

Investigating the selectivity of botanical and synthetic insecticides on *Doru luteipes*: there is no simple answer

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Abstract

To achieve sustainability in agricultural pest management, it is essential to integrate chemical and biological control through selectivity studies. In this study, we evaluated the lethal and sublethal effects of both botanical and synthetic insecticides used for controlling the fall armyworm, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae), on its natural enemy *Doru luteipes* (Scudder) (Dermaptera: Forficulidae). For this purpose, bioassays of mortality, behavior, and transgenerational effects were conducted. Botanical insecticides rich in acetogenins and limonoids caused less than 30% mortality in *D. luteipes* nymphs (2nd, 3rd, and 4th instars). In contrast, chlorantraniliprole-based insecticide (Premio®) was highly toxic, presenting mortality above 80% at all nymphal stages and reducing predation capacity by 55.97% and walking velocity by 28.44% compared to the control. Aqueous emulsion of the ethanolic extract from *Annona mucosa* seeds (ESAM) reduced the longevity of the adults to 88.88 days. Chlorantraniliprole resulted in the shortest pre-oviposition period (9.44 days), followed by a limonoids-based botanical insecticide (Azamax®) (11.00 days). Fourth-instar nymphs of the F₁ generation showed lower viability (54.54%) in the treatment with aqueous emulsion of the methanolic fraction from *Annona montana* leaves (EFAMON). The annonin-based commercial botanical insecticide (Anosom®) affected life table parameters, reducing the intrinsic growth rate ($r_m = 0.039$) and finite rate of increase ($\lambda = 1.04$). These findings indicate that botanical insecticides are less hazardous to the natural enemy *D. luteipes* than synthetic insecticide. This study provides important insights for improving pest control while preserving natural enemies.

Introduction

Combining chemical and biological control is a key precept in Integrated Pest Management (IPM) programs, and selectivity studies provide essential information for this achievement. In this context, a selective compound should effectively control the target pest while minimizing adverse effects on natural enemies (Castle and Naranjo 2009; Gentz et al. 2010; Bueno et al. 2017; Torres and Bueno 2018; Serrão et al. 2022). Recent efforts have focused on ensuring that newly introduced insecticide molecules are environmentally friendly, promoting a better agroecosystem balance. Examples of these new molecules include botanical insecticides, considered promising alternatives for pest management and less harmful to non-target organisms, along with organosynthetic compounds such as diamides (Ishaaya et al. 2007; Turchen et al. 2014; Gontijo et al. 2015; Ndakidemi et al. 2016; Bernardi et al. 2017; Rezende-Teixeira et al. 2022; Sparks and Bryant 2022; Cong et al. 2023; Schmidt-Jeffris 2023).

The fall armyworm, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae), is a significant agricultural pest that affects economically important crops, such as corn, cotton, soybean, rice, and wheat. Control efforts have been challenged by resistance to various chemical groups and *Bt* corn, which has intensified the search for new management alternatives (Montezano et al. 2018; Tay et al. 2023; Mota-Sanchez and Wise 2024). Among the promising options are botanical insecticides, such as extracts from *Annona mucosa* and *Annona montana* plants, and the commercial botanical insecticide Anosom®, derived from *Annona squamosa* plant. These compounds are rich in acetogenins, a class of

natural products consisting of long-chain fatty acids with a 2-propanol unit, and they exhibit distinct modes of action (Colom et al. 2009; Di Toto Blessing et al. 2012; Ansante et al. 2015, 2017; Costa et al. 2016; Hidalgo et al. 2020). Other registered compounds for controlling *S. frugiperda* include the commercial botanical insecticide Azamax®, which is based on azadirachtin and 3-tigloylazadiractol from the *Azadirachta indica* plant, and the commercial organosynthetic insecticide Premio®, whose active ingredient is chlorantraniliprole, belonging to the diamide group (MAPA, 2024).

Effective management of *S. frugiperda* requires that the use of chemical insecticides be compatible with the natural enemies involved in biological control, such as the predator *Doru luteipes* (Scudder) (Dermaptera: Forficulidae). This predator is commonly found in corn crops, feeding on eggs and larvae of *S. frugiperda*. Nymphs of *D. luteipes* can consume up to 10 eggs or larvae per day, while adults can consume up to 20 first- and second-instar larvae (Cruz et al. 1995; Cruz 2007; Pasini et al. 2010; Pacheco 2019). Additionally, as a polyphagous predator, *D. luteipes* is capable of preying on other agriculturally significant pests (Romero Sueldo and Virila 2009; Romero Sueldo et al. 2014; Silva et al. 2023).

However, achieving compatibility between chemical insecticides and natural enemies can be challenging. Selectivity depends on several factors, including the product's physicochemical composition, the target species, the method of exposure, the insect's developmental stages, its behavior, and its detoxification mechanisms (Godoy et al. 2010; He et al. 2019; Vázquez 2019; Silva et al. 2020; Potin et al. 2022). Although most of the insecticides mentioned earlier for controlling *S. frugiperda* are derived from botanical sources, and the organosynthetic insecticide (chlorantraniliprole) is reported to be selective towards non-target organisms, ecotoxicology studies need to be conducted considering the specific characteristics of the pest, the insecticide, the natural enemy, and the exposure method (Gontijo et al. 2015; Haddi et al. 2020; Serrão et al. 2022; Arroyo et al. 2023). Moreover, these studies should assess not only direct lethal effects but also sublethal effects, which may compromise the effectiveness of natural enemies and the sustainability of agricultural system (Haddi et al. 2020). (Desneux et al. 2007; Müller 2018).

Given that both synthetic and botanical insecticides, as well as biological control utilizing *D. luteipes*, are considered essential tools for *S. frugiperda* management in food production systems, this study tested the following hypotheses: (I) residues of botanical and synthetic insecticides may impact *D. luteipes* differentially across its life stages, (II) affect its behavior, and (III) cause transgenerational effects. Identifying selective products is fundamental to advancing the integrated management of *S. frugiperda* while safeguarding *D. luteipes*.

Materials and methods

Insect rearing

Adults of *D. luteipes* were collected from corn crops without insecticide application in an experimental field in Piracicaba, São Paulo, Brazil (22°42'49.1"S 47°37'32.8"W). Plastic containers (25.5 cm long × 18.5

cm wide × 8 cm high) covered with organza fabric and brown paper were used for laboratory maintenance. Inside the containers, accordion paper was provided as a refuge and shelter. Both adults and nymphs were fed ad libitum with an artificial diet developed by Guimarães et al. (2006), consisting of cat food (35%), wheat germ (27%), brewer's yeast (23%), powdered milk (14%), Nipagin (0.5%), and ascorbic acid (0.5%). Plastic straws (0.8 cm diameter) containing moistened cotton were used as substrates for oviposition (Naranjo-Guevara et al. 2017). As this species exhibits parental care, females were transferred with their respective eggs to new containers after oviposition. They remained there for fourteen days before being reintegrated into the colony. The nymphs were kept separate until adult emergence, with some used in experiments and others reserved for colony maintenance. The insects used in the bioassays belonged to the 14th laboratory-reared generations.

Spodoptera frugiperda larvae were obtained from a susceptible population maintained in the laboratory for over 20 years. The pupae and adults were kept in cages made of PVC tubes (20 cm in diameter × 20 cm in height), internally lined with white paper to facilitate oviposition. Neonate larvae were reared in 100 mL plastic cups containing an artificial diet (Greene et al. 1976). Upon reaching the third instar, they were individualized and kept until the pupal stage, at this point, they were transferred back to the cages. Adults were fed a 10% honey solution. All insect rearing and experiments were conducted in a climate-controlled room (temperature: $25 \pm 1^\circ\text{C}$, relative humidity: $60 \pm 10\%$, and photoperiod: 12 L:12 D).

Obtaining the treatments

The treatments involved two non-commercial botanical pre-formulations and three commercial insecticides, which consisted of two botanical and one organosynthetic. The pre-formulations included (1) an aqueous emulsion of the methanolic fraction from the ethanolic extract of *Annona montana* leaves (EFAMON) and (2) an aqueous emulsion of the ethanolic extract from *Annona mucosa* seeds (ESAM). The commercial insecticides comprised (1) Anosom® 1 EC, a botanical product based on acetogenins (annonin) (AgriLife SOM Phytopharma Ltda., Hyderabad, India); (2) Azamax® 1.2 EC, also a botanical insecticide containing limonoids (azadirachtin and 3-tigloylazadirachtol) (UPL Brasil Ltda., Campinas, São Paulo, Brazil); and (3) Premio® 200 SC, an organosynthetic insecticide with chlorantraniliprole as the active ingredient (FMC Química do Brasil Ltda., Campinas, São Paulo, Brazil). Distilled water and the solvents used in the pre-formulations were the control group.

Preparation of botanical pre-formulations

Botanical pre-formulations were prepared using ethanolic extracts from *A. montana* leaves and *A. mucosa* seeds. The leaves and seeds were dried in an oven at 38°C for 48h, ground in a knife mill, and the resulting powder was extracted with ethanol. After three days of rest, the material was filtered, and the solvent was removed in a rotary evaporator at 50°C and -600 mmHg . The *A. montana* extract was then subjected to liquid-liquid partition to separate phases with different chemical affinities and obtain the methanolic fraction, according to the procedure described by Lima et al. (2022).

Emulsions of the methanolic fraction of *A. montana* and the ethanolic extract of *A. mucosa* were prepared by adding the surfactants Triton® X-100 (0.1%, v v⁻¹) and Tween® 80 (1%, v v⁻¹), along with a mixture (1%, v v⁻¹) of acetone and methanol (1:1, v v⁻¹). These components helped to break the surface tension of the water, improve leaf adhesion, and facilitate the dilution of the emulsions.

Lethal concentration (LC) of *S. frugiperda*

Concentration-response curves were performed to determine the lethal concentration of treatments required to kill the pest *S. frugiperda*. Different treatment concentrations (at least eight) were incorporated into 100 g of artificial diet. After preparation, 1 mL of the contaminated diet was distributed into wells of 128-cell plastic plates (Advento do Brasil, São Paulo, Brazil) using a hypodermic syringe (BD Plastipak™). The plates were infested with neonate larvae (< 24 hours old) and sealed with transparent plastic lids for aeration. Mortality was assessed daily over seven days. For the EFAMON treatment, the concentrations, before being added to the diet, were pre-diluted in 6 mL of acetone and methanol (1:1, v/v). These same solvents were used in the control treatment. A completely randomized design was adopted, with four replicates per treatment and 16 insects per replicate. For the ESAM and Anosom®, as their concentration-response curves (LC) for *S. frugiperda* had already been determined in previous studies a mortality bioassay was conducted to confirm these values (ESAM LC₉₀ = 1,386.0 mg kg⁻¹; Anosom® LC₉₀ = 2,959.0 mg kg⁻¹) (Ansante et al. 2015, 2017). The bioassay followed the same procedures described above, confirming the toxicity of these two treatments against the pest.

Selectivity bioassays on *Doru luteipes*

Selectivity bioassays were conducted to assess the lethal and sublethal effects of treatments on *Doru luteipes*. The predator was exposed to tarsal contact with leaves treated with the lethal concentration (LC₉₀) previously determined for the pest. All experiments were performed in a completely randomized design and maintained in a climate-controlled room (temperature: 25 ± 1 °C, relative humidity: 60 ± 10%, and a photoperiod of 12 L: 12 D h).

Lethal effect on developmental stages

To evaluate the lethal effect of treatments on *D. luteipes* mortality, corn leaf discs (vegetative stage, V5) with 4.5 cm diameter were cut and dipped in the treatment solutions for 10 seconds. Distilled water and the solvents from pre-formulations were used as a control. After drying, the discs were placed in plastic containers (4.5 cm diameter and 5.0 cm height) containing carrageenan (2.5%, w v⁻¹) at the base to maintain moisture. Subsequently, insects were grouped by developmental stage (1st, 2nd, 3rd, 4th instars, and adults), all 24 hours old, and placed in containers containing treated leaves to allow tarsal contact with the treatments. An artificial diet was provided for feeding, along with moistened cotton to maintain adequate humidity. The containers were sealed with perforated lids to allow aeration, and

mortality was recorded 48 hours after exposure. Each treatment consisted of five replicates, with six insects per replicate (three males and three females for the adult stage).

Sublethal effects

Predation and walking behavior

For the sublethal effects assessment, adult *D. luteipes* were exposed to the LC₉₀ concentration of the treatments for 24 hours, following the same procedure described previously. This concentration was chosen because no significant mortality was observed in adults during the lethal effect bioassay.

After exposure, to evaluate predation, each insect was individually transferred to a plastic container (4.5 cm in diameter × 6.5 cm in height) and provided with 20 first-instar larvae of *Spodoptera frugiperda*. After 12 hours, the number of preyed larvae was recorded. The experiment included six treatments, ten replicates per treatment, consisting of five males and five females, with one insect per replicate, meaning one adult for every 20 larvae of *S. frugiperda*. All adults used in the experiment were no more than 24 hours old and had not received any artificial diet before the test.

The behavioral bioassay aimed to assess whether the treatments affected the movement activity of the insects. Each experimental unit consisted of four adult insects kept in plastic containers (25.5 cm length × 18.5 cm width × 8 cm height). After the exposure period (24h), each replicate was filmed for 10 minutes using a Sony Handycam camcorder (Hdr-cx405 Full Hd) positioned perpendicular to the insect container. The ambient light was covered with red cellophane. A total of six replicates per treatment were performed. Movement data were analyzed using an automated image capture system (EthoVision, Noldus et al. 2001). The variables analyzed were “Distance moved” (cm) and “Velocity” (cm s⁻¹). “Distance moved” was defined as the distance traveled by the subject's center of mass from the previous to the current sample. “Velocity” was defined as the distance traveled by the subject's center of mass per unit time (Cann et al. 2013). Both experiments were conducted at night (from 7 pm to 9 pm) when *D. luteipes* exhibits increased predatory activity (Naranjo-Guevara et al. 2017).

Biological parameters and transgenerational effects

To evaluate the sublethal effects of treatments on the biological parameters of *D. luteipes*, newly emerged adults (less than 24 hours old) were exposed to treated leaves for 24 hours. After exposure, ten couples/treatment were formed and placed individually in containers containing an artificial diet and moistened cotton. Daily observations recorded longevity and reproductive parameters, including the pre-oviposition period and fecundity. Once the females laid their eggs, the males were separated until the nymphs hatched, as *D. luteipes* exhibit parental care, and the presence of males could stress females during this period.

From these couples, 10 egg masses (30 eggs per mass) were selected to assess the development, viability, and sex ratio of the F_1 generation. The number of males and females was determined by differentiating the cerci: more curved cerci indicate males, while straight cerci indicate females. Subsequently, a fertility life table was constructed to investigate the effects of the treatment on the population growth. Biological data were collected, including x = average age of insects from egg stage, lx = survival rate, mx = specific fertility, and $lx.mx$ = total number of females born at age x . These data were used to estimate the life table parameters: net reproductive rate (R_0), mean generation time (T), intrinsic rate of increase (r_m), and finite rate of population increase (λ) (Maia et al. 2014). The experiment included six treatments, each with 10 replicates (one pair per replicate). Ten egg masses/couples containing 30 eggs each were used to assess egg cohorts.

Statistical analyses

The mortality data of developmental stages and predation capacity were analyzed by fitting a Generalized Linear Model (GLM) assuming binomial distribution. Behavior data were analyzed by fitting GLM with Gaussian distribution. In contrast, longevity, pre-oviposition period, fecundity, F_1 generation development, viability, and sexual ratio data were analyzed using GLM with Poisson distribution, with correction for overdispersion when necessary. For all models, the fit quality was assessed using a half-normal plot with simulated envelopes at the 95% level (Moral et al. 2017). The fertility life table was constructed using the `lifecycle.R` procedure described by Maia et al. (2014) using the Jackknife method, and parameters were analyzed using GLM with Gaussian distribution. For all fitted models, the deviance analysis was conducted, and when necessary, means were compared using Tukey's test ($p < 0.05$). All analyses were performed using R statistical software version 4.2.1 (R Core Team, 2021; <http://www.R-project.org>).

Results

Lethal concentration (LC) of *S. frugiperda*

The lethal concentration (LC) required for the non-commercial botanical pre-formulation EFAMON to cause 90% mortality in *Spodoptera frugiperda* larvae was 10,949.05 mg kg⁻¹ (Table 1, Fig. 1a). Among the commercial insecticides, the botanical product Azamax® (azadirachtin + 3-tigloylazadirachtol) showed an LC₉₀ of 65.429 mg kg⁻¹, while the synthetic insecticide Premio® (chlorantraniliprole) exhibited the lowest LC₉₀ value, 0.433 mg kg⁻¹ (Table 1, Fig. 1a). The toxicity bioassay confirmed that the lethal concentrations of the botanical pre-formulation ESAM (LC₉₀ = 1,386.0 mg kg⁻¹) and the botanical insecticide Anosom® (LC₉₀ = 2,959.0 mg kg⁻¹) effectively caused 90% mortality in the pest population (Fig. 1b).

Table 1

Lethal concentrations (LC₅₀ and LC₉₀) of botanical and synthetic insecticides for *Spodoptera frugiperda* larvae seven days after exposure

Treatments	<i>N</i> ^b	LC ₅₀ (mg kg ⁻¹) (95% CI) ^c	LC ₉₀ (95% CI)	Slope ± SE ^d	χ^2 (df ^e)	<i>P</i>
Pre-formulation EFAMON ^a	452	2,440.70 (1.960,76- 3.038,12)	10,949.05 (8,115.53- 14,771,89)	1.93 ± 0.20	9.39 (5)	0.094
Azamax® EC, 1.2g a.i L ⁻¹ (Azadirachtin + 3- tigloylazadirachtol)	442	10.619 (8.668– 13.010)	65.429 (44.279– 96.680)	1.62 ± 0.16	8.04 (4)	0.090
Premio® SC, 200g a.i. L ⁻¹ (Chlorantraniliprole)	523	0.176 (0.154– 0.201)	0.433 (0.371–0.506)	3.28 ± 0.28	2.58 (4)	0.630
^a EFAMON = Aqueous emulsion of the methanolic fraction of the ethanolic extract of <i>Annona montana</i> leaves. ^b <i>N</i> = Number of tested <i>S. frugiperda</i> . ^c CI = Confidence Interval at 95% error probability. ^d SE = Standard Error. ^e df = Degrees of freedom						

Lethal effect on developmental stages

The treatments affected the survival of *D. luteipes* at all nymphal stages ($\chi^2 = 46.633$, df = 20;120, $p < 0.001$) (Fig. 2). Premio® (chlorantraniliprole) caused the highest mortality, with values above 90% in the first instar and over 50% in other instars. However, this effect did not differ from that of ESAM (aqueous emulsion of the ethanolic extract of *Annona mucosa* seeds) and Anosom® (acetogenin annonin) in the first instar. For nymphs in the 2nd to 4th instars, botanical insecticides resulted in mortality below 30%. The tested treatments did not significantly affect mortality in the adult stage ($F = 1.547$, df = 5;120, $p = 0.1804$) (Fig. 2).

Predation and walking behavior

The interaction between the sex of adults and treatments was not significant ($F = 0.988$, df = 5;48, $p = 0.435$); however, the average predation capacity of males was higher than that of females ($F = 16.536$, df = 1;48, $p < 0.001$), with males preying on 89% and females on 77% of the prey offered. Regarding the treatments, there were differences in the predation of *S. frugiperda* by *D. luteipes* ($F = 33.967$, df = 5;48, $p < 0.001$), with adults exposed to leaves treated with the insecticide Premio® showing the lowest predation rate (40.5%). In comparison, predation was higher than 86% for other treatments, unlike the control with 92% predation (Fig. 3).

The walking behavior of adult *D. luteipes* was affected by the treatments, with analysis showing effects on velocity ($F = 4.89$, $df = 5;63$, $p < 0.001$) and distance ($F = 3.506$, $df = 5;63$, $p < 0.01$) (Fig. 4). A similar pattern was observed for both the “Velocity” and “Distance moved” variables, as expected due to the relationship between the variables. The adults contaminated with the synthetic insecticide Premio® exhibited lower locomotor activity, covering shorter distances (433.97 cm) and walking at a slower velocity (0.73 cm s^{-1}). However, compared to the other treatments, no significant differences in velocity were found between the synthetic insecticide Premio® (0.73 cm s^{-1}) and the commercial botanical insecticide Anosom® (0.98 cm s^{-1}) (Fig. 4a). No significant differences were observed in the distance moved between Premio® (433.97 cm), Anosom® (570.33 cm), and the negative control group (566.10 cm) (Fig. 4b).

Fig 4 Means ($\pm$ SE) of velocity **(a)** and distance moved **(b)** traveled by adult *D. luteipes* exposed to synthetic and botanical insecticides. Different letters among treatments indicate significant differences (Tukey, $p < 0.05$). EFAMON (aqueous emulsion of the methanolic fraction of the ethanolic extract of *Annona montana* leaves); ESAM (aqueous emulsion of the ethanolic extract of *Annona mucosa* seeds); Anosom® (acetogenin annonin); Azamax® (azadirachtin + 3-tigloylazadirachtol); Premio® (chlorantraniliprole)

Influence on longevity and reproductive parameters

Adults exposed to leaves contaminated with the treatments for 24 h showed differences in longevity (Table 2). The treatments constituted by ESAM and the synthetic insecticide Premio® reduced adult longevity to 88.88 and 95.17 days, respectively, differing from the control, which was 121.6 days. However, they did not differ from those of the other tested treatments. A similar pattern was observed for males, where Premio® reduced longevity to 71.56 days, differing from the control group, which was 112 days.

Table 2
Mean longevity ($\pm$ SE) of adult *D. luteipes* after exposure to synthetic and botanical insecticides

Treatments	Longevity (days)		
	Adult	Male	Female
Control	121.6 $\pm$ 4.75a	112.1 $\pm$ 6.83a	133.5 $\pm$ 3.48a
EFAMON	115.7 $\pm$ 5.97ab	106.7 $\pm$ 7.77ab	124.7 $\pm$ 8.22ab
ESAM	88.88 $\pm$ 6.78c	86.90 $\pm$ 9.47c	91.38 $\pm$ 10.3ab
Anosom®	105.3 $\pm$ 4.85abc	102.3 $\pm$ 6.8abc	108.4 $\pm$ 7.16ab
Azamax®	99.00 $\pm$ 5.67abc	104.1 $\pm$ 10.16bc	93.33 $\pm$ 4.06ab
Premio®	95.17 $\pm$ 8.50bc	71.56 $\pm$ 7.5abc	121.7 $\pm$ 9.41b
F	4.00	3.34	5.15
<i>p</i>	< 0.01	< 0.05	< 0.001

Means followed by the same letter in the column do not differ statistically (Tukey, $p < 0.05$). EFAMON (aqueous emulsion of the methanolic fraction of the ethanolic extract of *Annona montana* leaves); ESAM (aqueous emulsion of the ethanolic extract of *Annona mucosa* seeds); Anosom® (acetogenin annonin); Azamax® (azadirachtin + 3-tigloylazadirachtol); Premio® (chlorantraniliprole)

Among the reproductive parameters assessed, the pre-oviposition period was influenced by the treatments, with synthetic insecticide Premio® resulting in the shortest pre-oviposition period (9.44 days) compared to the control (13.10 days) and ESAM (13.00 days), indicating rapid sexual maturation of females exposed to this diamide. However, it did not differ statistically from botanical insecticides based on acetogenins (EFAMON and Anosom®) and limonoids (Azamax®). Mean fecundity values ranged from 31.20 to 57.10, with no significant statistical differences observed among the treatments (Table 3).

Table 3
Effects of synthetic and botanical insecticides on the reproductive parameters of *D. luteipes*

Treatments	Reproductive parameters	
	Pre-oviposition (days)	Fecundity (eggs/female)
Control	13.10 ± 0.54a	53.30 ± 8.75a
EFAMON	12.00 ± 0.70ab	53.10 ± 9.26a
ESAM	13.00 ± 1.21a	31.20 ± 10.37a
Anosom®	12.44 ± 0.80ab	39.30 ± 7.89a
Azamax®	11.00 ± 0.57ab	46.20 ± 5.98a
Premio®	9.44 ± 1.00b	57.10 ± 9.00a
F	3.14	1.56
<i>p</i>	< 0.05	0.18

Means followed (± SE) by the same letter in the column do not differ statistically from each other (Tukey $p < 0.05$). EFAMON (aqueous emulsion of the methanolic fraction of the ethanolic extract of *Annona montana* leaves); ESAM (aqueous emulsion of the ethanolic extract of *Annona mucosa* seeds); Anosom® (acetogenin annonin); Azamax® (azadirachtin + 3-tigloylazadirachtol); Premio® (chlorantraniliprole)

Biological parameters and transgenerational effects

The treatments did not affect the development time and sex ratio of the F_1 generation. The egg incubation and first- and fourth-instar nymph durations were approximately 8 days, while second- and third-instar nymphs lasted about 6 days. The complete cycle, from egg to adult, spanned 36–37 days, with a balanced sex ratio (close to 0.5) (Table 4). However, the viability of fourth-instar nymphs was affected by the treatments. EFAMON resulted in the lowest viability (54.54%), significantly lower than the control (91.91%), Anosom® (93.56%), and Premio® (86.87%) (Table 5). The treatments also influenced the demographic parameters highlighted in the fertility life table (Table 6). Anosom® presented the lowest intrinsic rate of growth ($r_m = 0.039$), significantly lower than the control (0.05), Azamax® (0.058), and Premio® (0.052). The net reproductive rate (R_0) increased in the following order: Anosom® (8.68), EFAMON (9.82), ESAM (10.64), control (15.77), Premio® (17.61), and Azamax® (21.66). A similar pattern was observed for the finite rate of increase (λ). No statistical differences were observed for the mean generation time (T).

Table 4

Mean ($\pm$ SE) development time and sex ratio of the F₁ generation of *D. luteipes* exposed to synthetic and botanical insecticides

Treatments	Time development (days)						Sexual
	Egg	1st	2nd	3rd	4th	Egg-adult	Ratio
Control	8.65 $\pm$ 0.18	8.00 $\pm$ 0.26	5.50 $\pm$ 0.26	6.37 $\pm$ 0.37	8.00 $\pm$ 0.32	36.50 $\pm$ 0.53	0.51 $\pm$ 0.05
EFAMON	8.62 $\pm$ 0.18	8.25 $\pm$ 0.16	6.14 $\pm$ 0.26	6.57 $\pm$ 0.52	7.20 $\pm$ 0.37	37.66 $\pm$ 0.84	0.61 $\pm$ 0.06
ESAM	8.5 $\pm$ 0.22	8.5 $\pm$ 0.22	6.0 $\pm$ 0.36	6.6 $\pm$ 0.49	7.83 $\pm$ 0.30	37.5 $\pm$ 0.5	0.51 $\pm$ 0.07
Anosom®	8.85 $\pm$ 0.14	8.57 $\pm$ 0.29	6.00 $\pm$ 0.21	5.85 $\pm$ 0.26	8.00 $\pm$ 0.30	37.28 $\pm$ 0.47	0.47 $\pm$ 0.04
Azamax®	8.50 $\pm$ 0.18	8.62 $\pm$ 0.18	5.25 $\pm$ 0.31	6.25 $\pm$ 0.31	8.00 $\pm$ 0.37	36.65 $\pm$ 0.26	0.52 $\pm$ 0.06
Premio®	8.42 $\pm$ 0.20	8.28 $\pm$ 0.18	5.71 $\pm$ 0.18	6.33 $\pm$ 0.33	7.16 $\pm$ 0.47	36.00 $\pm$ 0.25	0.51 $\pm$ 0.21
F	0.62	1.17	1.63	0.52	1.13	1.58	0.46
<i>p</i>	0.68	0.34	0.17	0.76	0.36	0.19	0.80

EFAMON (aqueous emulsion of the methanolic fraction of the ethanolic extract of *Annona montana* leaves); ESAM (aqueous emulsion of the ethanolic extract of *Annona mucosa* seeds); Anosom® (acetogenin annonin); Azamax® (azadirachtin + 3-tigloylazadirachtol); Premio® (chlorantraniliprole)

Table 5

Viability (%) of eggs and immature stages of the F₁ generation of *D. luteipes* exposed to synthetic and botanical insecticides

Treatments	Viability (%)					
	Egg	1st	2nd	3rd	4th	Total
Control	73.41 ± 7.23	87.01 ± 4.56	91.83 ± 3.12	95.52 ± 2.13	91.91 ± 4.76a	52.747.76
EFAMON	69.46 ± 6.60	89.68 ± 3.97	91.62 ± 3.60	76.46 ± 12.85	54.54 ± 11.50b	27.27 ± 8.25
ESAM	87.34 ± 5.50	86.31 ± 10.41	88.05 ± 4.12	84.07 ± 3.07	74.36 ± 5.96ab	39.5 ± 4.82
Anosom®	55.76 ± 11.76	91.29 ± 3.23	96.08 ± 1.74	97.17 ± 2.06	93.56 ± 4.78a	46.51 ± 11.35
Azamax®	77.29 ± 7.73	85.58 ± 4.46	96.91 ± 1.72	89.18 ± 4.67	74.31 ± 5.33ab	41.34 ± 5.18
Premio®	88.49 ± 6.58	90.92 ± 4.12	84.38 ± 5.40	85.71 ± 14.29	86.87 ± 6.32a	53.84 ± 12.81
F	2.24	0.22	1.92	0.89	4.74	1.30
p	0.07	0.95	0.11	0.49	< 0.01	0.28

Means (± SE) followed by the same letter in the column do not differ statistically from each other (Tukey $p < 0.05$). EFAMON (aqueous emulsion of the methanolic fraction of the ethanolic extract of *Annona montana* leaves); ESAM (aqueous emulsion of the ethanolic extract of *Annona mucosa* seeds); Anosom® (acetogenin annonin); Azamax® (azadirachtin + 3-tigloylazadirachtol); Premio® (chlorantraniliprole)

Table 6
Population parameters of *D. luteipes* (F₁) originated from adults exposed to botanical and synthetic insecticides

Treatments	Demographic parameter			
	r_m	R_0	T	λ
Control	0.050 ± 0.001ab	15.77 ± 1.70abc	55.183 ± 1.041a	1.051 ± 0.001ab
EFAMON	0.042 ± 0.002bc	9.82 ± 1.33c	54.603 ± 1.397a	1.043 ± 0.002bc
ESAM	0.041 ± 0.002bc	10.64 ± 2.11bc	58.310 ± 2.29a	1.042 ± 0.002bc
Anosom®	0.039 ± 0.003c	8.68 ± 1.74c	55.809 ± 2.141a	1.040 ± 0.004c
Azamax®	0.058 ± 0.001a	21.66 ± 1.70a	53.025 ± 0.87a	1.060 ± 0.001a
Premio®	0.052 ± 0.002a	17.61 ± 1.98ab	55.051 ± 1.263a	1.054 ± 0.002a
F	11.85	8.66	1.03	11.99
p	< 0.001	< 0.001	0.41	< 0.001
Means (± SE) followed by the same letter in the column do not differ statistically from each other (Tukey $p < 0.05$). (r_m = intrinsic rate of increase, R_0 = net reproductive rate, T = mean generation time and λ = finite rate of population increase). EFAMON (aqueous emulsion of the methanolic fraction of the ethanolic extract of <i>Annona montana</i> leaves); ESAM (aqueous emulsion of the ethanolic extract of <i>Annona mucosa</i> seeds); Anosom® (acetogenin annonin); Azamax® (azadirachtin + 3-tigloylazadirachtol); Premio® (chlorantraniliprole)				

Discussion

This study provides significant contributions to the understanding of the selectivity of botanical and organosynthetic insecticides on the predator *D. luteipes*. The synthetic insecticide Premio® (chlorantraniliprole) was found to cause high mortality in nymphs, an effect that was not observed in adults. Insecticide tolerance can vary significantly depending on insect's life stage. The immature stages of natural enemies tend to be more susceptible (Stecca et al. 2017; Freitas et al. 2017; Musa et al. 2021; Potin et al. 2022). This observation can be explained by two factors: 1) the higher ability of the insecticide to penetrate the bodies of nymphs, as their exoskeletons are less sclerotized compared to that of adults (Andersen 2010, 2012; Pang et al. 2023); 2) the high metabolic cost of detoxifying xenobiotics (Müller 2018; Khan et al. 2021). Insects have detoxification mechanisms to eliminate harmful compounds; however, nymphs may compromise vital resources for their physiological survival by allocating energy to detoxification (Ferreira et al. 2013; Luong and Horn 2017; Khan et al. 2021).

In a similar study conducted by Freitas et al. (2017), it was observed that nymphs (1st, 2nd, and 4th instars) of *D. luteipes* exposed to chlorantraniliprole residues at the label-recommended concentration had their survival affected, which is consistent with the results of our study. Another study by Campos et al. (2011) demonstrated that chlorantraniliprole does not cause adult mortality. In another species of

Dermaptera, *Euborelia annulipes*, chlorantraniliprole showed no toxicity in either adult or third-instar nymphs (Potin et al. 2022).

The botanical insecticides ESAM (aqueous emulsion of the ethanolic extract of *Annona mucosa* seeds) and Anosom® (acetogenin annonin) caused high mortality in first-instar nymphs. These two botanical insecticides are derived from plants of the Annonaceae family, known for biosynthesizing acetogenins (ACGs), which have documented insecticidal activities against various pest insect species (Ansante et al. 2015, 2017; Ribeiro, et al. 2015; Souza et al. 2017; Gomes 2018; Hidalgo et al. 2020). Their modes of action include inhibiting complex I of the mitochondrial electron transport system, preventing ATP production, and induction of programmed cell death (Alali et al. 1999; Tormo et al. 1999). Recent studies have also demonstrated the effects of acetogenins on the digestive system of certain insects, reducing nutrient absorption (Costa et al. 2016; Machado et al. 2021). Additionally, the interaction of acetogenins with cell membrane phosphates represents a novel site of action, leading to dehydration and causing irreversible damage to their structure and biological functions (Bombasaro et al. 2011; Blessing et al. 2012). Thus, first-instar nymphs may be more vulnerable to these compounds due to their lower exoskeleton sclerotization and high metabolic demand for detoxification, which may explain the higher observed mortality (Andersen 2010, 2012; Ferreira et al. 2013; Müller 2018; Pang et al. 2023).

Evaluating the sublethal impact of insecticides on the predation capacity and locomotion behavior of predators ensures that these biological control agents can continue to fulfill their ecological roles (Desneux et al. 2007). Although the survival of adult *D. luteipes* was not affected by the treatments, the synthetic insecticide Premio® (chlorantraniliprole) significantly reduced prey consumption and walking velocity. Predation is the primary ecological function of these insects, and changes in locomotion behavior may compromise their predatory efficacy, negatively affecting pest control capabilities (Campos et al. 2011; Freitas et al. 2017; He et al. 2019; Moreira et al. 2023; Silva et al. 2023).

Chlorantraniliprole is a ryanodine receptor modulator that affects muscle calcium release (Nauen 2006; Lahm et al. 2009). This insecticide can impact all muscles, including those related to locomotion, thus affecting insect movement and, consequently, their predation capability (He et al. 2019). Similar outcomes were observed in a study by Freitas et al. (2017), where the locomotion behavior of *D. luteipes* was reduced following exposure to chlorantraniliprole. Other studies support our findings regarding predation capacity. In *Euborellia annulipes* (Dermaptera: Anisolabididae), predation significantly decreases after chlorantraniliprole exposure (Potin et al. 2022). This impairment has also been observed in other natural enemies (Musa et al. 2021; Moreira et al. 2023). In contrast, Campos et al. (2011) showed that this insecticide does not affect the predation capacity of *D. luteipes*.

The botanical insecticide ESAM (aqueous emulsion of the ethanolic extract of *Annona mucosa* seeds) and Premio® (chlorantraniliprole) reduced the average longevity of adults. Regarding reproductive parameters, Premio® accelerated the pre-oviposition period, whereas fecundity was unaffected by any treatment. Literature suggests that reproduction is an energetically costly activity (Tallamy and Denno 1982; Soulages 2010; De Loof 2011). In this study, the number of eggs per female was not influenced;

however, insects exposed to ESAM and Premio® had shorter lives. This suggests a possible trade-off: insects exposed to these compounds had their physiological survival activities affected while maintaining reproductive capacity (Blacher et al. 2017; Jiang et al. 2023). Additionally, the stress caused by Premio® may accelerate the reproductive cycle in females. Similarly, this stress might respond to the toxic environment, inducing females to oviposit earlier as a survival strategy without necessarily reducing their total egg production (Fogel et al. 2016; Sial et al. 2018; Azhar and Khan 2024).

Freitas et al. (2017) demonstrated that only 20% of *D. luteipes* adults that came into contact with chlorantraniliprole residues survived up to 50 days. Other predators, such as *Chrysoperla* spp., also had their longevity affected by this insecticide (Wankhade et al. 2020). However, chlorantraniliprole did not affect the fecundity of *Coccinella septempunctata* (Coleoptera: Coccinellidae) at the lowest tested dose (Cong et al. 2023). Similar results were obtained for *Coleomegilla quadrifasciata* (Coleoptera: Coccinellidae) when exposed to dry chlorantraniliprole residues (Silva et al. 2020). In contrast, He et al. (2019) observed that applying the recommended field dose of chlorantraniliprole to *C. septempunctata* reduced fecundity. These results indicate that for *D. luteipes*, the lethal concentration (LC₉₀) of chlorantraniliprole-based insecticide used in this study did not cause changes in fecundity. In contrast, the effects may differ for other predator groups.

In addition to the direct impacts of insecticides, the effects of previous exposure can be transmitted to subsequent generations (Cheng et al. 2022; Afza et al. 2023). Therefore, selectivity studies must include an evaluation of the transgenerational effects (Le et al. 2021; Moreira et al. 2023). In this study, parameters such as developmental time, viability of developmental stages, sex ratio, and demographic parameters of the F₁ generation were evaluated.

The development time of each stage of *D. luteipes* and sex ratio were not influenced by the treatments. Similar results were found by Potin et al. (2022), who studied the effect of chlorantraniliprole on *Euborellia annulipes* (Dermaptera: Anisolabididae) and observed no impact on development time. Additionally, Abreu et al. (2024) investigated the selectivity of different aqueous extracts on the duration and fecundity of *Marava arachidis* (Dermaptera: Labiidae) and demonstrated that, at a concentration of 10% (w v⁻¹), the extracts did not affect this species.

Only the viability of the fourth-instar nymphs was influenced by the treatments, with the botanical insecticide EFAMON causing the lowest viability. No study has demonstrated the effects of *A. montana* derivatives on natural enemies. However, this plant belongs to the same genus as other *Annona* species, whose extracts have been shown to affect the viability of various insect species (Bernardi et al. 2017; Souza et al. 2019; Gonçalves et al. 2021). Much of this effect is attributed to the compounds in its composition, such as alkaloids, acetogenins, triterpenes, steroids, and lignans. Among these, acetogenins are particularly noteworthy, with their possible modes of action described earlier in this study (Gomes 2018; Hidalgo et al. 2020).

One approach to assess the impact of insecticides on insect population dynamics is to use fertility life tables (Amarasekare et al. 2016; Rossini et al. 2024). This study revealed that the F₁ generation, whose parents were exposed to Anosom® (acetogenin annonin) treatment, showed lower values of intrinsic growth rate (r_m) and finite rate of increase (λ) than the control. These parameters consider data on female fecundity and survival, which may indicate potential future effects on population dynamics, although not statistically different in this study (Kakde et al. 2014). The intrinsic population growth rate (r_m) reflects the maximum growth rate of a population, with higher values indicating a more significant potential for population success. The finite rate of increase represents the population growth rate from one generation to the next, and both parameters indicate whether the population is growing, stable, or declining (Maia et al. 2000). A similar result was observed by Morais (2020), who demonstrated a reduction in the fecundity of the predator *Cryptolaemus montrouzieri* (Coleoptera: Coccinellidae) when exposed to the same treatment. This suggests that Anosom® may have significant effects on population dynamics.

The results of this study indicate that the botanical insecticides, particularly a formulation based on limonoids (Azamax®) and an emulsion of the methanolic fraction of the ethanolic extract of *Annona montana* leaves (EFAMON), are less lethal to the natural enemy *D. luteipes* than synthetic insecticide chlorantraniliprole (Premio®). Chlorantraniliprole exhibited high toxicity, negatively impacting the mortality, predation capacity, and walking behavior of *D. luteipes*. These findings suggest that the botanical insecticides tested may be more compatible with conserving the studied natural enemy. However, there is no simple answer when it comes to selectivity studies. While botanical insecticides have demonstrated reduced effects on certain parameters, the results have varied for others. This lack of a definitive response is expected, as selectivity studies are complementary and build upon each other. Future investigations, particularly ecotoxicological assessments conducted under field conditions, can provide a more comprehensive understanding based on the results obtained. Therefore, the findings of this research offer significant contributions to filling the gaps in compatibility between chemical and biological control, promoting more sustainable agricultural practices.

Declarations

Author contributions

EDRS: conceptualization, methodology, statistical analysis, writing original draft; LVT: methodology, statistical analysis; MY, GSR, and IB: methodology and review; PTY: conceptualization, writing review and editing; LPR: writing review and editing. All authors reviewed and approved the manuscript.

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Conflict of interest

The authors declare no competing interests

Ethics approval

Not applicable

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Figures

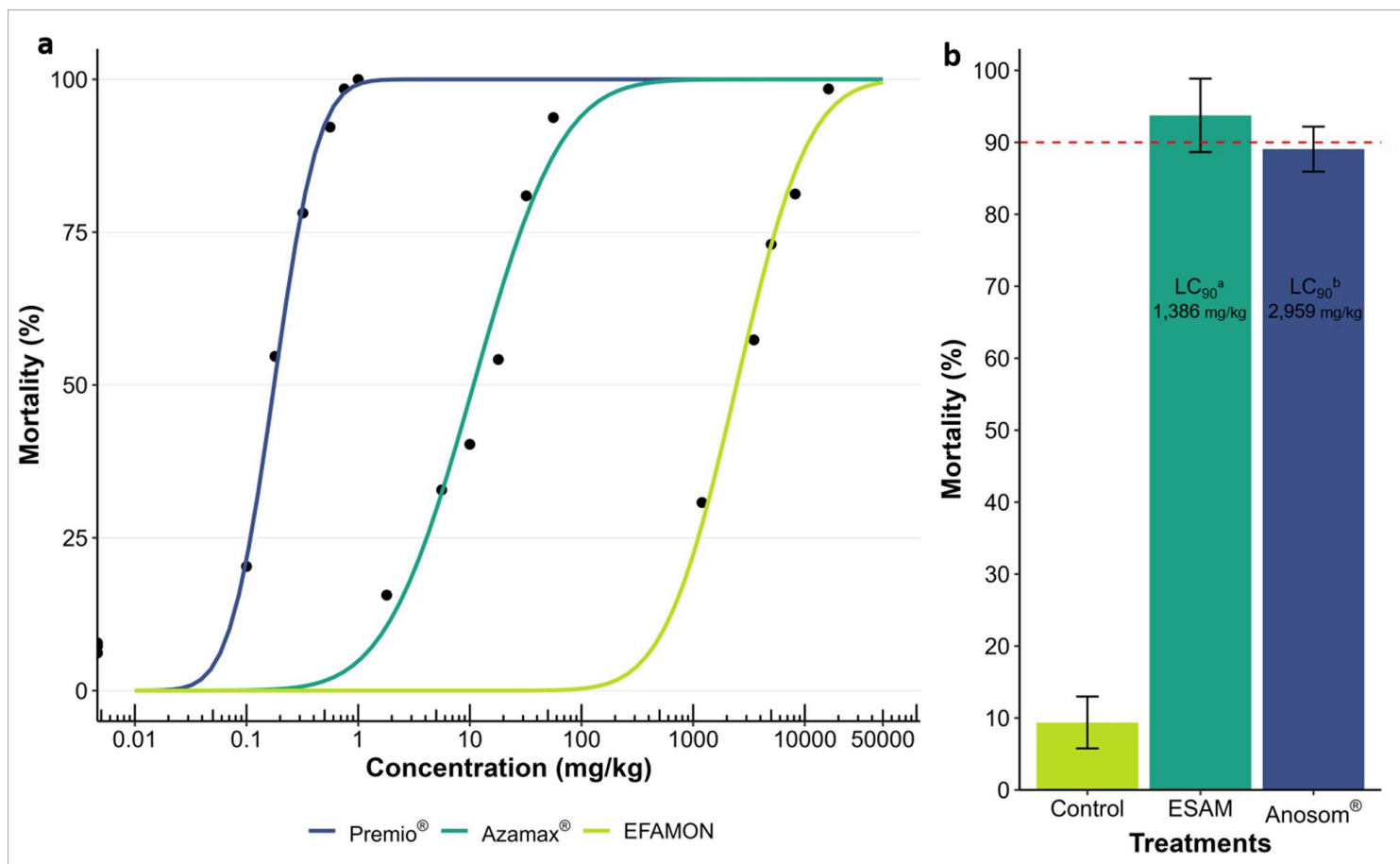


Figure 1

Estimated concentration-mortality curves for botanical and synthetic insecticides on *Spodoptera frugiperda* larvae seven days after exposure **(a)**. Mortality (%) of *S. frugiperda* larvae exposed to the lethal concentration (LC₉₀) of botanical pre-formulation ESAM (aqueous emulsion of ethanolic extract from *Annona mucosa* seeds) and Anosom[®] (acetogenin annonin) **(b)**. ^{a,b}LC₉₀ values from Ansante et al. (2015, 2017) and confirmed in this study

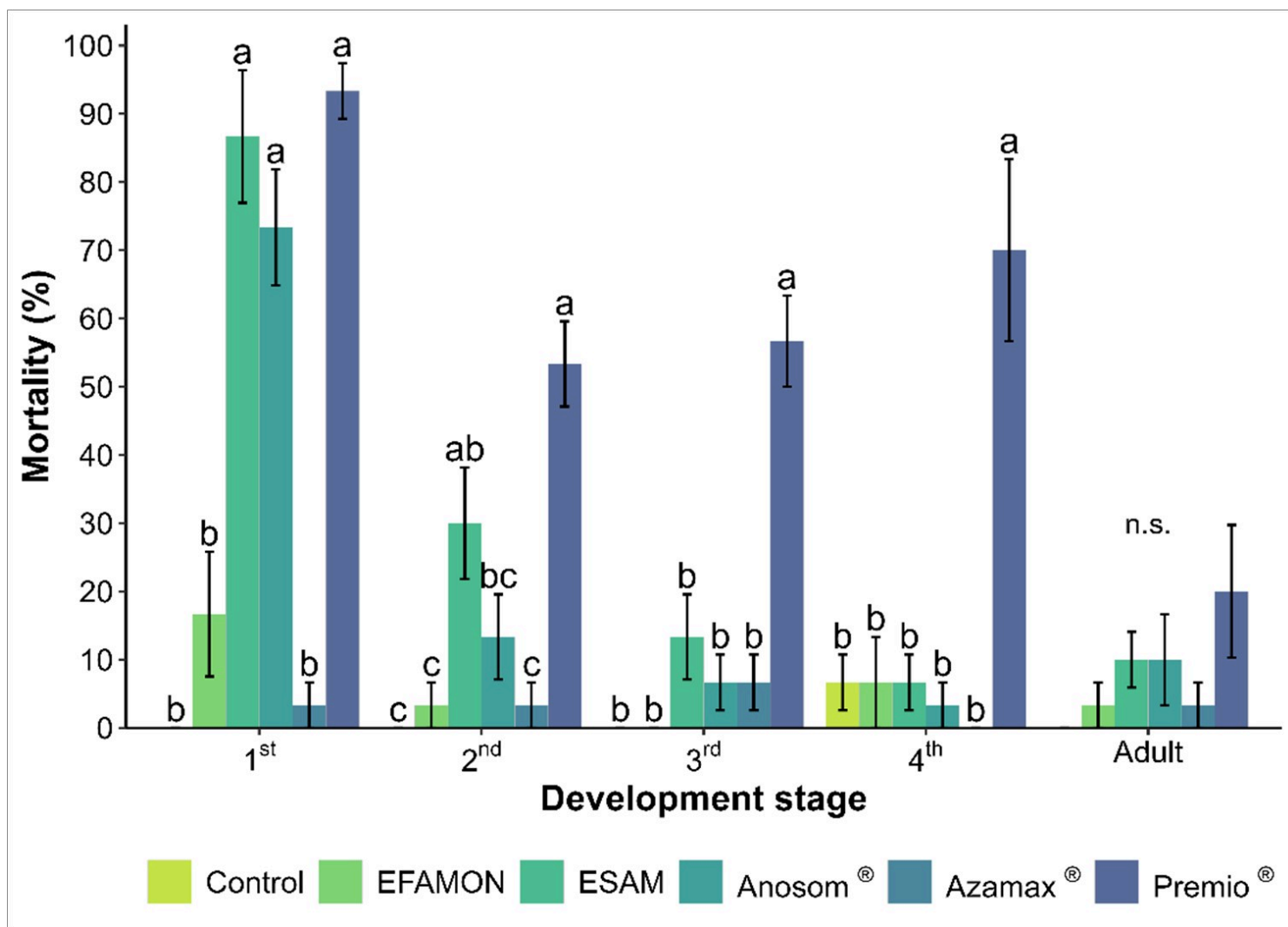


Figure 2

Mean mortality ($\pm$ SE) of *D. luteipes* developmental stages exposed to synthetic and botanical insecticides 48h after exposure. Different letters within each stage indicate significant differences (Tukey, $p < 0.05$). EFAMON (aqueous emulsion of the methanolic fraction of the ethanolic extract of *Annona montana* leaves); ESAM (aqueous emulsion of the ethanolic extract of *Annona mucosa* seeds); Anosom[®] (acetogenin annonin); Azamax[®] (azadirachtin + 3-tigloylazadirachtol); Premio[®] (chlorantraniliprole)

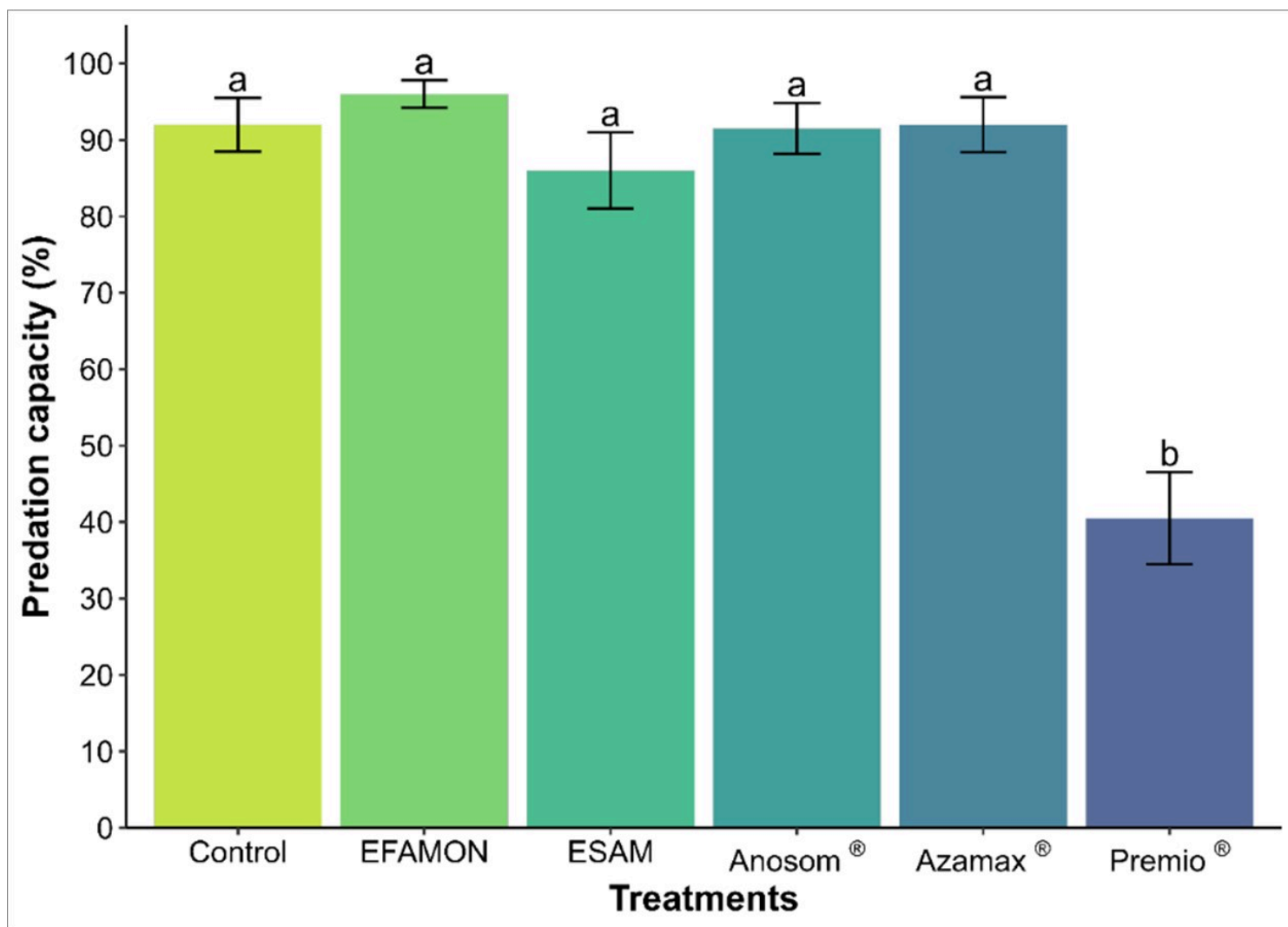


Figure 3

Predation capacity (%) $\pm$ SE of *Doru luteipes* adult on *S. frugiperda* larvae (1st instar) after exposure to synthetic and botanical insecticides. Different letters among treatments indicate significant differences (Tukey, $p < 0.05$; $\pm$ SE). EFAMON (aqueous emulsion of the methanolic fraction of the ethanolic extract of *Annona montana* leaves); ESAM (aqueous emulsion of the ethanolic extract of *Annona mucosa* seeds); Anosom® (acetogenin annonin); Azamax® (azadirachtin + 3-tigloylazadirachtol); Premio® (chlorantraniliprole)

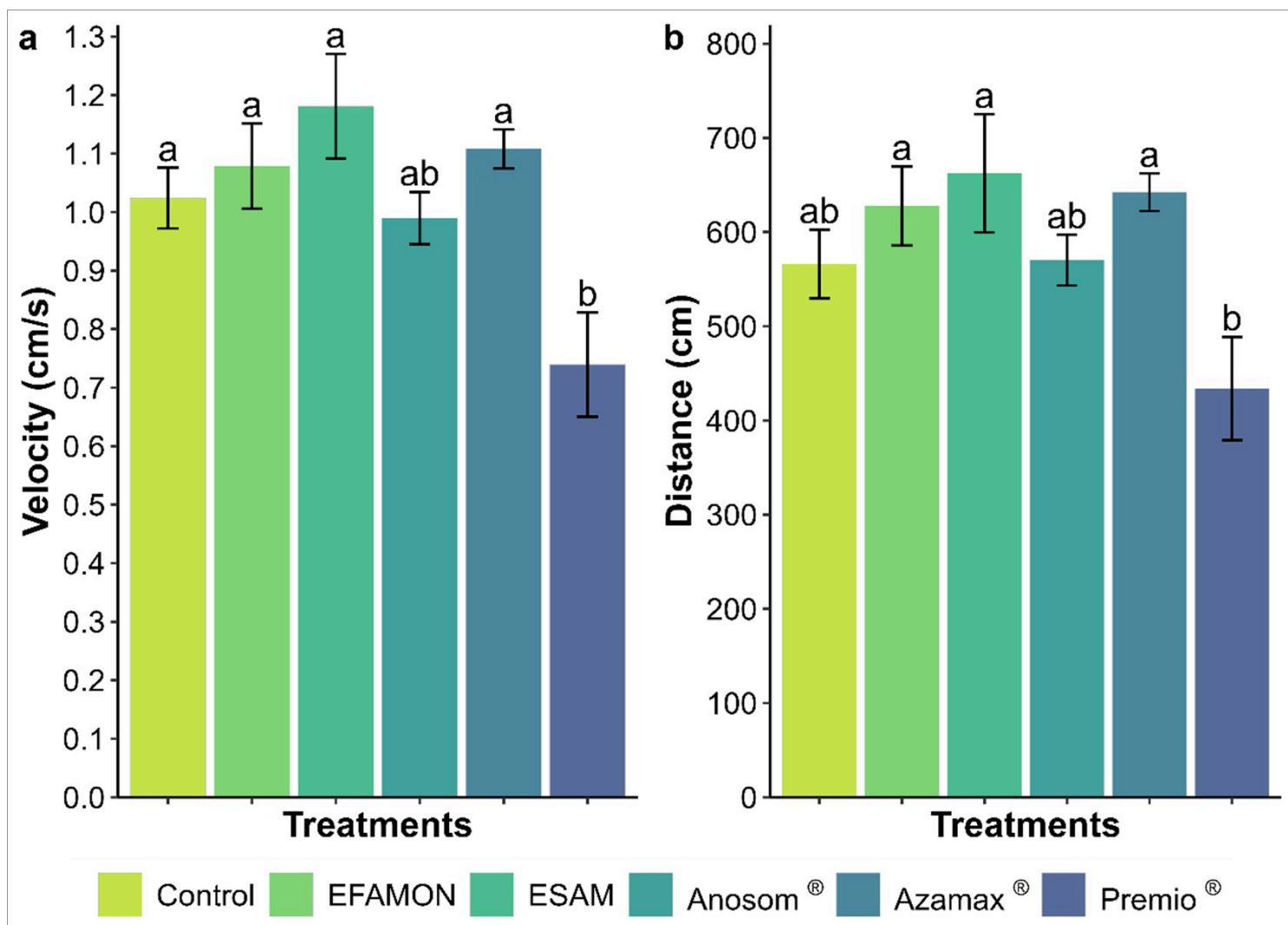


Figure 4

Means ($\pm$ SE) of velocity (**a**) and distance moved (**b**) traveled by adult *D. luteipes* exposed to synthetic and botanical insecticides. Different letters among treatments indicate significant differences (Tukey, $p < 0.05$). EFAMON (aqueous emulsion of the methanolic fraction of the ethanolic extract of *Annona montana* leaves); ESAM (aqueous emulsion of the ethanolic extract of *Annona mucosa* seeds); Anosom® (acetogenin annonin); Azamax® (azadirachtin + 3-tigloylazadirachtol); Premio® (chlorantraniliprole)