, was placed in a 50 mL plastic cup containing approximately 10 g of artificial diet, plus any additives according to the treatment. The insects remained in these conditions until pupation. The pupae were then placed individually in 50 mL plastic cups containing sterilized vermiculite, weighed at 24 h, and observed daily until emergence.

The parameters evaluated for each treatment were: larval survival, larval and pupal duration, pupal weight and viability, wing length (length from base to apex of the right anterior wing), and deformed adults (any deformation of the wings).

Seven pairings (mating arrangements) were formed with the adults obtained from the diet without propanone and oil (N), with propanone only (P), and with propanone and oil at 5,000 ppm (O5): (1) ♀ N and ♂ N; (2) ♀ N and ♂ P; (3) ♀ N and ♂ O5; (4) ♀ P and ♂ P; (5) ♀ P and ♂ N; (6) ♀ O5 and ♂ N; and (7) ♀ O5 and ♂ O5. Pairings 1 to 6 were successful, but pairing 7 did not produce eggs, so it was not included in the analyses.

DATA ANALYSES

A survival analysis was performed, and the curves compared by log-rank test. The lethal concentration was calculated by Probit analysis. Data were analyzed for normality by the Shapiro-Wilk test. Larval period duration, pupal viability, male longevity, fecundity, and fertility between treatments were compared by Kruskal-Wallis followed by the Dunn test. The pupal period duration, and pupal weight, wing length, adult deformation, and female longevity were evaluated by Analysis of Variance (ANOVA) followed by the Tukey test. The statistical program SPSS version 23 (IBM 2015) was used, at a significance level of 5%.

Results

The survival curves of *S. frugiperda* caterpillars (Fig. 1) was significantly reduced when feeding on neem oil, and with increasing concentrations (χ^2 = 216.22; df = 3; P < 0.001). The lethal concentration required to cause death of 50% and 90% of the population was 9,500 and 17,230 ppm, respectively (χ^2 = 34.88; Slope = 0.17; Intercept = −1.58; P < 0.005).

The *A. indica* oil, when mixed with the artificial diet, caused a significant increase in duration of larval (H = 12.78; df = 3; P < 0.01) and pupal (F = 4.06; df = 3; P < 0.05) periods in *S. frugiperda* (Table 1). Larval duration increased by approximately 76% in the highest concentration in relation to the control group, while the pupal period increased about 2 d on average between these 2 treatments (Table 1).

The oil decreased pupal weight (F = 12.46; df = 3; P < 0.01) by approximately 29% in the highest concentration in relation to the control group. However, the oil did not influence pupal viability (H = 4.83; df = 3; P = 0.185) (Table 1).

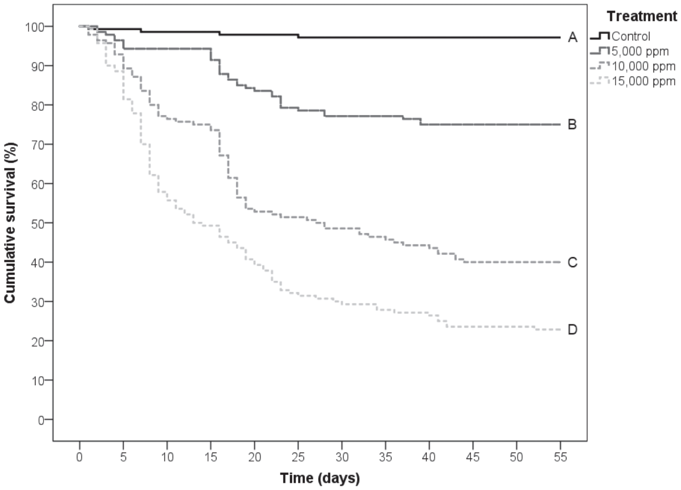

Fig. 1. Survival curves of immature *Spodoptera frugiperda* maintained on artificial diet containing propanone (control), or containing propanone and 3 concentrations (5,000, 10,000, or 15,000 ppm) of *Azadirachta indica* oil (27 ± 2 °C; 70 ± 10% RH; 14:10 h [L:D] photoperiod). Curves followed by different letters differ significantly by log-rank test ($P < 0.05$).

Wing length was reduced ($F = 4.67$; df = 3; $P < 0.05$) approximately 13% when the larvae developed on an artificial diet containing the highest concentration of this oil (Table 2). In addition, the percentage of deformed adults increased in the groups with diet containing neem at concentrations of 10,000 and 15,000 ppm ($F = 10.04$; df = 3; $P < 0.01$) by about 63% in the highest concentration in relation to control group (Table 2).

Mean fecundities (Table 3) were reduced when females or males developed on a diet containing 5,000 ppm oil in relation to control ($H = 38.24$; df = 5; $P < 0.001$). However, mean fertilities (Table 3) were not significantly altered when females or males developed on the diet with neem ($H = 7.36$; df = 5; $P = 0.20$). When both sexes developed on a diet containing neem oil at 5,000 ppm, females did not lay eggs; therefore, it was not possible to determine mean fertility. For this reason, they were not included in the analyses.

Mean longevities (Table 4), when immature insects were fed an oil-containing diet, were reduced for females by about 7.4 d ($F = 18.21$; df = 2; $P < 0.001$) and males by about 5.8 d ($H = 16.82$; df = 2; $P < 0.001$), in relation to the diet with only propanone.

Discussion

This is the first record of the effect that *A. indica* oil has on *S. frugiperda* adults when maintained on artificial diet containing oil during their larval period. The oil caused a reduction in longevity and fecundity of females, even when only the male had developed on an oil-containing diet. Effects of *A. indica* oil also were confirmed on the immature stages of *S. frugiperda*, as was previously reported (Lima et al. 2010; Roel et al. 2010; Campos & Boiça Jr 2012).

The larval mortality of *S. frugiperda* caused by *A. indica* oil at 15,000 ppm was about 77%; however, Roel et al. (2010) recorded a mortality of 100% at a much lower concentration (4,000 ppm). This is probably due to the use of newly hatched caterpillars by these authors, because, according to Yu (1983), younger *S. frugiperda* caterpillars are more susceptible to chemical insecticides. Lima et al. (2010) and Campos & Boiça Jr (2012) reported mortalities of 83%, similar to our findings, despite using a different methodology (the feeding substrates were different).

An increase in mortality related to the increase in oil concentrations recorded in this study was expected, because the effects of azadirachtin depend on both dose and exposure time (Mordue (Luntz) & Blackwell 1993). The death of these insects was probably caused by multiple factors, because the oil contains several compounds that are growth regulators, which indirectly reduce the production and release of ecdysteroids in the hemolymph, and consequently cause delay and abnormality of the molting that increases the mortality (Mordue (Luntz) 2004). In addition, azadirachtin stimulates cell death and blocks phagostimulating cells, which can lead to inhibition of feeding, and can culminate in starvation and death.

One of the main effects of neem oil is antifeedant activity, in which the insect has a low consumption or low utilization of the ingested food. Thus, some of our results, both for immature and adult, may have been due to antifeedant activity. According to Chapman (2013), the amount and quality of the diet consumed by the insect can affect their development, reproduction, and longevity.

Although dietary intake was not evaluated, individuals may not have fed adequately during the larval period on oil treatments, due to the presence of compounds such as meliantriol and salannin, which inhibit feeding (Campos et al. 2016). The reduction in food intake can result in a delay in achieving critical weight, i.e., when the insect reaches half the final weight of the larval stage and initiates hormonal regulation for metamorphosis (Nijhout et al. 2014). This delay would explain the long larval development times found in our study.

Another indication of antifeedant activity is the reduced pupal weight, because, as already discussed, after reaching critical weight, the insect irreversibly prepares for metamorphosis. As it cannot take advantage of the nutrients of the ingested food, the caterpillar cannot gain much weight before the juvenile hormone titres become depleted and the levels of ecdysteroids increase, consequently generating smaller pupae.

The reduction in pupal weight reported here also was observed by Roel et al. (2010), who noted that when the *S. frugiperda* caterpillars

Table 1. Mean (± SD) of larval and pupal development time, pupal weight, and pupal viability of *Spodoptera frugiperda* developing on an artificial diet containing propanone (control) or propanone plus various concentrations of *Azadirachta indica* oil (27 ± 2 °C; 70 ± 10% RH; 14:10 h [L:D] photoperiod). Numbers in parenthesis represent sample size.

	Development time (d)			
Treatment	Larval[a]	Pupal[b]	Pupal weight (mg)[b]	Pupal viability (%)[a]
Control	15.0 ± 0.53 A (137)	10.5 ± 0.54 A (108)	214.0 ± 3.25 A (136)	79.5 ± 9.15 A (136)
5,000 ppm	17.6 ± 1.61 AB (114)	11.1 ± 0.48 AB (51)	182.7 ± 23.02 B (104)	51.9 ± 26.09 A (104)
10,000 ppm	18.2 ± 1.38 AB (77)	11.0 ± 0.69 AB (19)	178.6 ± 16.70 BC (54)	42.3 ± 29.00 A (54)
15,000 ppm	26.5 ± 2.70 B (44)	12.3 ± 1.14 B (16)	151.4 ± 5.18 C (29)	55.4 ± 27.92 A (29)

[a]Means in the same column followed by different letters differ by the Dunn test ($P < 0.05$).
[b]Means in the same column followed by different letters differ by the Tukey test ($P < 0.05$).

Table 2. Mean (± SD) of wing length and percentage of deformation of *Spodoptera frugiperda* adults whose larval stage developed on an artificial diet containing propanone (control) or propanone and *Azadirachta indica* oil at different concentrations (27 ± 2 °C; 70 ± 10% RH; 14:10 h [L:D] photoperiod). Numbers in parentheses represent sample size.

Treatment	Wing length (mm)[a]	Deformed adults (%)[a]
Control	14.4 ± 0.21 A (97)	7.6 ± 3.26 A (110)
5,000 ppm	13.5 ± 0.29 AB (44)	28.6 ± 26.32 AB (51)
10,000 ppm	13.3 ± 1.02 AB (11)	44.4 ± 6.57 BC (19)
15,000 ppm	12.4 ± 0.88 B (4)	71.0 ± 19.77 C (16)

[a]Means followed by different letters in the columns differ by the Tukey test ($P < 0.05$).

Table 4. Mean (± SD) longevity of *Spodoptera frugiperda* adult males and females when larvae developed on an artificial diet without propanone and oil (N), with propanone only (P), or with both propanone and with *Azadirachta indica* oil at 5,000 ppm (O5) (27 ± 2 °C; 70 ± 10% RH; 14:10 h [L:D] photoperiod). Numbers in parentheses represent sample size.

	Longevity (d)	
Treatment	Females[a]	Males[b]
Diet N	11.9 ± 3.69 A (49)	9.4 ± 3.52 A (39)
Diet P	14.1 ± 4.99 A (24)	10.9 ± 5.15 A (28)
Diet O5	6.7 ± 4.18 B (20)	5.1 ± 2.82 B (14)

[a]Means in each column followed by different letters differ by the Dunn test ($P < 0.05$).
[b]Means in each column followed by different letters differ by the Tukey test ($P < 0.05$).

were exposed to artificial diet containing 500 ppm of *A. indica* oil, the pupal weight was approximately 19% lower than non-exposed insects. In contrast, Lima et al. (2010), with this same species, did not find any difference between the average pupal weight of the control group in relation to caterpillars developed on corn leaves with neem. The fact that the pupal weight was not reduced in this last work was probably because the authors exposed the caterpillars for only 48 h to plants immersed in neem; thus, by the time they reached the critical weight, their weight gain could have normalized.

The reduction of the average pupal weight in the group exposed to the concentration of 15,000 ppm also may have resulted in increased duration of the pupal period. Low nutritional reserves likely increase the duration of the pupal period, because during metamorphosis the larval reserves are used for pupal development, adult support, reproduction, and survival (Merkey et al. 2011). In addition to longer pupal period, reduction in pupal weight also was associated with shorter wing length. As noctuids with larger wings can travel faster and greater distances (Jones 2014), this effect can cause an impact in the field, reducing the dispersal capacity of adults and making it difficult to search for partners, food resources, and oviposition sites.

The difficulty of assimilating the nutrients during the larval period may be inferred by the reduced number of eggs when females or males were exposed to the diet containing neem oil. Arrese & Soulages (2010) stated that the amount of nutrients stocked during the larval period greatly impacts the adult and can reduce their fecundity. *Spodoptera eridania* Stoll (Lepidoptera: Noctuidae) females that emerged from smaller pupae had a lower fecundity than those that emerged from larger ones (Specht et al. 2015).

Longevity also was affected by exposure to *A. indica* oil. Lima et al. (2010) found that *S. frugiperda* caterpillars that had fed on corn leaves with neem oil had reduced longevity. Ingestion of the oil in adulthood also may reduce longevity, as observed by Pineda et al. (2009) in *Spodoptera littoralis* Boisduval (Lepidoptera: Noctuidae) when adults ingested water and honey solution containing different concentrations of azadirachtin. The shorter longevity can have an impact in the field, because it reduces the time to find a partner and the time that the female has to lay eggs.

Table 3. Mean (± SD) fecundity and fertility (%) from mated pairs of *Spodoptera frugiperda* that developed on an artificial diet without propanone and oil (N), with propanone only (P), or with both propanone and *Azadirachta indica* oil at 5,000 ppm (O5) (27 ± 2 °C; 70 ± 10% RH; 14:10 h [L:D] photoperiod). Numbers in parentheses represent sample size.

Pairings	Fecundity[a]	Fertility[a]
♀ N × ♀ N	904.4 ± 261.68 AB (22)	55.6 ± 16.55 A (22)
♀ N × ♀ P	729.2 ± 556.05 B (19)	36.4 ± 35.14 A (17)
♀ N × ♀ O5	223.5 ± 355.26 C (10)	50.4 ± 33.80 A (4)
♀ P × ♀ P	1216.1 ± 370.24 A (9)	47.9 ± 26.73 A (9)
♀ P × ♀ N	827.4 ± 585.64 AB (15)	48.5 ± 28.67 A (12)
♀ O5 × ♀ N	153.6 ± 244.57 C (16)	23.3 ± 29.58 A (7)

[a]Means in the same column followed by different letters differ by the Dunn test ($P < 0.05$).

About 45% of the caterpillars that developed on a diet containing neem oil at 5,000 ppm died in the prepupal stage in our study. Lima et al. (2010) attributed the mortality in this period to the reduction of ecdysone concentration, or the delay of its release in the hemolymph. Prepupal mortality has been reported for other noctuids such as *Spodoptera exempta* Walker (Tanzubil & McCaffery 1990), *S. frugiperda* (Lima et al. 2010), *S. litorallis* (Martinez & Van Emden 2001), *Spodoptera litura* F. (Gujar & Mehrotra 1983), and *Spodoptera mauritia* Boisduval (Jagannadh & Nair 1992) (all Lepidoptera: Noctuidae) when evaluating larval development in individuals exposed to neem oil, neem extract, or just azadirachtin.

The increased duration of the pupal period observed in this study was observed also by Roel et al. (2010) who evaluated the effect of *A. indica* oil in artificial diet on the same insect species. This increase may have been influenced by the 20E deficiency, triggered by some of the limonoids present in neem oil (Campos et al. 2016).

Hormonal dysfunctions also may have affected adults, even though they were only exposed to the active compounds during their larval stage. The increase in the percentage of deformation of adults also may be related to hormonal changes, as discussed by Schneider et al. (2017), who tested neem oil on *Diatraea saccharalis* F. (Lepidoptera: Crambidae), and recorded up to 50% of deformation in emerged adults. Martinez & Van Emden (2001), evaluating *S. littoralis* caterpillars exposed to the artificial diet containing azadirachtin, found that approximately 47% of the adults that emerged from the highest concentration tested (1 ppm azadirachtin in an artificial diet) had some type of deformation, such as twisted wings that did not expand fully. The authors commented that such abnormalities may result in changes in an insect's vital activities, such as feeding and locomotion, which makes it more vulnerable to several mortality agents or prevents it from damaging crops.

Martinez & Van Emden (2001) found a lower percentage of deformation in adults than in the present study; however, they evaluated the effect of only 1 of the compounds present in neem oil, azadirachtin. Campos et al. (2016) mentioned the existence of more than 100 biological compounds active in the oil besides azadirachtin, such meliantriol, nimbin, nimbidin, nimbinin, nimbolides, and salannin. Thus, synergistic effects may occur when more than 1 active compound is evaluated.

The results indicate that neem oil has both lethal and sublethal effects on *S. frugiperda*, and causes several developmental problems, which reduce the number and health of offspring generated, and increase the probability of death by natural enemies, thus reducing the persistence of this pest in the environment.

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