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Synergistic potential of dillapiole-rich essential oil with synthetic pyrethroid insecticides against fall armyworm

Potencial sinérgico do óleo essencial rico em dilapiol para inseticidas piretroides sintéticos frente à lagarta-do-cartucho

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ABSTRACT

The objective of this study was to evaluate the synergy and response homogeneity of the *Spodoptera frugiperda* larvae population to the *Piper aduncum* essential oil in combination with pyrethroid insecticides (alpha-cypermethrin, beta-cypermethrin, fenpropathrin, and gamma-cyhalothrin) compared to piperonylbutoxide (PBO) as positive control. Synergism (SF) comparisons were obtained using lethal concentration (LC₅₀) and lethal dose (LD₅₀) ratios of insecticides individually and in their respective synergistic combinations with essential oil and PBO. Dose/concentration-mortality slope curves were used to establish relative toxicity increase promoted by synergism. They also determined homogeneity response. Residual contact revealed significant potentiation for commercial insecticides formulated with beta-cypermethrin (SF=9.05-0.5) and fenpropathrin (SF=34.05-49.77) when combined with the *P. aduncum* essential oil. For topical contact, significant potentiation occurred only for alpha-cypermethrin (SF=7.55-3.68), fenpropathrin (SF=3.37-1.21), and gamma-cyhalothrin (SF=5.79-10.48) insecticides when combined with essential oil. With the exception of fenpropathrin and gamma-cyhalothrin, insecticides synergistic combinations presented homogeneous response by topical as well as residual contact at least with essential oil. The SF significance values of the *P. aduncum* essential oil combined with alpha-cypermethrin, beta-cypermethrin, fenpropathrin, and gamma-cyhalothrin insecticides indicated potential for this oil to be used as an alternative to PBO.

Key words: alpha-cypermethrin, beta-cypermethrin, fenpropathrin, gamma-cyhalothrin, insecticide resistance.

RESUMO

O objetivo deste trabalho foi avaliar a sinergia e homogeneidade de resposta de lagartas de *Spodoptera frugiperda* ao óleo essencial de *Piper aduncum*, em combinações com inseticidas do grupo dos piretroides: alfa-Cipermetrina,

beta-Cipermetrina, Fenpropatrina e gama-Cialotrina, em comparação ao butóxido de piperonila (PBO controle positivo). Por meio da relação das CL₅₀ e DL₅₀ dos inseticidas tomados isoladamente e de suas respectivas combinações sinérgicas com o óleo essencial e o PBO, foram obtidos os fatores de sinergismo (FS) para comparação entre si. O coeficiente angular das curvas de dose/concentração-mortalidade foi utilizado no estabelecimento do aumento de toxicidade relativa, promovida pelos sinérgicos e determinação da homogeneidade de resposta. Por contato residual, evidenciou-se significativa potencialização dos inseticidas comerciais formulados com beta-Cipermetrina (FS=9,05-0,5) e Fenpropatrina (FS=34,05-49,77), quando combinados com o óleo essencial de *P. aduncum*. Já por contato tópico, ocorreu significativa potencialização somente dos inseticidas alfa-Cipermetrina (FS=7,55-3,68), Fenpropatrina (FS=3,37-1,21) e gama-Cialotrina (FS=5,79-10,48) quando em combinação com o óleo essencial. Com exceção da Fenpropatrina e gama-Cialotrina, as demais combinações sinérgicas apresentaram homogeneidade de resposta tanto por contato tópico como residual, para pelo menos uma combinação sinérgica com o óleo essencial de *P. aduncum*. A significância dos valores do FS das combinações do óleo essencial de *P. aduncum* com os inseticidas a base de alfa-Cipermetrina, beta-Cipermetrina, Fenpropatrina e gama-Cialotrina pode indicar que esse óleo essencial é uma alternativa ao PBO.

Palavras-chave: alfa-Cipermetrina, beta-Cipermetrina, fenpropatrina, gama-Cialotrina, resistência a inseticidas.

INTRODUCTION

Synergistic action minimizes the quantity of chemical insecticides necessary to control insects because it acts as an alternative substrate, reacting

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with another site in the enzymatic system, thus preventing insecticide detoxification (CASIDA, 1970). Piperonyl butoxide (PBO) is the synergistic agent most used industrially (ROCHA & MING, 1999) and had been in commercial formulations of several synthetic pyrethroid insecticides for many years (FARNHAM, 1998). However, PBO has shown acute and chronic toxicity to non-target organisms (WALIA et al., 2004), resulting in reduced interest to use this synergistic agent.

As a possible replacement for PBO, lignans extracted from plants of the *Asteraceae* and *Piperaceae* families, which contain a methylenedioxyphenyl group, exhibited synergistic potential for conventional insecticides (BERNARD et al., 1995). Dillapiolol rich essential oil, obtained from *Piper aduncum* L. (*Piperaceae*), is an alternative to natural production of synergistic lignans on a commercial scale (FAZOLIN et al., 2006). This species, with its variable dillapiolol levels (MAIA et al., 1998), is abundant in the western Amazon (PIMENTEL et al., 1998), and is the most promising option to replace PBO (WALIA et al. 2004). Most dillapiolol-producing plants; however, have constraints with regard to cultivation at the industrial level, and availability on the market (TOMAR et al., 1979). To further investigate a promising alternative to PBO, this study compared PBO and *P. aduncum* essential oil effects (PAEO) in several insecticide formulations (alpha-cypermethrin, beta-cypermethrin, fenpropathrin, and gamma-cyhalothrin) on the synergy and response homogeneity of *Spodoptera frugiperda* (J.E. Smith, 1797) larvae.

MATERIALS AND METHODS

Obtaining *P. aduncum* essential oil

Adult *P. aduncum* plants were collected from the Embrapa Acre Active Germoplasm Bank (10.0226°S, 67.7088°W) in February 2013. Plants were cut to 0.4m above the soil and their leaves dried in an oven until to 30% moisture content. Essential oil was extracted via steam distillation using a diesel-heated boiler system, as previously described (PIMENTEL & SILVA, 2003).

Chromatographic analysis

Chromatographic analysis of essential oil was performed in a Hewlett Packard (HP) 5890 gas chromatograph (GC) equipped with an Agilent HP-5 fused silica column (30m × 0.32mm i.d. × 0.25µm of film thickness) using helium as the carrier gas at 1mL min⁻¹. The GC was additionally coupled with mass spectrometry (GC-MS). Chemical characterization

was performed by comparing mass spectra with those available in the mass spectrometry library database of the GC-MS, using authentic standards, data from scientific literature, and Kovats retention indices (ADAMS, 1995). A mixture of linear alkanes (C9 to C26) was injected into the chromatograph to determine the Kovats indices (THOMAZINI & FRANCO, 2000). Constituents were quantified by GC with a flame ionization detector (GC-FID) under the same operational conditions described for GC-MS.

Toxicology bioassays

Insecticide formulations with alpha-cypermethrin (Fastac®100 CS, BASF S.A.), beta-cypermethrin (Akito® 100 CS, Arysta Lifescience Do Brazil Ind.Quim. e Agropecuária Ltda.), fenpropathrin (Danimen® 300 EC, Sumitomo Chemical Company Ltda.), and gamma-cyhalothrin (Nexide®150 CS, Cheminova Brasil Ltda.) were acquired from pesticide stores. The piperonyl butoxide was 90% technical grade (Sigma Aldrich®).

Third-instar *S. frugiperda* larvae were used in all experiments, which were conducted at the Embrapa Acre Entomology Laboratory. Individuals were confined in Petri dishes (5.0cm × 1.5cm) and kept in biological oxygen demand (B.O.D) climatic chambers at 25 ± 2°C with a relative humidity of 70 ± 5% and a 12-hour photoperiod.

All bioassays used a completely randomized design, with four replicates of each concentration or combination evaluated. Ten larvae isolated in Petri dishes were used as replicates of each treatment, resulting in a total of 40 individuals for each treatment. The different concentrations of essential oils, insecticides, and synergistic combinations were obtained from stock solutions that were diluted in acetone (CORZO et al., 2012).

Preliminary bioassays

Response ranges corresponding to the dose intervals and levels that resulted in *S. frugiperda* larval mortality were obtained from close to zero to 100%. Narrower response ranges were obtained from this range of doses and levels according to the method described by FINNEY (1971). Seven different concentrations/doses and one control (the acetone used as a solvent) were prepared for the definitive toxicology evaluations.

Statistical analysis

Mortality values of the treatments were corrected according to the control-induced mortality using the formula by ABBOTT (1925).

Concentration-mortality curves were determined using Probit analysis in the Statistics Analysis Software (SAS) program (SAS Institute, 2001), which obtained the concentrations (LC_{50}) and doses (LD_{50}) with a 50% chance of causing larval mortality for PAEO, insecticides, and synergistic combinations.

Definitive bioassays for topical contact

Initially, LD_{50} was determined individually for *S. frugiperda* larvae subjected to treatment with PAEO and each commercial insecticide. Subsequently, combinations of sub-lethal doses of the essential oil (half and a quarter of the LD_{50}) and the sub-lethal doses (below LD_{40}) of commercial insecticides were prepared to evaluate the synergistic effect.

From each prepared treatment, 1.0 μ L was applied to the pronotum on the dorsal surface of *S. frugiperda* using a graduated microsyringe (AL-SARAR et al., 2006). Insects were fasted while exposed to the treatments for 24 hours. After this period, the mortality of all the individuals was evaluated.

The previously adopted procedure was used to evaluate the combinations of the lethal sub-doses of PAEO with insecticides, thus obtaining new doses of synergistic combinations with 50% chance of larval mortality (LD_{50}).

The same insecticide sub-doses used in the essential oil combinations were combined with PBO in 10:1 (PBO: insecticide) ratio (STEWART, 1998) for a comparison with the synergistic effects of the PAEO.

The synergism factor (SF) was calculated based on GUEDES et al. (1995) using the following equation: $SF = LD_{50}$ of the insecticide / LD_{50} of the insecticide + PAEO or PBO. The synergistic effect of the PAEO was considered significant when the SF values and their respective confidence intervals (CIs), calculated for each combination of a given insecticide, were greater than or equal to the SF and CI values obtained for the combination of the same insecticide with PBO.

The angular coefficient of the concentration-mortality curves was used to establish the increase in relative toxicity promoted by the PAEO and PBO. Higher angular coefficients indicated lower phenotypic variation in the insect population's response to these compounds (CHILCUTT & TABASHNIK, 1995).

Definitive bioassays for residual contact

From each treatment, 0.2 mL was applied to 5-cm wide filter paper for the residual contact evaluations. After drying under an exhaust hood, the filter paper was placed into Petri dishes, each of which received one *S. frugiperda* larva. Other

methodological procedures adopted were the same as those used in the topical contact bioassays.

RESULTS

Dillapiole was the major component of the PAEO at 71.9% (Table 1).

PAEO toxicity to the *S. frugiperda* larvae, evaluated by residual and topical contact, can be expressed by an LD_{50} of $1.2 \times 10^{-2} \mu$ L mg insect⁻¹ and an LC_{50} of $1.1 \times 10^{-4} \mu$ L cm⁻² (Table 2). These lethality values defined the following sub-doses for the synergistic combinations with the insecticides: $5.5 \times 10^{-5} \mu$ L cm⁻² ($\frac{1}{2} LC_{50}$ PAEO) and $2.8 \times 10^{-5} \mu$ L cm⁻² ($\frac{1}{4} LC_{50}$ PAEO) for residual contact evaluations and $6 \times 10^{-3} \mu$ L mg insect⁻¹ ($\frac{1}{2} LD_{50}$ PAEO) and $3 \times 10^{-3} \mu$ L mg insect⁻¹ ($\frac{1}{4} LD_{50}$ PAEO) for topical contact evaluations.

All insecticides in PAEO combinations exhibited toxicity to the *S. frugiperda* larvae via topical contact (Table 2). The SF values caused by PAEO were significant when the PAEO was combined in doses equivalent to $\frac{1}{2}$ and $\frac{1}{4}$ of the LD_{50} with the alpha-cypermethrin (SF= 7.55 and 3.68, respectively), fenpropathrin (SF= 3.37 and 1.21, respectively), and gamma-cyhalothrin insecticides (SF= 5.79 and 10.48, respectively). The same significance level was obtained when $\frac{1}{4}$ of the LD_{50} of the essential oil was combined with the beta-cypermethrin insecticide (SF= 5.15) (Table 2).

The SF values of the PAEO and pyrethroid combinations evaluated were higher than those obtained by GIST & PLESS (1985) when combining cypermethrin with PBO (SF between 1.31 and 3.1) and by BERNARD & PHILOGÈNE (1993) when combining gamma-cyhalothrin (SF= 3.5) with PBO, as well. Such results revealed the synergistic efficacy of the PAEO for this contamination pathway and insecticide group, regardless of sub-dose used.

The significance of the comparison between the SF values of fenpropathrin and PBO (SF= 4.21) was obtained within the confidence interval limits with a variation between 0.49 and 4.23. Piperonylbutoxide in combination with the synthetic pyrethroids evaluated exhibited relatively low SF values, ranging from 1.80 to 4.47. PBO acts in inhibiting the oxidases and esterases of *S. frugiperda* larvae, decreasing the detoxification capacity and consequently increasing the lethality of pyrethroids to this insect (USMANI & KNOWLES, 2001).

Angular coefficient values of the dose-mortality curves were considered low for topical contact in both synergistic combinations of the PAEO with the four insecticides evaluated. These

Table 1 - Chemical composition (%) of the *Piper aduncum* L. essential oil used as synergistic insecticide of the synthetic pyrethroid chemical group.

Chemical compounds	Percentage	Chemical compounds	Percentage
α -copaene	0.1	(E)- β -ocimene	0.1
α -cadinol	0.1	Elemicin	0.3
α -phellandrene	0.3	epi-cubebol	0.4
α -humulene	1.3	Humulene epoxide-II	0.4
α -muurolol	0.1	(E,E)- α -farnesene	0.4
α -pinene	1.8	Ω -terpinene	1.1
α -terpineol	0.1	γ -muurolene	0.1
α -terpinene	0.3	Germacrene D	0.3
Alloaromadendrene	0.3	Limonene	1.0
Apiol	0.4	Myrcene	0.2
β -caryophyllene	2.6	Myristicin	0.6
β -copaene	0.1	Ω -cadinene	0.3
β -cubebene	0.1	Caryophyllene oxide	1.1
β -elemene	0.3	p-cymene	0.4
β -ocimene	0.2	Perylene	0.2
β -pinene	1.5	Pentadecane	0.8
β -Santalene	0.1	Piperitone	4.6
β -silenene	0.2	Terpinolene	0.2
Cyclosativene	0.2	Trans-cadine-1(2), 4-diene	0.1
Cubebol	0.8	Viridiflorol	0.4
Dillapiol	71.9	4-Terpineol	1.3
(E)-Nerolidol	0.2	Unidentified	2.7

values, however, were higher than the respective angular coefficients of the insecticides considered individually in the case of both alpha-cypermethrin ($\frac{1}{2}$ LD₅₀ of the PAEO) and beta-cypermethrin (Table 2). Fenpropathrin and gamma-cyhalothrin insecticides, conversely, exhibited higher angular coefficients than those synergistic combinations with PAEO.

As a result of this response homogeneity, reduced selection pressure for this larval population's resistance is expected for the PAEO synergistic combinations with alpha-cypermethrin and beta-cypermethrin via topical contamination.

The effect occurred through residual contact, expressed by the LC₅₀ of the synergistic combinations of the PAEO with the insecticides evaluated, and also indicated sufficient toxicity for promoting *S. frugiperda* larval mortality (Table 2).

For this contamination pathway, there were significant SF values for the two PAEO sub-doses combinations with beta-cypermethrin (SF=9.05 for $\frac{1}{2}$ LC₅₀ PAEO and 0.5 for $\frac{1}{4}$ LC₅₀ PAEO) and fenpropathrin (SF = 34.05 for $\frac{1}{2}$ LC₅₀ PAEO and 49.77 for $\frac{1}{4}$ LC₅₀ PAEO) (Table 2). The maximum SF value (1,141.57) stands out and, was yielded by combination of $\frac{1}{4}$ of the LC₅₀ of the PAEO with alpha-cypermethrin. A significant SF value was

obtained for beta-cypermethrin combined with $\frac{1}{2}$ of the LC₅₀ of the PAEO (9.10) compared with the value for PBO (SF=6.17), within the confidence interval limits ranging from 0.14 to 5.94.

The SF values were insignificant for the synergistic combinations of the alpha-cypermethrin with $\frac{1}{2}$ of the LC₅₀ of the PAEO and for the two synergistic PAEO combinations with gamma-cyhalothrin via residual contact. These results indicated synergistic inefficiency associated with this contamination pathway, since SF was significant for insecticides via the topical pathway, regardless of the proportion of PAEO used.

Contrary to what was expected, higher SF values were observed for the combinations of PAEO used at $\frac{1}{4}$ of the LC₅₀ or LD₅₀ and/or lethal doses than at half of the respective LC₅₀ or LD₅₀ for most of the insecticides via both contamination pathways evaluated (Table 2). Such results may be attributed to the responses to the different proportions of the PAEO combinations with the insecticides, which met the equivalence index that classified the combinations as additive, synergistic, or antagonistic (RAMAKRISHNAN & JUSKO, 2001).

Angular coefficient values of the concentration-mortality curve were low for residual contact. However, the angular coefficients of the

Table 2 - Toxicity of the PAEO combinations with synthetic pyrethroid insecticides *Spodoptera frugiperda* (J.E.Smith, 1797) larvae via topical and residual contact.

Insecticide combinations	LD ₅₀ (μL mg insect ⁻¹) and LD ₅₀ (μL cm ⁻²) - (95% CI)	SF	N	HF	Angular coefficient±MSE
PAEO	1.2×10 ⁻² (7.1 ×10 ⁻³ -1.8 ×10 ⁻²)	-----	280	1.31	0.33 ± 0.04
	1.1×10 ⁻⁴ (6.3 ×10 ⁻⁵ -1.6 ×10 ⁻⁴)	-----	160	1.69	0.52 ±0.04
α-cypermethrin	1.9×10 ⁻³ (1.2 ×10 ⁻³ -2.8 ×10 ⁻³)	-----	190	0.90	0.59 ± 0.07
	1.6×10 ⁻⁶ (1.2 ×10 ⁻⁶ -1.9 ×10 ⁻⁶)	-----	200	0.77	0.38 ±0.02
α-cypermethrin + ½ LD ₅₀ PAEO	7.9×10 ⁻⁶ (2.8 ×10 ⁻⁶ -1.5 ×10 ⁻⁵)	7.6 (7.5-7.9)*	200	0.92	0.65 ± 0.09
	2.1×10 ⁻⁷ (1.6 ×10 ⁻⁷ -2.5 ×10 ⁻⁷)	243.3 (192.2-442.8) ^{ns}	200	0.95	0.25 ±0.05
α-cypermethrin + ¼ LD ₅₀ PAEO	1.7×10 ⁻⁶ (1.3 ×10 ⁻⁶ -2.2 ×10 ⁻⁶)	3.7 (3.4-3.7)*	220	1.32	0.57 ± 0.06
	4.2×10 ⁻⁷ (3.3 ×10 ⁻⁷ -5.6 ×10 ⁻⁷)	1141.6 (969.9-1294.4)*	280	1.10	0.47 ±0.05
α-cypermethrin + BPO	3.0×10 ⁻⁶ (9.2 ×10 ⁻⁷ -5.3 ×10 ⁻⁶)	2.4 (0.1-5.6)	200	0.55	0.15 ± 0.03
	6.5×10 ⁻⁷ (2.2 ×10 ⁻⁷ -1.4 ×10 ⁻⁵)	638.1 (533.7-1353.1)	200	1.15	0.27 ±0.05
β-cypermethrin	1.5×10 ⁻² (2.3 ×10 ⁻⁴ -2.0 ×10 ⁻²)	-----	280	0.48	0.45 ± 0.03
	1.5×10 ⁻⁶ (1.2 ×10 ⁻⁶ -1.9 ×10 ⁻⁶)	-----	240	1.52	0.52 ±0.07
β-cypermethrin+ ½ LD ₅₀ PAEO	1.7×10 ⁻³ (1.0 ×10 ⁻³ -2.7 ×10 ⁻³)	1.0 (0.8-1.0) ^{ns}	200	0.96	0.62 ± 0.03
	1.6×10 ⁻⁶ (1.2 ×10 ⁻⁶ -2.4 ×10 ⁻⁶)	9.1 (0.2-7.4)*	160	1.71	0.54 ±0.07
β-cypermethrin + ¼ LD ₅₀ PAEO	2.8×10 ⁻³ (2.0 ×10 ⁻³ -3.7 ×10 ⁻³)	5.2 (5.2-5.3)*	200	0.35	0.67 ± 0.04
	2.9×10 ⁻⁷ (2.2 ×10 ⁻⁷ -3.7 ×10 ⁻⁷)	0.6 (0.1-5.3)*	160	1.44	0.58 ±0.07
β-cypermethrin + BPO	2.5×10 ⁻³ (1.7 ×10 ⁻³ -3.3 ×10 ⁻³)	4.5 (4.4-4.5)	200	0.29	0.56 ± 0.07
	3.4×10 ⁻⁷ (2.6 ×10 ⁻⁷ -4.2 ×10 ⁻⁷)	6.2 (0.1-5.9)	160	1.51	0.54 ±0.12
Fenpropathrin	2.2×10 ⁻³ (1.9 ×10 ⁻³ -2.6 ×10 ⁻³)	-----	160	0.49	0.72 ± 0.06
	6.2×10 ⁻⁷ (4.0 ×10 ⁻⁷ -8.4 ×10 ⁻⁷)	-----	230	0.80	0.59 ±0.08
Fenpropathrin F + ½ LD ₅₀ PAEO	6.4 ×10 ⁻⁵ (3.6 ×10 ⁻⁵ -9.5 ×10 ⁻⁵)	3.4 (2.7-3.8)*	200	1.11	0.50 ± 0.09
	1.8×10 ⁻⁷ (1.5 ×10 ⁻⁷ -2.2 ×10 ⁻⁷)	34.1 (27.4-51.2)*	200	0.45	0.35±0.03
Fenpropathrin + ¼ LD ₅₀ PAEO	4.4×10 ⁻⁵ (2.1 ×10 ⁻⁵ -6.8 ×10 ⁻⁵)	1.2 (1.1-1.1)*	200	1.38	0.67 ± 0.09
	5.1×10 ⁻⁷ (3.8 ×10 ⁻⁷ -7.5 ×10 ⁻⁷)	49.8 (38.2-87.0)*	200	1.01	0.28 ±0.04
Fenpropathrin + BPO	1.2×10 ⁻⁴ (2.1 ×10 ⁻⁵ -2.8 ×10 ⁻⁴)	4.2 (0.5-4.2)	200	1.06	0.77 ± 0.08
	1.5×10 ⁻⁷ (1.2 ×10 ⁻⁷ -1.7 ×10 ⁻⁶)	17.9 (9.0-90.3)	160	1.70	0.43 ±0.08
γ-cyhalothrin	1.1×10 ⁻³ (8.5 ×10 ⁻⁴ -1.4 ×10 ⁻³)	-----	200	1.09	0.86 ± 0.07
	1.9×10 ⁻⁷ (1.6 ×10 ⁻⁷ -2.2 ×10 ⁻⁷)	-----	320	1.45	0.56 ±0.04
γ-cyhalothrin + ½ LD ₅₀ PAEO	1.1×10 ⁻⁴ 7.3 ×10 ⁻⁵ -1.6 ×10 ⁻⁴)	5.8 (0.6-5.9)*	160	0.22	0.45 ± 0.03
	3.3×10 ⁻⁸ (2.7 ×10 ⁻⁸ -3.8 ×10 ⁻⁷)	9.7 (8.5-11.7) ^{ns}	280	1.38	0.40 ±0.04
γ-cyhalothrin + ¼ LD ₅₀ PAEO	1.8×10 ⁻⁴ (1.3 ×10 ⁻⁴ -2.1 ×10 ⁻⁴)	10.5 (9.7-11.7)*	280	1.27	0.58 ± 0.06
	1.8×10 ⁻⁸ (1.4 ×10 ⁻⁸ -2.3 ×10 ⁻⁸)	6.2 (6.3-6.5) ^{ns}	200	1.54	0.54 ±0.10
γ-cyhalothrin + BPO	6.3×10 ⁻⁵ (5.1 ×10 ⁻⁵ -7.9 ×10 ⁻⁵)	1.8 (1.7-18.3)	280	0.35	0.52 ± 0.05
	1.8×10 ⁻⁸ (1.6 ×10 ⁻⁸ -2.1 ×10 ⁻⁸)	17.1 (16.8-17.3)	320	0.90	0.49 ±0.04

Note: LD₅₀ and LC₅₀ = lethal doses and concentrations that cause 50% insect mortality; 95% CI = confidence interval at 95% probability; and SF = synergism factors calculated as a function of lethal doses and concentrations. (*) indicates significant difference between SF values of the combination and BPO; N = total number of insects to obtain the curve; HF = heterogeneity factor (p>0.1); and MSE = mean standard error.

insecticides considered individually for the alpha-cypermethrin (¼ LD₅₀ PAEO) and beta-cypermethrin (½ and ¼ LD₅₀ PAEO) combinations were even lower (Table 2).

As a result of this response homogeneity, corroborating previously obtained results for topical contact, reduced selection pressure for this larval population's resistance is expected for the synergistic combinations of PAEO with alpha- and beta-cypermethrin.

Prior to this study, the efficacy of dillapiole as a pyrethroid synergist had already been reported (WILKINSON et al., 1966; MUKERJEE et al., 1979; BERNARD et al., 1990). This secondary compound acts to detoxify insects through a combination of lignans with the methylenedioxyphenyl group, characteristic of *Piperaceae* and considered as a cytochrome P450 monooxygenase inhibitor.

As an additional tool for managing resistance to insecticides, PAEO has the potential

to reduce commercial insecticide doses and PBO in particular (WALIA et al., 2004).

CONCLUSION

The efficacy of commercial insecticides such as beta-cypermethrin and fenpropathrin was significantly enhanced when combined with *P. aduncum* essential oil via residual contact.

Only the alpha-cypermethrin, fenpropathrin, and gamma-cyhalothrin insecticides showed significantly enhanced efficacy via topical contact. The remaining insecticides, with the exception of fenpropathrin and gamma-cyhalothrin, exhibited response homogeneity for at least one synergistic combination with the *P. aduncum* essential oil both via topical and residual contact.

Significance of the SF values of the combinations of PAEO with the alpha-cypermethrin, beta-cypermethrin, fenpropathrin, and gamma-cyhalothrin insecticides indicates a potential for this essential oil to be used as an alternative to PBO.

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