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Combining the essential oil of *Piper aduncum* L. with commercial insecticides

Combinação do óleo essencial de *Piper aduncum* L. com inseticidas comerciais

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Abstract

The use of synergists is important in minimizing the amount of chemical insecticide required for insect control. Their use can contribute to reducing environmental contamination and preserving beneficial insects. To further investigate a promising alternative to the synergist piperonyl butoxide (PBO), we compared the synergistic effects of PBO and *Piper aduncum* L. essential oil (PAEO) when combined with several insecticides (cypermethrin, zeta-cypermethrin, permethrin, and esfenvalerate) on the larvae of *Spodoptera frugiperda* (J. E. Smith, 1797). Initially, we determined the lethal doses and concentrations (LD_{50} and LC_{50}) for *S. frugiperda* larvae subjected to separate treatments with PAEO and with each commercial insecticide. Subsequently, in order to evaluate the synergistic effect, combinations of sublethal doses or sublethal concentrations of the essential oil (½ and ¼ of the LD_{50} or LC_{50}, respectively) were prepared with sublethal doses or sublethal concentrations of the insecticides (below the LD_{40} or LC_{40}). To confirm the evidence of the synergistic efficacy of the PAEO, the same reduced concentrations and doses of the insecticides that were previously used in combinations with the oil were also combined with PBO at a ratio of 10:1 (PBO:Insecticide). Through the relationship between the CL_{50} and DL_{50} of the insecticides taken separately and in their synergistic combinations with the PAEO and PBO, synergism factors (SF) were calculated for the various combinations. With residual contact, there was a significant enhancement of the commercial insecticides formulated with cypermethrin (SF = 73.03), zeta-cypermethrin (SF = 16.51), and permethrin (SF = 8.46-17.22) when combined with the PAEO; by contrast, with topical application there was a significant enhancement only for zeta-cypermethrin (SF = 0.40-4.26), permethrin (SF = 2.10-4.79), and esfenvalerate (SF = 3.80) when combined with the essential oil. With the exception of esfenvalerate, the other synergistic combinations showed homogeneous responses for topical application and residual contact for at least one synergistic combination with PAEO. The significance of the SF values from combining PAEO with cypermethrin, zeta-cypermethrin, permethrin, and esfenvalerate insecticides may indicate that this essential oil is an effective alternative to PBO.

Key words: Botanical synergistics. Cytochrome P-450. Esterases. Piperaceae.

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Resumo

A importância da utilização de sinergistas está relacionada à minimização da quantidade de inseticida químico necessária para o controle de insetos, podendo contribuir com a diminuição da contaminação ambiental e preservação de insetos benéficos. Na busca de uma alternativa ao butóxido de piperonila (PBO), o estudo comparou os efeitos do PBO e do óleo essencial de *Piper aduncum* L. combinados com os inseticidas cipermetrina, permetrina e esfenvalerato, quanto ao efeito sinérgico e homogeneidade de resposta de larvas de *Spodoptera frugiperda* (J. E. Smith, 1797). Inicialmente foram determinadas as doses e concentrações letais (DL_{50} e CL_{50}) para larvas de *S. frugiperda* submetidas ao tratamento com o OEPA assim como para cada inseticida comercial considerados de forma isolada. Posteriormente, para avaliação do efeito sinérgico, foram realizadas combinações das doses e concentrações sub-letais com o óleo essencial (metade e um quarto da DL_{50} ou CL_{50}, respectivamente) com as doses e concentrações sub-letais dos inseticidas comerciais (abaixo das DL_{40} ou CL_{40}, respectivamente). Para complementar a comprovação da eficácia sinérgica do OEPA, foram utilizados como tratamentos adicionais as mesmas sub-concentrações e sub-doses dos inseticidas utilizadas anteriormente nas combinações com o óleo, passando a ser combinadas com o PBO na proporção de 10:1 (PBO: Inseticida). Por meio da relação das CL_{50} e DL_{50} dos inseticidas tomados isoladamente e de suas respectivas combinações sinérgicas com o OEPA e o PBO, foram obtidos os fatores de sinergismo (FS) para comparação entre si. Por contato residual foi evidenciada significativa potencialização dos inseticidas comerciais formulados com cipermetrina (FS= 73,03), zeta-cipermetrina (FS= 16,51) e permetrina (FS= 8,46-17,22), quando combinados com o OEPA. Já por contato tópico ocorreu significativa potencialização somente dos inseticidas zeta-cipermetrina (FS= 0,40-4,26), permetrina (FS= 2,10-4,79) e esfenvalerato (FS= 3,80) quando em combinação com o OEPA. Com exceção do esfenvalerato, as demais combinações sinérgicas apresentaram homogeneidade de resposta tanto por contato tópico como residual, para pelo menos uma combinação sinérgica com o OEPA. A significância dos valores do FS das combinações do óleo essencial de *P. aduncum* com os inseticidas à base de cipermetrina, zeta-cipermetrina, permetrina e esfenvalerato podem indicar ser este óleo essencial uma opção ao PBO.

Palavras-chave: Citocromo P-450. Esterases. PIPERACEAE. Sinérgico botânico.

Introduction

The fall armyworm *Spodoptera frugiperda* (J. E. Smith, 1797) (Lepidoptera: Noctuidae) is considered to be the pest that is most damaging to corn crops, and it can cause significant reductions in productivity (FARINELLI; FORNASIERI FILHO, 2006).

In Brazil, synthetic pyrethroids are still widely used to control *S. frugiperda*. Some products approved for use on corn in Brazil are formulated with cypermethrin, zeta-cypermethrin, permethrin, and esfenvalerate. With the exception of zeta-cypermethrin, *S. frugiperda* has been found to be resistant to these synthetic pyrethroids (APRD, 2013).

One of the tactics used to deal with insecticide resistance, as cited by Guedes and Oliveira (2002), is combining two insecticides, or combining an insecticide with a synergistic, a compound that in sublethal doses increases the insecticide's lethality.

The synergistic reduces the amount of chemical insecticide required to control insects either because it acts as an alternative substrate, protecting the insecticide from detoxification, or because it reacts with another site in the enzyme system, preventing the insecticide from becoming detoxified (CASIDA, 1970).

Piperonyl butoxide (PBO) is the synergistic that is most used industrially (ROCHA; MING, 1999). It is obtained by synthesis from safrole and is used in commercial formulations with pyrethrins, cypermethrin, deltamethrin, and fenvalerate (FARNHAM, 1998). PBO acts to inhibit the oxidases and esterases of *S. frugiperda* larvae, thereby increasing the lethality of the pyrethroids (USMANI; KNOWLES, 2001).

Lignans from the methylenedioxyphenyl group that are extracted from plants of the Piperaceae family also present synergistic potential with conventional insecticides, since they inhibit the same enzymatic group as PBO (BERNARD et al., 1995). Walia et al. (2004) highlighted dillapiole as the product that is most likely to replace PBO.

Oil that is rich in dillapiole, obtained from *Piper aduncum* L. (Piperaceae) (FAZOLIN et al., 2006), is a potential source of synergistic lignans. Commercial-scale production of many dillapiole-producing plant species may present constraints (TOMAR et al., 1979), but *P. aduncum*, in addition to containing high levels of dillapiole (MAIA et al., 1998), is abundant in the Western Amazon and its commercial-scale production is feasible (SÁ et al., 2002). It is important also note that, despite being found practically throughout the entirety of Brazilian territory (GUIMARÃES; GIORDANO, 2004), the bioprospected chemotypes in the Western Amazon contain higher levels of dillapiole (MAIA et al., 1998).

To further investigate this promising alternative to PBO, we compared the synergistic effects of PBO and *P. aduncum* essential oil (PAEO) in combination with several insecticides (cypermethrin, zeta-cypermethrin, permethrin, and esfenvalerate) on the response homogeneity of *Spodoptera frugiperda* (J. E. Smith, 1797) larvae.

Material and Methods

Obtaining P. aduncum essential oil

Adult *P. aduncum* plants were collected from the Active Germplasm Bank of Embrapa Acre (IBAMA Permits: 02001.050950/2011-61 for scientific research and 02000.000460/2013-96 for bioprospecting). These plants were cut at a height of 0.4 m aboveground and the leaves were removed for processing and drying. The essential oil was obtained in an extractor, using the steam distillation principle in a diesel-heated boiler system, adapted from Pimentel and Silva (2000).

Chromatographic analysis

The PAEO chromatographic analysis was conducted on a HP5890 gas chromatograph that was equipped with an HP5 fused silica capillary column (30 mm × 0.32 mm diameter × 0.25 m film thickness), and with helium used as the distilled gas at 1 mL min^{-1}. Quantification of the substances was performed by electronic integration of the signals. The oil obtained during this process was found to contain dillapiole as its major component (71.9%).

Toxicology bioassays

Insecticide formulations based on cypermethrin (Cypermethrin Nortox® 250EC), zeta-cypermethrin (Fury® 180EW), permethrin (Pounce® 384EC), and esfenvalerate (Sumidan® 25EC) were acquired from commercial stores. The PBO was acquired from Sigma Aldrich® and had a 90% technical grade.

The experiments were performed at Embrapa Acre's Entomology Laboratory and the toxicological evaluations of *S. frugiperda* followed the methodology used by Estrela et al. (2006). Third instar larvae were used in all experiments as the target insect (authorization for breeding, SISBIO: 13464-2). The larvae were placed in Petri dishes (5.0 cm × 1.5 cm) and kept in climatic chamber at 25° C ± 2° C, with a relative humidity of 70 ± 5%, and a photophase of 12 h.

Preliminary bioassays

Preliminary tests were performed to define the experimental patterns for the following variables: insect exposure time to the compounds (24 h without feeding); volumes to be applied (0.2 mL for residual contact and 1.0 µL for topical application); and number of insects per treatment (40 total, 10 for each treatment repetition).

Definitive bioassays for topical and residual contact

Initially, the lethal doses and lethal concentrations (LD_{50} and LC_{50}) for *S. frugiperda* larvae subjected to treatment with the PAEO were determined, with each commercial insecticide being evaluated separately.

Subsequently, in order to evaluate the synergistic effect, we prepared combinations of sublethal doses or sublethal concentrations of the essential oil (½ and a ¼ of the LD_{50} or LC_{50}, respectively) and sublethal doses or sublethal concentrations of the insecticides (below the LD_{40} or LC_{40}, respectively).

All bioassays were conducted under a completely randomized design, with four replicates for each evaluated concentration or combination. Ten individualized larvae were used in Petri dishes as replicates for each treatment. The different concentrations of essential oil or insecticide, or a synergistic combination, were obtained from stock solutions that had been subjected to serial dilutions in acetone (CORZO et al., 2012).

Overall response ranges were determined from the concentrations and doses that caused low mortality (near zero) on the one hand, and high mortality of the *S. frugiperda* larvae (near 100%) on the other. From this wide range of concentrations and doses, narrower response ranges were determined, following the methodology described by Finney (1971). Seven concentrations were subsequently established from this methodology for the final toxicological evaluations, in addition to one control (acetone solvent).

The mortality values from the treatments were corrected based on the mortality of the control (ABBOTT, 1925). The concentration-mortality curves were determined by Probit analysis, using the SAS program (SAS INSTITUTE, 2001). This analysis made it possible to obtain concentrations and doses likely to result in 50% larval mortality (LC_{50} and LD_{50} values, respectively) with the PAEO, the insecticides, and the evaluated synergistic combinations.

The PAEO, insecticides, and synergistic combinations were evaluated toxicologically with respect to topical application and residual contact. In the topical case, 1.0 μL of each concentration or synergistic combination was applied in the dorsal side of a *S. frugiperda* larva's pronotum, with the aid of a graduated microsyringe (AL-SARAR et al., 2006).

To evaluate the effects of residual contact, filter papers, 5 cm in diameter, impregnated with 0.2 mL of the different concentrations of essential oil, insecticide, or synergistic combination were employed. The impregnated filter papers were dried in a fume hood for about 5 min until the solvent had completely evaporated (ESTRELA et al., 2004). Subsequently, these papers were placed on Petri dishes that received a third instar larva of *S. frugiperda* that was then left unfed for a 24 h period, after which the mortality of the larvae was evaluated.

Statistical Analysis

In order to evaluate the combinations of different concentrations of PAEO with insecticides, we employed the procedure previously adopted for correcting the mortality of the treatments (ABBOTT, 1925). Similarly, concentration-mortality curves were determined by Probit analysis using the SAS program (SAS INSTITUTE, 2001). With this analysis, it was possible to determine the projected concentrations, doses, and synergistic combinations that would be likely to result in a mortality of 50% of the larvae (LC_{50} and LD_{50} value, respectively).

To confirm the evidence of the synergistic efficacy of PAEO , the same sub-lethal concentrations and doses of the insecticides that were previously used in combination with the oil were also combined with PBO at a ratio of 10:1 (PBO: Insecticide) (STEWART, 1998).

The synergistic efficacies of PAEO and PBO were evaluated by calculating the Synergy Factor

(SF), in accordance with Guedes et al. (1995) (SF = LD_{50} or LC_{50} of the insecticide/LD_{50} or LC_{50} of the insecticide + PAEO or PBO), which revealed the relative potency of the synergistic combinations of the lethal doses or concentrations of the insecticides taken separately, and in combination with the synergistic compounds. The synergistic effect of the PAEO was considered significant when the SF values and their respective confidence intervals (CI, calculated for each combination of a given insecticide) were higher than or equal to the values of the SF and the CI obtained from combining the same insecticide with PBO.

Another variable considered in the evaluation of the synergistic behavior of the combinations was the angular coefficient of the concentration-mortality curve obtained from the Probit analysis, which was used for establishing the relative toxicity increase caused by PAEO and PBO. The angular coefficient, according to Chilcuit and Tabashnik (1995), is the inverse of the standard deviation of the phenotypic distribution of the tolerance to an insecticide or to a synergistic combination. Thus, greater angular coefficients indicate less phenotypic variation in the insect population's response to these compounds.

Results

Toxicity of the PAEO for the larvae of S. frugiperda

The toxicity values of the PAEO for the evaluated *S. frugiperda* larvae in relation to residual contact and topical application were LC_{50} = 1169.70 ppm and LD_{50} = 1.07 µL mg insect^{-1} (Table 1).

Table 1. Lethal doses and concentrations (LD_{50} and LC_{50}) of *Piper aduncum* oil for *Spodoptera frugiperda* third instar larvae (J. E. Smith, 1797) through residual and topical contact (n = 280).

LD_{50} (95% CI) (µL x mg insect $^{-1}$)	LC_{50} (95% CI) ppm	χ^2	DF	Prob.	Angular coefficient ± SEM
1.07 (6.31-1.59)	-----	33.9	26	0.14	0.33 ± 0.04
-----	1169.70 (698.40-1755.40)	23.7	20	0.06	0.52 ± 0.04

n = total number of insects submitted to the test, DL_{50} = lethal doses and CL_{50} = lethal concentrations, causing 50% of mortality of insects; 95% CI = confidence interval with 95% of probability; χ^2 = Chi-square, DF = degrees of freedom, Prob. = probability and SEM = standard error of the mean.

These lethality values for the synergistic combinations with the commercial insecticides corresponded to the following proportions: for residual contact, ½ and ¼ of the LC_{50} of essential oil, corresponding to 584.85 ppm (½ LC_{50} PAEO) and 292.43 ppm (¼ LC_{50} PAEO), respectively; and for topical application, 421.55 (½ DL_{50} PAEO) and 210.78 (¼ DL_{50} PAEO) µL x mg insect $^{-1}$, corresponding to ½ and ¼ of the DL_{50} value, respectively.

Toxicological assessments of topical application

In the synergistic evaluations of topical application, all insecticides combined with PAEO were toxic to the *S. frugiperda* larvae (Table 2). However, the synergy factor (SF) values obtained from the PAEO were significant when combined in doses equivalent to ½ and ¼ of its LD_{50} with zeta-cypermethrin insecticides (SF = 4.26 and 0.40, respectively) and permethrin (SF = 4.79 and 2.10, respectively). The same significance was obtained when ½ the DL_{50} value of the oil was combined with esfenvalerate insecticide (SF = 3.80) (Table 2).

As regards the esfenvalerate, the significance of the SF value in relation to the PBO (SF = 5.22) was obtained within the limits of its confidence interval, which ranged from 1.59 to 5.97.

The PBO combined with cypermethrin presented a high SF value (3288.10), which indicated that the combination had high synergy. Due to the high SF value provided by the PBO, the SF values of 5.97 and 4.76 that were obtained by synergistic combinations of cypermethrin and PAEO using ½ and ¼ of its lethal dose, respectively, were not considered significant.

Using the angular coefficient of the dosage-mortality curve for each of the two synergistic PAEO dosages when combined with the four evaluated insecticides as a reference, notably high values were observed for zeta-cypermethrin combined with ¼ of PAEO LD_{50} (0.99), and for esfenvalerate combined with ½ the DL_{50} of the PAEO (0.83) (Table 2).

The combination of permethrin with ¼ of the DL_{50} of PAEO had a low angular coefficient (0.40), but it was above that observed for the insecticide separately considered (0.32) (Table 2).

Toxicological assessments of residual contact

The residual contact effect, expressed by the CL_{50} of the synergistic PAEO combinations with the evaluated insecticides, presented sufficient toxicity to promote the mortality of *S. frugiperda* larvae (Table 3).

With respect to the synergism factors for this application method (Table 3), significant values were observed for the different PAEO combinations with three insecticides: cypermethrin and permethrin combined with ½ of the CL_{50} of essential oil (SF = 73.03 and 17.22, respectively), and zeta-cypermethrin combined with ¼ of the CL_{50} of essential oil (SF = 16.51).

There was no significant difference in the SF of the synergistic combinations of esfenvalerate with the PAEO under the residual contact method.

Permethrin, when combined with ¼ of the CL_{50} of the essential oil, showed a significant SF value in relation to PBO (SF = 8.46), a value that was within the limits of the confidence interval (8.07-10.82). Thus, for this application method, the synergistic effect of PAEO and permethrin was very close to the effect provided by the PBO.

Upon comparing the values of the angular coefficient of the concentration-mortality curve that were obtained from the larvae under residual contact, only the separate esfenvalerate values were higher when compared to all the other synergistic combinations. With the exception of combinations of ½ of the CL_{50} of PAEO with cypermethrin, and ¼ of the CL_{50} of PAEO with zeta-cypermethrin, the other combinations all presented values above those observed in the evaluations of the isolated products. It is also important to note that the high value of the slope (0.95) for the combination of cypermethrin with ¼ of the CL_{50} of PAEO (Table 3) confirms a homogeneous response to this synergistic combination from the larval population.

Table 2. Lethal doses of synergistic combinations of synthetic pyrethroid with *Piper aduncum* oil to *Spodoptera frugiperda* third instar larvae (J. E. Smith, 1797) through topical contact (n = 280).

Insecticide combinations	LD50 (95% CI) (µL x mg insect -1)	SF	χ 2	DF	Prob.	Angular Coefficient ± SEM
Cypermethrin	5.53×10^{-2} (4.70×10^{-2}-1.59×10^{-2})	------	20.9	20	0.29	0.84 ± 0.63
Cypermethrin + ½ LD_{50} OPA	9.26×10^{-3} (6.58×10^{-3}-1.29×10^{-2})	5.97 (5.52-7.14) ns	11.3	22	0.88	0.54 ± 0.05
Cypermethrin + ¼ LD_{50} OPA	1.16×10^{-2} (9.34×10^{-3}-1.42×10^{-2})	4.76 (4.64-5.03) ns	15.8	20	0.33	0.79 ± 0.10
Cypermethrin + BPO	1.68×10^{-5} (9.91×10^{-6}-2.58×10^{-5})	3288.09 (2547.38-4739.49)	23.9	28	0.99	0.15 ± 0.01
Zeta-Cypermethrin	7.33×10^{-4} (4.77×10^{-4}-1.08×10^{-3})	------	17.8	22	0.47	0.38 ± 0.05
Zeta Cypermethrin + ½ LD_{50} OPA	1.71×10^{-4} (6.35×10^{-5}-3.10×10^{-4})	4.26 (3.50-7.51) *	19.0	20	0.39	0.28 ± 0.04
Zeta Cypermethrin + ¼ LD_{50} OPA	1.85×10^{-3} (1.57×10^{-3} -2.19×10^{-3})	0.40 (0.30-0.50) *	16.4	22	0.29	0.99 ± 0.09
Zeta Cypermethrin + BPO	7.36×10^{-3} (3.30×10^{-3}- 8.76×10^{-1})	0.10 (0.04-0.14)	11.6	20	0.64	0.12 ± 0.05
Permethrin	3.27×10^{-4} (2.10×10^{-4}-5.75×10^{-4})	------	19.3	20	0.15	0.32 ± 0.06
Permethrin + ½ LD_{50} OPA	6.82×10^{-5} (4.11×10^{-5}-1.09×10^{-4})	4.79 (4.89-5.25) *	19.6	22	0.61	0.27 ± 0.03
Permethrin + ¼ LD_{50} OPA	1.56×10^{-4} (1.17×10^{-4}-2.25×10^{-4})	2.10 (1.72-2.55) *	16.6	20	0.55	0.40 ± 0.03
Permethrin + BPO	1.53×10^{-3} (9.84×10^{-4}-2.85×10^{-3})	0.21 (0.20-0.22)	19.2	22	0.38	0.37 ± 0.04
Esfenvarelate	2.00×10^{1} (6.89×10^{2}-3.90×10^{1})	------	26.6	22	0.23	0.23 ± 0.04
Esfenvarelate + ½ LD_{50} OPA	5.25×10^{2} (4.33×10^{-2}-6.53×10^{-2})	3.80 (1.59-.97) *	12.0	20	0.61	0.83 ± 0.07
Esfenvarelate + ¼ LD_{50} OPA	1.95×10^{-1} (1.4×10^{-1}-2.72×10^{-1})	1.02 (0.49-1.44) ns	17.9	22	0.12	0.58 ± 0.06
Esfenvarelate + BPO	3.82×10^{-2} (3.13×10^{-2}- 4.75×10^{-2})	5.22 (2.20-8.21)	19.2	20	0.38	0.46 ± 0.07

n = total number of insects submitted to the test, LD_{50} = lethal doses causing 50% of mortality of insects; 95% CI = confidence interval with 95% of probability; SF (LD_{50}) = synergistic factor calculated regarding the lethal doses; (*) indicates a significant difference regarding the SF of the combination with BPO; χ^2 = Chi-square, DF = degrees of freedom, Prob. = probability and SEM = standard error of the mean.

Table 3. Lethal concentrations of synergistic combinations of synthetic pyrethroids with *Piper aduncum* oil to *Spodoptera frugiperda* third instar larvae (J. E. Smith, 1797) for residual contact (n = 280).

Insecticide combinations	LC_{50} (95% CI) (ppm)	SF	χ^2	DF	Prob.	Angular coefficient ± SEM
Cypermethrin	256.70 (170.20-3677.00)	----------	22.7	20	0.20	0.41 + 0.04
Cypermethrin + ½ LC_{50} OPA	3.52 (2.18-5.33)	73.03 (68.91-78.25)*	24.3	26	0.56	0.29 + 0.03
Cypermethrin + ¼ LC_{50} OPA	66.30 (49.70-90.60)	3.87 (3.42-4.05) ns	28.3	20	0.06	0.95 + 0.14
Cypermethrin + BPO	13.70 (7.89-26.00)	18.74 (14.12-21.59)	22.4	20	0.32	0.24 + 0.03
Zeta-Cypermethrin	747.80 (609.40-904.50)	----------	25.1	22	0.29	0.59 + 0.06
Zeta Cypermethrin + ½ LC_{50} OPA	617.00 (412.80-887.80)	1.21 (1.02-1.48) ns	26.7	20	0.06	0.59 + 0.08
Zeta Cypermethrin + ¼ LC_{50} OPA	45.30 (34.10-57.50)	16.51 (15.73-17.87)*	27.5	26	0.38	0.36 + 0.05
Zeta Cypermethrin + BPO	93.00 (65.50-129.10)	8.04 (7.01-9.30)	21.2	20	0.07	0.75 + 0.12
Permethrin	246.20 (112.50-525.10)	----------	34.4	22	0.06	0.22 + 0.04
Permethrin + ½ LC_{50} OPA	14.30 (7.17-24.30)	17.22 (15.70-21.61)*	27.1	26	0.40	0.30 + 0.02
Permethrin + ¼ LC_{50} OPA	29.10 (10.40-57.90)	8.46 (8.07-10.82)*	31.4	22	0.09	0.32 + 0.02
Permethrin + BPO	25.20 (6.58-61.30)	9.77 (8.57-17.09)	27.5	20	0.07	0.24 + 0.04
Esfenvarelate	48756.10 (41444.60-55764.90)	----------	12.6	20	0.56	1.31 + 0.20
Esfenvarelate + ½ LC_{50} OPA	3640.70 (2248.50-5415.20)	13.39 (10.30-18.43) ns	41.3	28	0.08	0.36 + 0.03
Esfenvarelate + ¼ LC_{50} OPA	100148.10 (66822.40-43634.70)	0.49 (0.16-0.62) ns	19.9	20	0.34	0.40 + 0.12
Esfenvarelate + BPO	1130.80 (717.30-1695.50)	43.11 (32.88-57.80)	36.8	28	0.12	0.29 + 0.03

n = total number of insects submitted to the test, LC_{50} = lethal concentrations causing 50% of mortality of insects; 95% CI = confidence interval with 95% of probability; SF (LC_{50}) = synergistic factor calculated regarding the lethal concentrations; (*) indicates a significant difference regarding the SF of the combination with BPO; χ^2 = Chi-square, DF = degrees of freedom, Prob. = probability and SEM = standard error of the mean.

For permethrin and zeta-cypermethrin, in both the evaluated exposure methods, significantly higher SF values were observed for the PAEO in combination with ¼ of the CL_{50} or DL_{50} of these insecticides (Tables 2 and 3).

Discussion

The toxicity of the PAEO to the larvae of *S. frugiperda* was comparable to that reported by Lima et al. (2009) using PAEO containing considerable amounts of dillapiole.

In comparison, in experimental conditions similar to our assessment by topical application, the SF values of synergistic combinations of PAEO with permethrin, with those obtained by Gist and Pless (1985) combining the insecticide with PBO (SF between 1.60 and 2.90), has proven the effectiveness of synergic PAEO for permethrin, regardless of the used dose.

In this same method of exposure, the combination of the PBO with cypermethrin presented a high SF value (3288.10), indicating high synergy in the combination. This synergy affects the inhibition of oxidases and esterases in the *S. frugiperda* larvae, decreasing their detoxifying ability and thereby increasing the lethality of cypermethrin to this insect (USMANI; KNOWLES, 2001).

The SF values obtained from the combination of the PAEO with cypermethrin, incorporating ½ of the DL_{50} (SF = 5.97) and ¼ of the DL_{50} (SF = 4.76), were not considered significant due to the high value that resulted from combining this insecticide with PBO. However, results obtained by Gist and Pless (1985) (SF between 1.10 and 3.10), using the same active insecticide combined with PBO, were similar to our results with PAEO.

For the insecticides permethrin and zeta-cypermethrin, in both evaluated exposure methods, the significantly elevated SF values for the combinations using ¼ of the LC_{50} or LD_{50} of PAEO (Tables 2 and 3) can be related to the differently proportioned responses of combining these insecticides with the PAEO. According to Ramakrishnan and Jusko (2001), this follows the equivalence index in which the combinations are classified as additive, synergistic, or antagonistic. In this situation, complementary evaluations are necessary to define the isobolograms of these combinations, thereby avoiding the decrease in the efficiency of PAEO when used as a synergistic at ½ of the LC_{50} or LD_{50}, with the manifestation of the antagonistic effect of its association with these insecticides.

The toxicity of esfenvalerate through topical application - which presents a significant SF value in relation to the PBO, even within the bounds of its confidence interval - allows the synergistic effect of the PAEO, because the effect of this insecticide using this exposure method is very close to the effect provided by the PBO.

Considering in a general way the homogenous response to the synergistic combinations of PAEO with all the evaluated insecticides that was revealed by the values of the angular coefficients, a decrease in the selection pressure for resistance is expected in this population, both through topical application and residual contact.

The performance of dillapiole as a synergistic of pyrethroid insecticides found in this research has already been reported (WILKINSON et al., 1966; MUKERJEE et al., 1979; BERNARD et al., 1990), since this secondary compound acts in the detoxifying process through its association of lignans to the methylenedioxyphenyl group. According to these earlier reports, the production of this metabolite in this association is characteristic of Piperaceae, which are considered important inhibitors of monooxygenases dependent on the P450 cytochrome.

It is possible that dillapiole inhibits other detoxifying enzymes such as esterases, since, in observations made by Gunning et al. (1996), the PBO, the lignan of a molecular structure and the synergistic action, similar to dillapiole, inhibited the esterases of noctuids such as *S. frugiperda*.

Synergists such as PBO and dillapiole can potentially reduce commercial doses of insecticides and be used as an additional tool to deal with resistance to such insecticides. Synergists generally act by inhibiting detoxifying or enhancing enzymes that are activated by insecticides.

Conclusions

The residual-contact effects on *S. frugiperda* third instar larvae of commercial insecticides formulated with cypermethrin, zeta-cypermethrin, and permethrin, when combined with the PAEO, were significantly enhanced. The only significant enhancement of topical application was found with commercial insecticides formulated with zeta-cypermethrin, permethrin, and esfenvalerate when combined with the PAEO. Synergistic PAEO combinations with the evaluated insecticides, with the exception of esfenvalerate, presented a homogenous response in both topical application and residual contact, at least for one synergistic combination with the essential oil. The significance of the SF values from combining PAEO with cypermethrin, zeta-cypermethrin, permethrin, and esfenvalerate-based insecticides may indicate that this essential oil is an alternative option to PBO.

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