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Sublethal effects of growth-regulating insecticides of synthetic and botanical origins on the biological parameters of *Spodoptera frugiperda* (Lepidoptera: Noctuidae)

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Abstract

The objective of this study was to evaluate the effects of growth-regulating insecticides of synthetic (e.g., Certero 480 SC, Intrepid 240 SC, Match EC and Mimic 240 SC) and botanical origins (e.g., Azamax 1.2 EC, Agroneem 850 EC, Azact 2.4 EC and Fitoneem 850 EC) on the biological parameters and fertility life table of *Spodoptera frugiperda* (J.E. Smith) under laboratory conditions. Larvae were fed insecticides that were incorporated into artificial diets. To develop the fertility life table, the following biological parameters were evaluated: survival at 7 days after infestation (d.a.i) and survivorship at adult eclosion, duration of the neonate-to-adult eclosion period, larval and pupal weights and total fecundity (number of total eggs per female). The results indicated that *S. frugiperda* neonates surviving LC_{25} or LC_{50} concentrations of the evaluated insecticides showed longer larval and egg-to-adult periods, lower larval and pupal weights and reduced fecundity, when compared to the control treatment. Larvae exposed to Azamax at LC_{25} or LC_{50} concentrations showed the greatest increase in generation duration (75 d). In addition, *S. frugiperda* adults emerged from pupae when larvae reared on an artificial diet containing growth regulating insecticides of synthetic and botanical origins produced fewer females per female per generation (R_o). As well as, lower rates of natural population increase per day (r_m) compared to insects fed the control diet. Our findings indicated that, neem-derived products and growth-regulating insecticides of synthetic origin may be employed within integrated management strategies that aim to keep populations of *S. frugiperda* below levels that cause economic damage. Similarly, they offer alternatives for insecticide resistance management programs.

Introduction

The fall armyworm, *Spodoptera frugiperda* (J.E. Smith, 1797) (Lepidoptera: Noctuidae), is currently considered one of the most important agricultural pests in the main commodity-producing regions of the world (Goergen *et al.*, 2016; Bateman *et al.*, 2018; Sun *et al.*, 2021). In the Brazilian agricultural landscape, *S. frugiperda* stands out for its very high biotic potential and generates approximately eight generations per year (Montezano *et al.*, 2019). In addition, this species has a high capacity for dispersion, migration, and polyphagia, which favors their occurrence in different host plants of economic importance (for example maize, soybean, and rice) (Montezano *et al.*, 2019).

In Brazil and other places where *S. frugiperda* occurs, the main strategies used for its management in corn crops are applications of synthetic insecticides (e.g., pyrethroids, diamides, spinosyns, carbamates, and organophosphates, among others) (Carvalho *et al.*, 2013; Burtet *et al.*, 2017). Additionally, genetically modified plants express insecticidal proteins from *Bacillus thuringiensis* Berliner (*Bt*) (Bernardi *et al.*, 2012; Yano *et al.*, 2015; Marques *et al.*, 2017). However, inappropriate use of these technologies has contributed to the rapid evolution of *S. frugiperda* resistance to insecticides (Diez-Rodríguez and Omoto, 2001; Carvalho *et al.*, 2013; Nascimento *et al.*, 2016; Okuma *et al.*, 2018; Bolzan *et al.*, 2019) and *Bt* proteins (Bernardi *et al.*, 2016; Horikoshi *et al.*, 2016). This scenario has prompted new studies aimed at developing new strategies and approaches, as well as alternative methods for the management of fall armyworm (Paredes-Sánchez *et al.*, 2021).

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Using products that have low toxicity to beneficial insects and nontarget organisms and have reduced environmental impact are important strategies to be incorporated into integrated pest management (IPM) programs (Dhadialla *et al*., 1998; Lira *et al*., 2020). In this context, insecticides that regulate insect growth (IRGs) may play an important role in the management of *S. frugiperda* (Toscano *et al*., 2012), including both those of synthetic and natural origins.

Among products with natural origins, botanical insecticides have expanded their market significantly in recent years (Isman, 2020). However, their contribution is still small compared to that of synthetic insecticides. Botanical insecticides are produced from substances produced by plant secondary metabolisms (allelochemicals); they generally have a broad spectrum of action against pest arthropods and may cause lethal toxicity or behavioral changes, such as repellency and deterrence of feeding and oviposition (Ribeiro *et al*., 2016; Bernardi *et al*., 2017; Tak and Isman, 2017; Geisler *et al*., 2019). In addition, the rapid degradation of these compounds in the environment (photodegradable) causes these products to be easily accepted by the community as safer and more sustainable (Amoabeng *et al*., 2014; Isman, 2020).

Among the most well-known and widespread botanical insecticides are those produced from neem, *Azadirachta indica* (Juss) (Meliaceae), whose main active ingredient is the tetranortriterpenoid, azadirachtin (Campos *et al*., 2016; Isman, 2020). Azadirachtin may exert anti-feeding (acting on insect chemoreceptors) and repellent actions (Isman, 2006; Qin *et al*., 2019). In addition, it can interfere with the development of endocrine glands that control insect metamorphosis by compromising the ecdysis process (Mordue (Luntz) and Blackwell, 1993). Studies have demonstrated high acute lethal toxicity levels of this compound on arthropod-pests with different feeding habits, such as leafminers (fly and moth larvae); suckers (mites, thrips, aphids, and bed bugs); and chewers (larvae and scarabs) (Adhikari *et al*., 2020), including effects on larvae and adults of *S. frugiperda* (Duarte *et al*., 2019). Despite these promising bioactive effects, the use of IRGs has not been widespread in IPM programs due to the incessant search for products with fast action (knock-down effects, especially) in pest control (Singh and Suri, 2013).

In addition to their lethal effects, neem-based insecticides and growth regulators of synthetic origin can produce sublethal effects at certain concentrations, such as increases in larval and pupal periods, reductions in pupal weight and viability and increases in the percentage of adults with malformations (Lima *et al*., 2010; Roel *et al*., 2010; Duarte *et al*., 2019). These effects can directly influence insect population dynamics in future generations and favor management strategies (Cabodevilla *et al*., 2011). Based on this assumption, the objective of this study was to evaluate the sublethal effects of growth regulating insecticides of synthetic and botanical origins on the biological parameters and fertility life table of *S. frugiperda*, under controlled laboratory conditions.

Material and methods

Tested insects

Specimens used in the tests came from a population of *S. frugiperda* collected from conventional (non-Bt) corn during the 2012/2013 season in Mogi Mirim, São Paulo, Brazil (22°28′31″ S and 46°54′21″ W). In the laboratory, the larvae were maintained for more than 25 generations on an artificial diet (Kasten *et al*., 1978). For adult feeding, a 10% ($v.v^{-1}$) honey solution was used. The moths were kept in cylindrical PVC cages (24.0 cm height × 14.5 cm diameter), which were internally lined with newsprint as a substrate for oviposition, and closed at the top with voile fabric.

Concentration-response curves

To characterize the concentration-response curves for each product (Table 1), bioassays using the method of incorporating an artificial diet as proposed by the Insecticide Resistance Action Committee (Method 20, IRAC International) were used (IRAC 2011). For this purpose, six concentrations of each insecticide (range: 312.5–10,000 $mg\,kg^{-1}$), diluted in distilled water, were used (IRAC 2011). As a negative control, distilled water was used. For the bioassays, we used the artificial diet proposed by Kasten *et al*. (1978), which is commonly used for rearing *S. frugiperda*. The insecticide concentrations were incorporated into the diets at temperatures between 45 and 50°C, with the aid of a vortex-type shaker for 2 min. Subsequently, the diets were distributed in 16-well plastic plates (Advento do Brasil, São Paulo, Brazil) (4 ml per well). After cooling the diets, one *S. frugiperda* neonate larva (0–24 h old) was added to each well using a fine brush. The plates were closed and maintained in a chamber at 25 ± 1°C, 60 ± 10% RH, and a photoperiod of 14:10 (L:D) h. For each concentration, 8 repetitions (wells) were used, each repetition consisted of 5 larvae, totaling 80 larvae per concentration. Larval mortality was evaluated at 7 days. Larvae that survive beyond the first instar were also considered dead.

Table 1. Botanical and synthetic growth regulating insecticides evaluated against *Spodoptera frugiperda*

Active ingredient	Commercial name	Chemical group	Manufacturer
Purified limonoids (azadirachtin + 3-tigloyl-azadiractol)	Azamax 12 EC	Tetranortriterpenoid	UPL Brasil
neem crude oil	Azact 2.4 EC	Tetranortriterpenoid	Lacsa Company
Neem crude oil	Fitoneem 850 EC	Tetranortriterpenoid	Dalneem
Neem crude oil	Agroneem 850 EC	Tetranortriterpenoid	Agrovant
Lufenuron	Match EC	Benzoylurea	Syngenta
Methoxyfenozide	Intrepid 240 SC	Diacylhydrazine	Corteva
Triflumuron	Certero 480SC	Benzoylurea	Bayer
Tebufenozide	Mimic 240 SC	Diacylhydrazine	Iharabras

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Sublethal effects insecticides on Spodoptera frugiperda

To evaluate the sublethal effects of the insecticides on *S. frugiperda*, LC_{25} and LC_{50} concentration of each insecticide (Table 2) were used in bioassays when incorporated in artificial diets (IRAC 2011). For this, the defined concentrations were added to the artificial diet as described by Kasten *et al.* (1978) when the temperature of the diet reached 45–50°C. The incorporations were performed with a vortex tube shaker for 2 min. Diets containing the incorporated products were distributed in glass containers (8.0 cm height × 2.4 cm diameter, with a total volume of 64.84 cm^2) containing 4 ml diet $tube^{-1}$ with the aid of a repeat dosing pipette. After gelling and cooling the diets and with the aid of a fine brush, neonate *S. frugiperda* larvae were inoculated (1 larva < 24 h age) in each tube. The tubes were sealed with hydrophilic cotton to allow gas exchange with the external environment, and kept in a climatized room (temperature 25 ± 1°C, relative humidity 60 ± 10% and photophase 14:10 h L:D). For each insecticide concentration (LC_{25} or LC_{50}), 10 replicates (tubes) were used, with 16 larvae/repetition, totaling 160 larvae per insecticide.

The following biological parameters of the immature stages were evaluated: survival (%) at 7 days after infestation (d.a.i) and survivorship at adult eclosion, duration (days) of neonate-to-adult eclosion, larval weight (mg) at 7 d postinfection (d.p.i.), and pupal weight (mg) 24 h after pupal formation. The total fecundity (number of total eggs per female) was evaluated by forming 20 couples/treatment of *S. frugiperda*, which were paired separately in PVC cages (23 cm height × 10 cm diameter), which were internally coated with white paper and closed with sheer fabric. The number of eggs was counted daily and the mortality of adults was recorded.

Statistical analyses

To estimate the lethal concentrations (LC_{25} and LC_{50}) and respective CIs, the concentration-mortality data of each product were subjected to Probit analysis (PROC PROBIT, SAS® Institute 2000, Cary, NC). A likelihood ratio test was conducted to test the hypothesis that the LC values were equal. If the hypothesis was rejected, pairwise comparisons were performed, and significance was declared if the CIs did not overlap (Robertson *et al.*, 2007). To evaluate the sublethal effects of the insecticides on *S. frugiperda* receiving artificial diets, data from all biological parameters evaluated [survival at 7 d.a.i and survivorship at adult eclosion (%), duration of neonate-to-adult eclosion, larval weight, pupal weight and total fecundity of *S. frugiperda*] were evaluated for normality by the Shapiro-Wilk test and homoskedasticity by the Hartley and Bartlett test. Subsequently, data were subjected to a two-way ANOVA using the PROC GLM procedure in SAS® 9.1 (SAS Institute, 2011). Factor A was represented by the insecticide concentrations (LC_{25} or LC_{50}). Factor B was composed of insecticides (e.g., Certero, Match, Intrepid, Mimic, Azamax, Agroneem, Azact and Fitoneem) and untreated controls. The insecticide concentrations and interactions were used as fixed factors in the model. Mean differences were calculated by the Least-Square Means Statement (LSMEANS option of PROC GLM) using a Tukey-Kramer adjustment test ($P < 0.05$) in SAS® 9.1 (SAS Institute, 2011).

Survivorship, development time, and reproductive data were used to estimate the population growth parameters, such as the mean length of one generation (T), net reproductive rate (R_o; average number of female offspring that would be born to a cohort of females), and intrinsic rate of population increase (r_m; daily production of females per parental female). Fertility life table parameters were obtained using the jackknife technique by applying the '*lifetable.sas*' procedure developed by Maia *et al.* (2000) in SAS® 9.1 (SAS Institute, 2011).

Results

Concentration-response curves

Overall, *S. frugiperda* larvae were more susceptible to growth-regulating insecticides than compared to neem-based insecticides (Table 2). When comparing the susceptibilities to the neem-based insecticides, it was observed that Azact was more toxic to larvae and had the lowest LC_{25} and LC_{50} values ($LC_{25} = 102.4$ and $LC_{50} = 210.5$ – Table 2) compared to Agroneem ($LC_{25} = 653.7$ and $LC_{50} = 1{,}115.3$), Azamax ($LC_{25} = 271.3$ and $LC_{50} = 700.5$) and Fitoneem ($LC_{25} = 125.2$ and $LC_{50} = 198.3$) (Table 2). The values for the growth-regulating insecticides Certero ($LC_{25} = 37.8$ and $LC_{50} = 69.4$ – Table 2), relative to the growth-regulating insecticides, Intrepid ($LC_{25} = 66.9$

Table 2. Concentration-mortality response (LC; g or ml of commercial product) of *S. frugiperda* neonates exposed to the artificial diet treated with different concentrations of botanical and synthetic growth regulating insecticides

Treatments	*Slope* ± SE	LC_{25} (95% FL)[a,b]	LC_{50} (95% FL)[a,b]	χ^2[c]	d.f[d]
Agroneem	3.97 ± 0.98	653.7 (612.4–693.2)a	1115.3 (1100.2–1170.3)a	6.7	6
Azact	4.12 ± 0.25	102.4 (95.3–112.4)d	210.5 (198.4–232.9)c	5.3	6
Azamax	3.82 ± 0.81	271.3 (245.8–290.9)b	700.5 (654.3–847.8)b	7.5	6
Fitoneem	3.14 ± 0.79	125.2 (117.6–132.5)c	198.3 (175.3–211.9)c	2.4	6
Certero	3.72 ± 0.39	37.8 (29.1–45.6)g	69.4 (60.1–82.3)f	8.9	6
Intrepid	4.07 ± 0.97	66.9 (54.2–78.5)f	134.7 (125.4–150.2)e	4.2	6
Match	3.90 ± 0.45	89.4 (85.3–94.3)e	165.4 (160.3–169.7)d	6.3	6
Mimic	3.96 ± 0.11	43.2 (39.4–52.6)g	74.5 (69.4–82.5)f	7.4	6

[a] LC_{25} and LC_{50}: concentration of commercial product (g or ml per 100 l^{-1} of water) required to kill 25 and 50% of larvae in the observation period of 7 days, respectively.
[b] LC_{25} and LC_{50} values designated by different letters within a column are significantly different from each other through non-overlap of 95% fiducial limits. Significance of differences among slopes determined by likelihood ratio test of equality followed by pairwise comparisons using non-overlapping fiducial limits.
[c] χ^2 significant.
[d] Degrees of freedom.

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and $LC_{50} = 134.7$), Match ($LC_{25} = 89.4$ and $LC_{50} = 165.4$) and Mimic ($LC_{25} = 43.2$ and $LC_{50} = 74.5$) (Table 2) were verified.

Survivorship

The larval survival of *S. frugiperda* neonates was significantly affected from the use of the LC_{25} or LC_{50} concentration values of the products at 7 d.p.i. (LC_{25}: $F = 96.0$; df = 8, 81; $P < 0.0001$ and LC_{50}: $F = 84.1$; df = 8, 81; $P < 0.0001$, respectively). Azamax 12 EC caused the lowest larval survival of *S. frugiperda* at 7 d.a.i, which was similar to the results for growth-regulating insecticides of synthetic origin (e.g., Certero 480 SC, Match EC, Intrepid 240 SC, and Mimic 240 SC) at the LC_{25} (variations in survival between 54.2 to 59.6%) and LC_{50} (variations in survival between 51.1 to 55.3%) concentrations (Table 3). In contrast, for the other products of botanical origin (e.g., Agroneem 850 EC, Azact 1.4 EC, and Fitoneem 850 EC), neonate survival was equal to or greater than 80% at LC_{25} and above 66% at the LC_{50} concentration (Table 3). In the same way, there were significant interactions between the artificial diets and concentrations evaluated (LC_{25} or LC_{50}) ($F = 112.9$; df = 8, 81; $P < 0.0001$ and $F = 111.3$; df = 8, 81; $P < 0.0001$) regarding survivorship at 7 d (fig. 1a) and for survivorship at adult eclosion for all products (fig. 1b). However, Azamax produced the lowest rates of survival to adulthood for both the LC_{25} (46.2%) and LC_{50} (42.2%) concentrations, which were statistically similar to treatments with growth-regulating insecticides of synthetic origin (Table 3).

Table 3. Survivorship (% ± SE) of *S. frugiperda* neonates developing on artificial diet treated with different concentrations of botanical and synthetic growth regulating insecticides

Treatments	Larval survivorship 7 d.a.i (%)[a]	Survivorship at adult eclosion (%)[a]
LC_{25} values for each product[b]		
Control	90.6 ± 2.89a	84.1 ± 3.10a
Agroneem	80.4 ± 2.57b	67.2 ± 2.57b
Azact	80.1 ± 2.63b	67.8 ± 2.63b
Azamax	59.6 ± 2.60c	55.3 ± 2.60c
Fitoneem	79.0 ± 2.63b	70.4 ± 2.57b
Certero	58.7 ± 2.70c	53.9 ± 2.71c
Intrepid	54.2 ± 2.65c	54.5 ± 2.65c
Match	56.2 ± 2.41c	51.1 ± 2.91c
Mimic	56.7 ± 2.73c	53.5 ± 2.68c
LC_{50} values for each product[b]		
Control	91.4 ± 3.11a	87.3 ± 2.85a
Agroneem	68.3 ± 2.68b	54.1 ± 3.11b
Azact	66.8 ± 2.64b	57.9 ± 2.45b
Azamax	46.2 ± 2.91c	42.2 ± 2.85c
Fitoneem	69.0 ± 3.10b	56.8 ± 2.45b
Certero	41.2 ± 1.87c	43.8 ± 2.11c
Intrepid	41.9 ± 1.98c	41.5 ± 3.10c
Match	43.2 ± 1.62c	43.2 ± 2.41c
Mimic	41.2 ± 2.01c	45.4 ± 2.92c

[a]Means ± SE within a column followed by the same letter in each at concentration evaluated are not significantly different (LSMEANS followed by Tukey-Kramer test; $P > 0.05$).
[b]LC_{25} or LC_{50} concentrations used against *S. frugiperda* neonates.

Development

Regardless of the product or concentration tested (LC_{25} or LC_{50}), incorporation of insecticides based on limonoids extracted from neem and growth-regulating insecticides of synthetic origin in an artificial diet resulted in a significant increase in duration of the larval stage and the duration (neonate to adult) of *S. frugiperda* compared to the negative control (diet only) (Table 4). The botanical insecticide, Azamax, was associated with the longest duration (longer than 70 days) of the neonate to adult period in relative to all other treatments, regardless of the concentration used (Table 4 – LC_{25}: $F = 96.8$; df = 8, 119; $P < 0.0001$ or LC_{50}: $F = 69.4.82$; df = 8, 119; $P < 0.0001$). However, larvae fed crude neem oil (e.g., Agroneem, Azact, and Fitoneem) had shorter neonate to adult periods (∼46 days for LC_{25} and ∼56 days for LC_{50} concentrations) (Table 4). However, *S. frugiperda* larvae fed artificial diets treated with growth-regulating insecticides of synthetic origin from the benzoylurea (e.g., Certero and Match) and diacylhydrazine (e.g., Intrepid and Mimic) groups had neonate-to-adult durations of ∼52 to 57 days at LC_{25} and ∼ 60 days at LC_{50} concentrations (Table 4). In turn, larvae fed untreated diets (negative control) had developmental periods of 30 days (Table 4). The neonate-to-adult periods for larvae exposed to LC_{50} concentrations were consistently longer than those for larvae fed artificial diets treated with LC_{25} concentrations for all products evaluated (fig. 1c). The weights of larvae (7 days) were also significantly affected when neem-based products or synthetic growth regulators were added to the artificial diet (Table 4). The lowest larval weights were observed in treatments consisting of products of synthetic origin (LC_{25} or LC_{50}) (Table 4 – LC_{25}: $F = 117.12$; df = 8, 84; $P < 0.0001$; LC_{50}: $F = 99.4$; df = 8, 80; $P < 0.0001$). By comparing the results when LC_{25} and LC_{50} concentrations were used in the artificial diets it was found that larvae fed artificial diets containing LC_{50} insecticide concentrations consistently had significantly lower weights (fig. 1d). In addition, pupal weights (24 h) were also significantly affected by all treatments tested relative to the control treatment (Table 4 – LC_{25}: $F = 103.4$; df = 8, 81; $P < 0.0001$; LC_{50}: $F = 117.9$; df = 8, 81; $P < 0.0001$). The surviving larvae fed artificial diets treated with LC_{50} concentrations of the insecticides produced pupae with lower weights than those that developed with LC_{25} concentrations (fig. 1e). However, there were no significant differences among insecticides based on limonoids extracted from neem and growth-regulating insecticides of synthetic origin (Table 4).

Reproduction and population growth

Regardless of the concentration used (LC_{25} or LC_{50}) and product tested, *S. frugiperda* females receiving such treatments showed lower total fecundities (number of eggs/female) in relation to control (Table 4 – LC_{25}: $F = 169.8$; df = 8, 136; $P < 0.0001$; LC_{50}: $F = 197.3$; df = 8, 136; $P < 0.0001$). Greatest reductions were observed in females from larvae fed an artificial diet content benzoylurea triflumuron (Certero) in LC_{25} or LC_{50} concentrations. However, females from larvae that developed on artificial diets treated with the LC_{50} concentration had significantly lower fecundities than those exposed to the LC_{25} concentrations (fig. 1f).

According to the estimated life table parameters (Table 5), all larvae maintained on artificial diets containing growth-regulating

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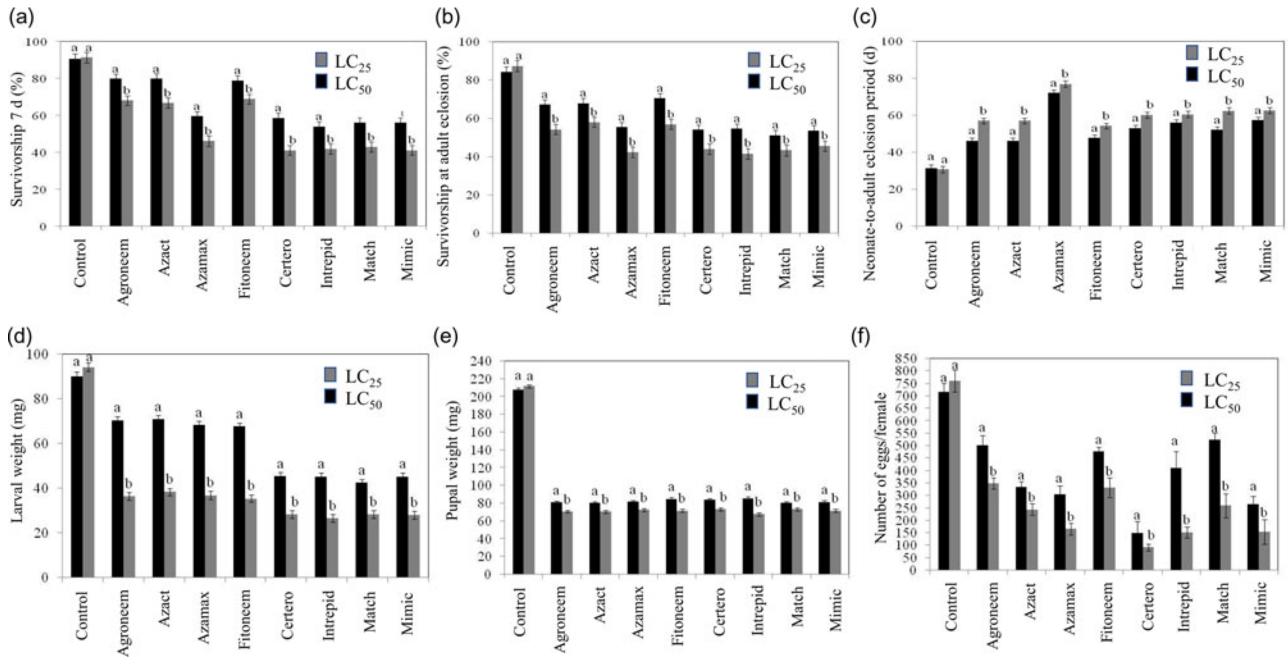

Fig. 1. Survivorship (a), Survivorship at adult eclosion (b), neonate-to-adult eclosion period (c), larval weight (d), pupal weight (e) and number of eggs/female (f) of *S. frugiperda* developing on artificial diet treated with different concentrations of botanical and synthetic growth regulating insecticides. Pairs of bars (± SE) with the same letter are not significantly different (LSMEANS followed by Tukey test; $P > 0.05$).

Table 4. Biological parameters (mean ± SE) of *S. frugiperda* developing on artificial diet treated with different concentrations of botanical and synthetic growth regulating insecticides

Treatments	Neonate-to-adult eclosion period (d)[a]	Larval weight at 7 d (mg)[a]	Pupal weight (mg)[a]	Number of eggs/female[a]
LC_{25} values for each product[b]				
Control	31.3 ± 1.89d	95.2 ± 1.89a	207.7 ± 1.93a	716.1 ± 32.4a
Agroneem	46.2 ± 1.63c	70.3 ± 1.57b	80.6 ± 1.63b	500.5 ± 39.6b
Azact	46.0 ± 1.60c	70.8 ± 1.63b	80.1 ± 1.44b	332.1 ± 23.6c
Azamax	72.1 ± 1.59[a]	68.2 ± 1.63b	81.5 ± 1.92b	303.6 ± 33.9c
Fitoneem	47.7 ± 1.62c	67.5 ± 1.60b	84.6 ± 1.87b	475.4 ± 18.5b
Certero	53.0 ± 1.70b	45.2 ± 1.70c	83.4 ± 1.32b	148.1 ± 47.6d
Intrepid	56.0 ± 1.65b	45.1 ± 1.65c	85.3 ± 1.24b	409.4 ± 67.3b
Match	52.0 ± 1.65b	42.3 ± 1.63c	80.2 ± 1.76b	522.1 ± 27.4b
Mimic	57.3 ± 1.68b	45.1 ± 1.68c	81.1 ± 1.40b	263.7 ± 33.9c
LC_{50} values for each product[b]				
Control	30.6 ± 1.89d	98.1 ± 1.80a	211.3 ± 2.27a	760.3 ± 44.8a
Agroneem	56.9 ± 1.63c	36.3 ± 1.60b	70.4 ± 1.65b	348.6 ± 21.4b
Azact	56.9 ± 1.64c	38.2 ± 1.64b	70.1 ± 1.85b	243.6 ± 22.9c
Azamax	76. 9 ± 1.84[a]	36.7 ± 1.84b	72.3 ± 1.42b	165.3 ± 23.5d
Fitoneem	54.2 ± 1.60c	35.2 ± 1.60b	71.4 ± 1.32b	330.6 ± 39.1b
Certero	60.1 ± 1.73b	28.1 ± 1.73c	72.8 ± 1.87b	90.8 ± 14.0e
Intrepid	60.4 ± 1.76b	26.3 ± 1.76c	67.3 ± 1.90b	151.2 ± 21.8d
Match	62.3 ± 1.81b	28.2 ± 1.34c	72.9 ± 1.63b	258.6 ± 47.5c
Mimic	62.5 ± 1.72b	27.9 ± 1.55c	71.2 ± 1.32b	153.2 ± 49.1d

[a]Means ± SE within a column followed by the same letter in each at concentration evaluated are not significantly different (LSMEANS followed by Tukey-Kramer test; $P > 0.05$).
[b]LC_{25} or LC_{50} concentrations used against *S. frugiperda* neonates.

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Table 5. Fertility life table parameters of *Spodoptera frugiperda* developing on artificial diet treated with sublethal concentrations (LC_{25} or LC_{50}) of different growth regulating insecticides of synthetic and botanical origin

	Fertility life table parameters[a,b]		
Treatments	*T* (days)	R_o (♀/♀)	r_m (♀/♀*day)
LC_{25} values for each product			
Control	36.6 ± 0.46e	253.5 ± 5.44a	0.151 ± 0.002a
Agroneem	51.2 ± 0.39c	154.6 ± 4.11b	0.094 ± 0.003c
Azact	44.6 ± 0.39d	100.6 ± 6.10d	0.108 ± 0.002b
Azamax	75.2 ± 1.28a	84.8 ± 3.12e	0.063 ± 0.001e
Fitoneem	52.9 ± 0.53c	130.1 ± 2.10c	0.094 ± 0.005d
Certero	47.7 ± 0.33d	138.0 ± 1.17c	0.085 ± 0.003d
Intrepid	56.3 ± 0.32c	135.7 ± 3.89c	0.086 ± 0.003d
Match	66.0 ± 0.47b	139.8 ± 4.24c	0.085 ± 0.001d
Mimic	51.0 ± 0.46c	133.8 ± 1.80c	0.088 ± 0.002d
LC_{50} values for each product			
Control	36.4 ± 0.61f	250.5 ± 7.11a	0.155 ± 0.003a
Agroneem	61.8 ± 0.59c	91.1 ± 2.10b	0.073 ± 0.003b
Azact	60.3 ± 0.51c	84.9 ± 3.11c	0.071 ± 0.003bc
Azamax	80.9 ± 0.44a	45.4 ± 3.12e	0.045 ± 0.005d
Fitoneem	59.5 ± 0.54c	59.2 ± 2.67d	0.068 ± 0.002bc
Certero	55.0 ± 0.93d	51.4 ± 3.14d	0.067 ± 0.002bc
Intrepid	62.4 ± 0.46c	54.7 ± 2.80d	0.061 ± 0.001c
Match	69.4 ± 0.39b	54.3 ± 2.90d	0.064 ± 0.006c
Mimic	55.8 ± 1.04d	56.3 ± 2.10d	0.078 ± 0.004b

[a] *T*, mean length of a generation (d); R_o, net reproductive rate (females per female per generation); and r_m, intrinsic rate of population increase (per day).
[b] Means ± SE within a column followed by the same letter in each concentration (LCs) are not significantly different (*t*-test for pairwise group comparisons, $P > 0.05$).

insecticides of synthetic and botanical origins showed significant effects on their fertility life table parameters compared to the negative control (Table 5). The botanical insecticide, Azamax caused the greatest increases in duration of each generation, which were (T) ~75 and 80.9 d when the larvae were exposed to artificial diets treated with LC_{25} or CL_{50} concentrations respectively. In addition, fewer than ~84.8 and 45.4 females/newborn female/generation (R_o) were produced, respectively, whereas the progeny survivors in the control group produced approximately 250 females/female over ~36 d (Table 5). The female progeny of the survivors of the diet containing Azamax exhibited natural population increase rates (r_m) lower than 0.063 and 0.045 on LC_{25} at LC_{50} concentrations respectively, indicating an approximately 60 to 70% lower capacity for population increase when compared to the control treatment (Table 5). Certero, Intrepid, Match and, Mimic at LC_{25} and LC_{50} concentrations also showed significant reductions in the R_o and r_m values relative to the control treatment (Table 5).

In all comparisons between artificial diets treated with LC_{25} insecticide concentrations and diets with LC_{50} insecticide concentrations, the population growth parameters were always lower for artificial diets treated with LC_{50} levels (Table S1). These findings indicate that the more pronounced sublethal effects of insecticides at LC_{50} levels provide a greater population suppression of *S. frugiperda* over time.

Discussion

The results obtained in the present study demonstrated the potential sublethal effects of growth-regulating insecticides of botanical and synthetic origins on the developmental parameters of *S. frugiperda*. Additionally, this study demonstrated the potential for using these insecticides as an alternative strategy to manage this important pest species. The main bioactive effects observed were on the viability of the developmental stages (larval and pupal) and duration of the biological cycle (egg-adult). These aspects can considerably influence the population dynamics and demographic growth of the studied pest species under field conditions. This fact is already demonstrated by the estimated parameters of the life table and fertility.

Limonoid-rich neem-derived products (especially azadirachtin) have multiple modes of action against target species (Isman, 2020). In addition to direct action on the production and release of ecdysteroids in hemolymph, the insecticide also blocks phago-stimulating cells, which leads to inhibition of feeding and delayed development (Mordue (Luntz), 2004; Duarte *et al.*, 2019). In turn, the effects caused by growth-regulating insecticides of synthetic origin are based, in the case of benzoylureas, on interrupting the formation of structures composed of chitin, which consequently leads to abnormal endocuticular deposition (Mulder and Gijswijt, 1973). On the other hand,

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diacylhydrazines act as agonists of ecdysteroid receptors accelerating the molting process and by preventing satisfactory replacement of the old integument (Carlson *et al.*, 2001). Although the effects of such products are already widely reported, our study indicates for the first time the effects of such products at sublethal concentrations on the fertility life table parameters. These parameters are important indicators of the population growth of a pest species under field conditions.

Among all botanical insecticides tested, Azamax was the most promising product, which stems from its higher azadirachtin and 3-tigloilazadiractol contents (previously azadirachtin A and B), which are considered the main active components of neem formulations (Isman, 2017). On the other hand, the other neem products tested, notably crude oils with very diversified limonoid profiles, showed promising effects but to lesser extents when compared to Azamax.

By incorporating the tested insecticides into the diets, it was possible to visually observe reductions in diet intakes and consequently, weight decreases in larvae and pupae. Feeding reductions directly affect the critical weights of exposed organisms (Nijhout *et al.*, 2014), as reported in studies by Duarte *et al.* (2019), which corroborates the results found in the present study. Roel *et al.* (2010) reported that during metamorphosis process, insects do not fully utilize the nutrients contained in their diets, so the larvae do not gain weight before juvenile hormones are depleted and ecdysteroid levels increase and consequently, smaller pupae result. However, Lima *et al.* (2010) did not observe reductions in pupal weight, a fact that must be related to the shorter exposure time (48 h) of the larvae to neem derivatives in that study, which made it impossible to find potential postingestive effects.

Low levels of nutritional reserves increase the duration of the pupal period, as nutrients support successful metamorphosis, directly influencing the reproduction and survival of individuals (Merkey *et al.*, 2011). Toscano *et al.* (2012) reported increased mortality of *S. frugiperda* larvae exposed to neem and lufenuron compared to pyroligneous extracts. Assimilation of nutrients during the larval period directly affects fecundity (Arrese and Soulages, 2010). In the present study, significant reductions in total fertility were observed when comparing the control group with the other treatments. Research involving the genus *Spodoptera* found an association between small-sized pupae and fecundity rates (Specht *et al.*, 2015; Duarte *et al.*, 2019), reducing longevity (Pineda *et al.*, 2009; Lima *et al.*, 2010), with a significant impact on pest demographics in the field as well as on mating timing (Duarte *et al.*, 2019). In our study, we observed that the duration of each generation for all insecticides was longer compared to the negative control. However, we emphasize that the net reproduction rates were lower, thus causing smaller offspring.

Spodoptera exigua larvae (Hübner, 1808) (Lepidoptera: Noctuidae) fed methoxyfenozide and lufenuron exhibited reductions in larval and pupal weights, prolongation of larval and pupal development, and significant reductions in fertility, which also corroborated the results obtained in the present study (Chein *et al.*, 2019). Likewise, several authors report reductions in pupal weight and increases in larval and pupal development times for lepidopterans exposed to hexaflumuron, diflubenzuron, flufenoxuron and bistrifluron (El-Ghar *et al.*, 2010; Mahmoudvand *et al.*, 2011; Zhu *et al.*, 2012; Hafeez *et al.*, 2021). These results demonstrate that *S. frugiperda* larvae exposed to neem-based insecticides or synthetic growth regulators exhibited suppression of large populations of this pest over time. The results indicate that neem-derived products, as well as growth-regulating insecticides of synthetic origin, cause sublethal effects on *S. frugiperda*, reducing their ability to reproduce and contributing to integrated management strategies that aim to keep populations below damaging levels. Thus, these insecticides are considered as environmentally safer alternatives for managing *S. frugiperda* in different agricultural production systems.

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Author contribution. LNM: Conceptualization, Methodology, Investigation, Data Curation, Writing – Original Draft. FCSG, MBA and DTTA: Investigation, Writing – Review & Editing. MR: Formal Analysis, Data Curation, Writing – Review & Editing. APSAdR and LPR: Resources, Supervision, Funding acquisition, Writing – Review & Editing. DB: Resources, Supervision, Project administration, Funding acquisition, Writing – Review & Editing.

Conflicts of interest. The authors declare no competing interests.

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