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Scientific Article

Bioactivity of commercial botanical insecticides against *Duponchelia fovealis* (Lepidoptera: Crambidae)

Bioatividade de inseticidas botânicos comerciais contra *Duponchelia fovealis* (Lepidoptera: Crambidae)

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ABSTRACT – Botanical insecticides have emerged as valuable tools for integrated pest management, offering particularly appealing ecological advantages in strawberry cultivation. Restrictions in the phytosanitary schedule pose challenges, such as controlling *Duponchelia fovealis* Zeller (Lepidoptera: Crambidae), a significant pest for this crop. This study aimed to evaluate the toxicity and lethal concentrations of two commercial botanical insecticides, one based on *Sophora flavescens* extract and the other on *Azadirachta indica* oil, against *D. fovealis* under laboratory conditions. Seven concentrations were tested for each botanical insecticide: *S. flavescens* extract (oxymatrine; Matrine®) at 6%, 4%, 2%, 1.5%, 1%, 0.75%, and 0.5%; and *A. indica* (azadirachtin; Fitoneem®) at 8%, 6%, 4%, 1.5%, 1%, 0.75%, and 0.5%). The synthetic insecticide chlorfenapyr (Pirate®) was used as a positive control (0.0104%, 0.0139%, 0.0186%, 0.0440%, 0.0587%, 0.0789%, and 0.1042%), while distilled water was used as a negative control. Topical applications were performed on third-instar larvae of *D. fovealis* to determine lethal concentrations through bioassays. The *S. flavescens*-based botanical insecticide exhibited the highest toxicity against *D. fovealis*, resulting in a mortality rate of 95.9% at a 6% concentration. *A. indica* at a 6% concentration resulted in a maximum mortality of 66.3%. The lethal concentrations (LC_{50} and LC_{90}) observed were 1.97% and 5.84% for *S. flavescens* and 3.93% and 30.57% for *A. indica*, respectively. Therefore, the botanical insecticide based on *S. flavescens* extract shows high potential as an alternative for controlling *D. fovealis*, a significant pest in strawberry cultivation.

Keywords: European pepper moth. Toxicity. Neem. *Sophora flavescens*. Sustainability.

RESUMO – Os inseticidas botânicos têm se destacado como ferramenta no manejo integrado de pragas, cujas características ecológicas são particularmente atraentes para o morangueiro. Devido as restrições na sua grade fitossanitária, um dos desafios está no controle da praga-chave *Duponchelia fovealis* Zeller (Lepidoptera: Crambidae). Este estudo teve como objetivo avaliar a toxidade e as concentrações letais de dois inseticidas botânicos comerciais a base de extrato de *Sophora flavescens* e óleo de *Azadirachta indica* contra *D. fovealis*, em condições de laboratório. Utilizaram-se sete diferentes concentrações (*Sophora flavescens* [Oximatrine / Matrine®]: 6%, 4%, 2%, 1,5%, 1%, 0,75% e 0,5% e *Azadirachta indica* [Azadiractina Fitoneem®]: 8%, 6%, 4%, 1,5%, 1%, 0,75% e 0,5%) Como controle positivo foi testado Clorfenapir (Pirate®) (0,0104%, 0,0139%, 0,0186%, 0,0440%, 0,0587%, 0,0789% e 0,1042%). Como controle negativo, utilizou-se água destilada. Em bioensaios para determinar concentrações letais, foram realizadas aplicações tópicas sobre lagartas do terceiro instar da *D. fovealis.* Os resultados demonstraram que o inseticida botânico *S. flavescens* apresentou a mais alta toxicidade contra *D. fovealis,* alcançando uma taxa de mortalidade de 95,9% das lagartas a uma concentração de 6%. Azadiractina apresentou uma mortalidade máxima de 66,3% a uma concentração de 6%. As concentrações letais de *S. flavescens* foram CL_{50}: 1,97% e CL_{90}: 5,84% e de Azadiractina CL_{50}: 3,93% e CL_{90}: 30,568%. O inseticida botânico formulado a partir de *S. flavescens* apresenta elevado potencial como ferramenta alternativa no controle de *D. fovealis*, uma praga significativa em morangueiro.

Palavras-chave: Lagarta-da-coroa. Toxicidade. Nim. *Sophora flavescens*. Sustentabilidade.

Conflict of interest: The authors declare no conflict of interest related to the publication of this manuscript.

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INTRODUCTION

Chemical control remains the primary method for managing pests in strawberry (*Fragaria* × *ananassa* (Weston) Duchesne) cultivation; however, there is a growing emphasis on adopting more sustainable management practices to reduce potential environmental impacts and pesticide residues on fruits. Furthermore, strawberries are considered a minor crop in Brazil, limiting the availability of approved pesticides for their cultivation (BRASIL, 2024). In this context, controlling *Duponchelia fovealis* Zeller (Lepidoptera: Crambidae), a newly identified key pest in strawberry fields in Brazil, has been significantly challenging, as no approved insecticides targeting this pest are available in the national market.

The European pepper moth (*D. fovealis*) is a polyphagous pest native to southern Europe and the eastern Mediterranean, now widespread across several continents (ZAWADNEAK et al., 2023). In South America, it was first observed in 2010 in São José dos Pinhais, Paraná, Brazil (ZAWADNEAK et al., 2017). Recently, *D. fovealis* has been detected in strawberry production areas in Mexico and Costa Rica (GONZÁLEZ-FUENTES et al., 2023). This species feeds on leaves and fruits at various maturation stages of the strawberry cycle (ZAWADNEAK et al., 2023).

The damage caused by *D. fovealis* has driven the search for new control alternatives for this pest (GONÇALVES et al., 2022). Biological control agents have been tested against this species, including parasitoids of the genus *Trichogramma* (Trichogrammatidae) (ALANO et al., 2021), as well as the compatibility of entomopathogenic fungi with the predatory stink bug *Podisus nigrispinus* Dallas (Hemiptera: Pentatomidae) and the ladybug *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae) (ARAUJO et al., 2020); and promising results with the predator *Chrysoperla externa* (Hagen) (GONÇALVES et al., 2024). Among the entomopathogenic microorganisms tested are the bacterium *Bacillus thuringiensis* (LUZ et al., 2024), fungi (STUART et al., 2023), and actinobacteria (PORSANI et al., 2022).

Additionally, botanical insecticides have long been regarded as attractive alternatives to synthetic chemical insecticides for pest management due to their specific toxicity to pest insects, selectivity for natural enemies, rapid molecular degradation, low environmental toxicity, and human safety (ISMAN, 2006; DIVEKAR, 2023). Thus, botanical insecticides with multiple modes of action are useful for crop protection, providing residue-free food (DIVEKAR, 2023). These ecological characteristics are particularly appealing for strawberry cultivation, where daily harvesting requires fruits free of chemical residues. Few bioassays have been conducted to evaluate the mortality of *D. fovealis* caused by botanical formulations. Ataide et al. (2020) evaluated the lethal concentrations (LC_{50} and LC_{90}) of ginger (*Zingiber officinale* Roscoe (Zingiberaceae)) essential oil against *D. fovealis* and reported toxicity to eggs and first, second, and third-instar larvae. Gonçalves et al. (2022) tested ethanolic extracts from *Annona* species and observed larval mortality, histological damage to tissues and organs, and a significant feeding inhibition rate. Zawadneak et al. (2022) reported that seed extracts from *Annona* species, although exhibiting low lethality against *D. fovealis*, inhibited larval development.

Thus, the reported efficacy against various lepidopteran pests (IDRESS et al., 2023) has encouraged studies on botanical insecticides, especially those already approved for use in several crops. In this sense, the present study aimed to evaluate the toxicity of two commercial botanical insecticides, one based on *Sophora flavescens* extract and the other on *Azadirachta indica* oil, against *D. fovealis* under laboratory conditions.

MATERIAL AND METHODS

Rearing of *Duponchelia fovealis*

The *D. fovealis* rearing was initiated from larvae and adults collected from commercial strawberry fields in São José dos Pinhais, Paraná, Brazil (25°37'S, 49°04'E; 900 m altitude). The insects were maintained under controlled laboratory conditions (25 ± 2 °C, 70 ± 10% relative humidity, and a 14-hour photoperiod), following the protocols described by Zawadneak et al. (2017). *D. fovealis* adults were kept in cages (16.5 cm in high × 12 cm in diameter) and fed an artificial solution containing 0.5 g nipagin, 0.5 g sorbic acid, 30 g sugar, 10 mL honey, 170 mL beer, and 500 mL distilled water (ZAWADNEAK et al., 2017). The cages were lined with paper towels (Mili®, Curitiba, Brazil) as an oviposition substrate. The paper towels were replaced every 24 hours, cut into egg-containing strips, and placed in 100 mL plastic containers with an artificial diet (ZAWADNEAK et al., 2017) for larval feeding. After pupation, larvae were transferred to Gerboxes containing moistened filter paper. Emerging adults were transferred to cages to maintain the stock colony. Voucher specimens were deposited in the Coleção Entomológica Padre Jesus Santiago Moure (DZUP) at the Federal University of Paraná, Curitiba, Paraná.

Determination of lethal concentrations

Seven concentrations of two commercial botanical insecticides were tested in a completely randomized experimental design, as follows: 6%, 4%, 2%, 1.5%, 1%, 0.75%, and 0.5% of *Sophora flavescens* extract (oxymatrine; Matrine®, Dinagro Agropecuaria Ltda, Ribeirão Preto, Brazil); and 8%, 6%, 4%, 1.5%, 1%, 0.75%, and 0.5% of *Azadirachta indica* oil (azadirachtin; Fitoneem®, Dalneem Brasil Comércio de Produtos Agropecuários Ltda; Itajai, Brazil). The synthetic insecticide chlorfenapyr (Pirate®) was used as the positive control at concentrations of 0.0104%, 0.0139%, 0.0186%, 0.0440%, 0.0587%, 0.0789%, and 0.1042%. All solutions were diluted in distilled water. Distilled water was used as the negative control. The tested commercial insecticide concentrations were determined based on preliminary assays, with the highest concentrations selected according to the manufacturers' recommendations.

Ten replicates were used for each treatment in all bioassays. Each replicate consisted of a 9 cm diameter Petri dish containing 10 third-instar larvae (n = 100) and a leaflet of the strawberry cultivar San Andreas. Before testing, the larvae were kept in a Gerbox and subjected to 24-hour food deprivation.

Treatments were applied using micropipettes (NichipetEX, 100–1000 µL; KASVII basic, P2 0.2–2 µL; P20 2–20 µL). After dilution in water, the solutions were vortexed for 40 seconds. A Sagyma SW776 airbrush (10 lb pol^{-1}) was used to spray 2 mL of the solution onto leaves and larvae in the Petri dishes. After spraying, the dishes were sealed with polyvinyl chloride film to prevent larval escape while allowing gas exchange. Leaflets were replaced as needed based on consumption.

The insects were maintained under controlled conditions (25 ± 2 °C, 70 ± 10% relative humidity, and a 14-hour photoperiod). Larval mortality was assessed over five days. Larvae that did not exhibit movement when touched with a brush were considered dead.

Statistical analysis

All mortality data were corrected using Abbott's formula (1925) and subjected to analysis of variance (ANOVA). Means were compared using the Scott-Knott test at a 5% significance level with SISVAR software (FERREIRA, 2011). Lethal concentration data were analyzed using Probit and Logit methods (FINNEY, 1971) with Polo Plus software (LeOra Software Company).

RESULTS AND DISCUSSION

The *Sophora flavescens*-based botanical insecticide at a 6% concentration showed the highest efficacy after 120 hours (F = 27.45, DF = 4, *p*-value < 0.0001), with a mean mortality rate of 95.9% (Table 1). *S. flavescens* at a 4% concentration resulted in 74.5% mortality, which did not significantly differ from the positive control (chlorfenapyr) at the highest concentrations (0.0789% and 0.1042%), with mortality rates of 76.5% and 84.7%, respectively. In contrast, the *Azadirachta indica*-based botanical insecticide at the highest concentrations (4–8%) reached a maximum mortality rate of 66.3%, demonstrating lower efficacy than *S. flavescens*.

Table 1. Mortality rate (mean ± standard error [SE]) of third-instar *Duponchelia fovealis* larvae treated with botanical insecticides at different concentrations, evaluated using the contact method after 120 hours.

Treatments	Concentration (%)	Number of individuals tested	Mean (%)		SE	
	6	100	95.9	±	2.34	a
	4	100	74.5	±	6.84	b
	2	100	58.2	±	4.69	c
Sophora flavescens extract	1.5	100	28.6	±	4.84	d
	1	100	19.4	±	2.69	e
	0.75	100	15.3	±	5.54	e
	0.5	100	10.2	±	2.69	e
	8	100	57.1	±	5.10	c
	6	100	66.3	±	5.59	c
	4	100	54.1	±	2.85	c
Azadirachta indica oil	1.5	100	25.5	±	3.65	d
	1	100	16.3	±	5.99	e
	0.75	100	11.2	±	4.87	e
	0.5	100	5.1	±	3.14	e
	0.1042	100	84.7	±	3.27	b
	0.0789	100	76.5	±	3.88	b
	0.0587	100	64.3	±	3.95	c
Positive control (chlorfenapyr)	0.044	100	32.7	±	4.87	d
	0.0186	100	39.8	±	3.53	d
	0.0139	100	28.6	±	3.14	d
	0.0104	100	24.5	±	6.20	d

Means followed by same letter within columns are not significantly different from each other by the Scott-Knott test at a 5% significance level.

S. flavescens extract showed an LC_{50} of 1.97% (Table 2), indicating a higher potential than *A. indica* oil (LC_{50} = 3.93%). Additionally, it exhibited the steepest concentration-response curve compared to *A. indica* oil and chlorfenapyr. However, the synthetic insecticide chlorfenapyr was the most toxic to *D. fovealis* larvae, with the lowest LC_{50} value (0.035%).

The major compound in the evaluated *S. flavescens* extract, oxymatrine, resulted in the highest mortality rate for third-instar *D. fovealis* larvae. Additionally, an anti-feeding effect was observed with this botanical insecticide. Similar findings were reported by Mina et al. (2021) when testing oxymatrine on the fall armyworm *Spodoptera frugiperda* (Lepidoptera: Noctuidae). Tian and Zhang (2023) observed an anti-feeding effect and an LC_{50} of 46.77 mg L^{-1} when testing *S. flavescens* extract against the worker ant *Solenopsis invicta* Buren (Hymenoptera: Formicidae), a higher LC_{50} value compared to that observed in the present study for *D. fovealis* larvae (3.753 mg L^{-1}; 1.97%). Conversely, Afrapoli et al. (2022) reported an LC_{50} of 2.87 mg L^{-1} for second-instar larvae of *Cydalima perspectalis* (Lepidoptera: Crambidae), similar to that observed in the present study after 120 hours. These results confirm the efficacy and high toxicity of *S. flavescens* for pest control.

Azadirachta indica oil was less toxic to *D. fovealis* than *S. flavescens* extract. According to Reddy and Amtwi

(2016), azadirachtin has a significant potential as a control agent against the early stages of *Dargida diffusa* (Lepidoptera: Noctuidae), serving as a hatching inhibitor, larvicide, and feeding deterrent. Conversely, this compound was more efficient than oxymatrine in controlling fall armyworms (*S. frugiperda*) (IDREES et al., 2023). Additionally, azadirachtin demonstrated high toxicity against the coffee red mite *Oligonychus ilicis* (Acari: Tetranychidae) (SILVA; BATISTA; BRITO, 2009).

Table 2. Slope of concentration-response curves and lethal concentrations (LC_{25}, LC_{50}, LC_{75}, and LC_{90}) of botanical insecticides on third-instar *Duponchelia fovealis* larvae.

Treatment	No.	B	S	X²	DF	CL_{25} (%)	CL_{50} (%)	CL_{75} (%)	CL_{90} (%)
Sophora	700	2.757*	0.252*	13.554*	5	1.145	1.97	3.393	5.84
flavescens		4.658**	0.439**	12.897**		(0.673–1.530)	(1.418–2.547)	(2.630–5.065)	(4.146–11.561)
Azadirachta	700	1.504*	0.133*	13.162*	5	1.42	3.93	10.989	30.568
indica		2.473**	0.230**	14.747**		(0.708–2.153)	(2.70–6.46)	(6.535–31.377)	(14.258–166.774)
Positive control (chlorfenapyr)	700	1.475*	0.149*	26.231*	5	0.012	0.035	0.102	0.295
		2.393**	0.251**	26.373**		(0.002–0.023)	(0.018–0.064)	(0.058–0.605)	(0.122–8.827)

No. = number of individuals tested; B = estimated slope coefficient; S = standard deviation; X² = chi-square test; DF = degrees of freedom. Lethal concentrations are presented at a 95% confidence level with their respective confidence intervals and the statistical test used: Probit (*) and Logit (**), for *Duponchelia fovealis* larvae exposed through topical application.

Bernardi et al. (2011) observed a high mortality rate of *Bonagota salubricola* (Meyrick) (Lepidoptera: Tortricidae) larvae exposed to a 0.2% concentration of an azadirachtin-based commercial product (NeemAzal-T/S®), a result that contrasts with the findings for the similar product tested against *D. fovealis* in the present study. Zawadneak et al. (2022) reported that an azadirachtin-based botanical insecticide (Azamax) inhibited the *D. fovealis* larval development but exhibited low lethality. These findings suggest that, despite a low larvicidal effect, azadirachtin-based insecticides warrant further investigation to elucidate their sublethal effects. Nevertheless, they represent a promising strategy within integrated pest management (IPM) programs due to their low residue levels and minimal environmental impact.

Azadirachtin affects the neurosecretory system and leads to insect death by disrupting the regulation of ecdysteroids, a crucial substance for insect molting and metamorphosis (PAVANA et al., 2023). Additionally, even small amounts of azadirachtin can persist in the insect's body during the pre-pupal and pupal stages, causing death and the formation of intermediate larval-pupal instars. This indicates significant sublethal effects even when mortality rates are low.

The toxic effects of botanical insecticides, including those based on *S. flavescens* and *A. indica*, can vary, ranging from lethal to sublethal, or serving merely as repellent, such as antifeedants and egg deterrents (ISMAN, 2006). These insecticides can cause morphological alterations, act as growth regulators, and induce mortality at various developmental stages. Additionally, they can reduce longevity, fecundity, and fertility (VENDRAMIM; RIBEIRO; BALDIN, 2023), as well as induce sterility and behavioral alterations (FORMENTINI; ALVES; SCHAPOVALOFF, 2016). The compounds in these botanical insecticides offer the advantages of low environmental persistence, rapid pest control, and minimal toxicity to natural enemies (VENDRAMIM; RIBEIRO; BALDIN, 2023). According to the regulations of the Brazilian Ministry of Agriculture and Livestock, a product must achieve a mortality rate of at least 80% to be approved as a botanical insecticide (FILOMENO et al., 2017). These characteristics suggest the potential of using *S. flavescens* and *A. indica* compounds for controlling *D. fovealis* in strawberry cultivation.

CONCLUSIONS

Botanical insecticides based on *Sophora flavescens* extract or *Azadirachta indica* oil demonstrate significant potential for controlling *Duponchelia fovealis* due to their high toxicity to third-instar larvae. However, further studies are crucial to evaluate their sublethal effects and field applicability for effectively incorporating these botanical insecticides into integrated pest management (IPM) programs.

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