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Toxicity and synergism of the essential oil of *Piper aduncum* L. in populations of *Sitophilus zeamais* (Coleoptera: Curculionidae)[1]

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ABSTRACT

Researches on the use of essential oil of *Piper aduncum* L. (EOPA) as a synergist in stored grain pests are still incipient. This study aimed to determine the toxicity of deltamethrin and EOPA, as well as to study the effect of the binary mixture of EOPA + deltamethrin, on five *Sitophilus zeamais* Brazilian populations. The toxicity was investigated based on concentration-mortality data from deltamethrin and EOPA in thirteen *S. zeamais* populations. The standard susceptibility population of ¼ LC_{20} was used with the deltamethrin combinations to generate lethal concentrations capable of causing 50 and 95 % of mortality (LC_{50} and LC_{95}). A binary mixture of EOPA x deltamethrin was also tested in five *S. zeamais* populations to detect the synergistic effect with the deltamethrin. The concentration-mortality curves were generated, and the residual toxicity values after 24 h of exposure ranged 0.003-0.08 µL cm^{-2} for deltamethrin, 0.04-2.038 µL cm^{-2} for EOPA and 0.0016-0.014 µL cm^{-2} for the binary mixture. The *S. zeamais* populations showed an uneven toxicity in response to the deltamethrin. The Jacarezinho (Paraná state) population showed resistance, with toxicity ratios for LC_{50} increasing by 3.06 and 4.13 times, when compared to susceptible populations, for EOPA and deltamethrin, respectively. The Barbacena (Minas Gerais state) population was considered as the susceptibility standard in the EOPA toxicity bioassays. The binary mixture suppressed the resistance mechanism of the tested *S. zeamais* populations due to the synergistic action of the bioinsecticide with the deltamethrin.

KEYWORDS: Maize weevil, deltamethrin, botanical insecticide.

RESUMO

Toxicidade e sinergismo de óleo essencial de *Piper aduncum* L. em populações de *Sitophilus zeamais* (Coleoptera: Curculionidae)

Estudos sobre o uso de óleo essencial de *Piper aduncum* L. (OEPA) como sinérgico em pragas de grãos armazenados ainda são incipientes. Objetivou-se determinar a toxicidade de deltametrina e OEPA, bem como estudar o efeito da mistura binária de OEPA + deltametrina, em cinco populações brasileiras de *Sitophilus zeamais*. A toxicidade foi investigada com base em dados de concentração-mortalidade de deltametrina e OEPA em treze populações de *S. zeamais*. A população padrão de suscetibilidade de ¼ CL_{20} foi utilizada com as combinações de deltametrina para gerar concentrações letais capazes de causar mortalidade de 50 e 95 % (CL_{50} e CL_{95}). Uma mistura binária de OEPA x deltametrina também foi testada em cinco populações de *S. zeamais* para detectar o efeito sinérgico com a deltametrina. As curvas concentração-mortalidade foram geradas e os valores de toxicidade residual após 24 h de exposição variaram 0,003-0,08 µL cm^{-2} para deltametrina, 0,04-2,038 µL cm^{-2} para OEPA e 0,0016-0,014 µL cm^{-2} para a mistura binária. As populações de *S. zeamais* apresentaram toxicidade desigual em resposta à deltametrina. A população de Jacarezinho (PR) apresentou resistência, com os índices de toxicidade para CL_{50} aumentando 3,06 e 4,13 vezes, em comparação às populações suscetíveis para OEPA e deltametrina, respectivamente. A população de Barbacena (MG) foi considerada padrão de suscetibilidade nos bioensaios de toxicidade de OEPA. A mistura binária suprimiu o mecanismo de resistência das populações de *S. zeamais* testadas devido à ação sinérgica do bioinseticida com a deltametrina.

PALAVRAS-CHAVE: Gorgulho do milho, deltametrina, inseticida botânico.

INTRODUCTION

Sitophilus zeamais Motschulsky (Coleoptera: Curculionidae), also known as maize weevil, is an insect pest that causes significant post-harvest damage both in the field and during storage, affecting maize on a worldwide scale. Adults and larvae feed on healthy grains. However, depending on the extent of damage, this pest can compromise the entire stored output (López-Castilho et al. 2018).

Conventional pest management approaches rely on synthetic pesticides, with chemical control

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showing the highest effectiveness, as it provides a fast response and decreases pests down to harmless levels, from the economic perspective (Freitas et al. 2016). Synthetic pesticides, on the other hand, are often not very selective, and their indiscriminate use has harmed human health and the environment, contributing to the growth of resistant insect populations. In this scenario, certain plant-derived substances (e.g., essential oils) have been proposed as sustainable alternatives for the problem posed by artificial products (Patiño-Bayona et al. 2021, Achimón et al. 2022).

Essential oils are complex mixtures of volatile, biodegradable organic molecules isolated from various portions of aromatic plants and composed of terpenes, phenylpropanoids and their derivatives, observed in the phytochemical profile of these by-products (Singh et al. 2021). Essential oils have been shown to have a variety of biological effects (e.g., acute and chronic toxicity, repellent activity, and suppression of oviposition, growth, feeding and development) on insect pest species (Yang et al. 2020). Furthermore, the insecticidal effectiveness of several essential oils against various agricultural pests has been thoroughly documented (Benelli & Pavella 2018, Campolo et al. 2018).

Piper aduncum L. is a Piperaceae that has been widely studied due to its chemical compounds with verified insecticidal activity against many insect orders. The toxicity of the essential oil of *P. aduncum* (EOPA) has been previously observed in several insect taxa, including Diptera (Misni et al. 2011), Hymenoptera (Souto et al. 2012), Coleoptera (Estrela et al. 2006), Lepidoptera (Fazolin et al. 2016) and Hemiptera (Turchen et al. 2016). Furthermore, because of its synergistic activity in mixtures with other insecticides, EOPA has the potential to be employed as an alternative to synthetic insecticides (Fazolin et al. 2016).

The idea of combining essential oils with conventional insecticides is interesting, as it could expand prospects for innovative insect pest management tactics, while reducing synthetic insecticide inputs (Faraone et al. 2015). These products are key instruments for overcoming insecticide resistance and increasing the availability of insecticide residues in the insect's body, in addition to reducing the use of commercial pesticides (Norris et al. 2018).

Given the economic importance of *S. zeamais* as a pest that affects maize and other stored cereals, research with botanical insecticides compatible with integrated pest management is timely, aiming at mitigating the negative effects of the indiscriminate use of synthetic insecticides (Coitinho et al. 2011). From this perspective, this study aimed to investigate the toxicity of deltamethrin and EOPA, as well as to assess the effect of a binary mixture of EOPA + deltamethrin, on *S. zeamais* Brazilian populations.

MATERIAL AND METHODS

The essential oil of *Piper aduncum* L. (EOPA) extraction was performed at the laboratory of the Fundação de Tecnologia do Estado do Acre, while the toxicity and synergism bioassays were performed at the Universidade Federal do Acre (UFAC), in Rio Branco, Acre state, Brazil.

Thirteen Brazilian populations of *Sitophilus zeamais* were collected from Barbacena, Juiz de Fora, Machado and Viçosa (Minas Gerais state); Crixás and Uirapuru (Goiás state); Jacarezinho and Londrina (Paraná state); Rio Branco and Plácido de Castro (Acre state); Picos (Piauí state); Recife (Pernambuco state); and Tunápolis (Santa Catarina state). The individuals were sourced from laboratory stock colonies and placed in 1.5-L glass flasks capped with perforated plastic lids and lined inside with organza fabric, in order to allow gas exchange at constant conditions of temperature (27 ºC), relative humidity (75 %) and scotophase (24 h). Following the methods developed by Sousa et al. (2009), maize grains with water content of 13 % on a wet basis were employed as food substrate, previously fumigated with phosphine (PH_3) and stored at -18 ºC to avoid reinfestation.

For synthetic insecticides bioassays, the active ingredient deltamethrin, trade name K-Obiol®25 EC (Bayer Vapi Private Limited/Plot nº 306/3, II Phase, GIDC Vapi 396 195 Gujarat/India), chemical group pyrethroids, toxicological class category 4 - low toxic, was employed.

Naturally occurring, *P. aduncum* adult plants were collected at the UFAC (9º57'29"S and 67º48'36"W). The plant material was collected in the morning during August 2021. Initially, botanical samples were gathered from 0.4 m of soil and dried to 30 % of moisture at 45 ºC. After drying, the plant material was placed in plastic bags to extract the essential oils. The *P. aduncum* specimen was deposited at the UFAC herbarium (voucher nº UFACPZ 20.646). Dr. Elsie Franklin Guimarães, of the Rio de Janeiro Botanical Garden Herbarium (RB Herbarium), was the professional who identified the species.

The hydrodistillation technique or water vapor dragging was employed to extract the essential oil. The extraction system was set up using a heating mantle to assign a volumetric flask coupled to a Clevenger apparatus, which was then connected to a refrigeration system. For each extraction, 150 g of dry biomass were passed through a funnel into a 5-L volumetric flask and calibrated with distilled water. After extraction, the oil was dried in a decantation funnel, using anhydrous sulfate. Subsequently, the essential oil was bottled in amber vials and stored in a refrigerator at 4 ºC.

Preliminary studies were conducted to estimate the minimum and maximum concentrations of essential oil that could cause insect mortality within a 5 to 95 % range. Based on this information, five distinct concentrations of EOPA and deltamethrin were established for the exposure bioassays of toxicity with the thirteen *S. zeamais* populations.

The following concentrations were used for definitive deltamethrin toxicity bioassays: 0.0027, 0.0046, 0.0093, 0.0173 and 0.0519 L cm^{-2}, except for the Crixás population, whose values were different (0.0027, 0.0046, 0.0056, 0.0093 and 0.0173 L cm^{-2}). Increasing levels of EOPA were used for the toxicity assay, as indicated in early testing (Table 1).

In order to assess the synergistic effect of the EOPA + deltamethrin mixture, preliminary tests were conducted with the sublethal concentration of EOPA (one-quarter of the LC_{20}: 0.0208 L cm^{-2} of the susceptibility standard population) combined with the synthetic commercial insecticide concentrations to estimate the minimum and maximum concentrations of insect mortality within a 5 to 95 % interval in five populations of *S. zeamais*. The EOPA toxicity test revealed the standard susceptibility population (Barbacena).

According to the methods modified by Almeida et al. (2017), the same sublethal concentration of EOPA was coupled with deltamethrin concentrations. The binary mixture (EOPA + deltamethrin) bioassay concentrations were 0.002, 0.005, 0.006, 0.009 and 0.014 L cm^{-2}. Only five populations were chosen for the synergism bioassays based on the populations with the highest and lowest LC_{50} in the deltamethrin bioassays. These populations were the ones from Jacarezinho, Plácido de Castro, Recife, Uirapuru and Viçosa.

The definitive toxicity and synergism bioassays were performed in Petri dishes (9.0 x 1.5 cm) coated with filter paper using 50 non-sexed insects aged 1 to 25 days. With an automatic pipettor, 1 mL of the tail volume was applied to the filter sheets according to the concentrations of the commercial insecticide and EOPA diluted in acetone (solvent), and 1 mL of acetone (solvent that did not impact the *S. zeamais* survival) for the control treatment. After the solvent had completely evaporated (approximately 5 min), 50 adult insects per plate were infested in four replications, and mortality was assessed after 24 hours.

In order to prevent the insects from escaping, the internal side surfaces of the Petri dishes were treated with inert talcum powder, in order to hinder the insects'

Table 1. Toxicity of the essential oil of *Piper aduncum* L. concentrations (C) used in toxicity bioassays of *Sitophilus zeamais* Motschulsky, 1855 (Coleoptera: Curculionidae) populations.

Population (city/state)	Concentrations (µL cm^{-2})				
	C1	C2	C3	C4	C5
Barbacena (MG)	0.0393	0.0786	0.6052	0.9416	1.1648
Crixás (GO)	0.0393	0.0786	0.6052	0.9416	1.1648
Jacarezinho (PR)	0.1179	0.2358	1.1648	1.4557	2.0389
Juiz de Fora (MG)	0.0393	0.1179	0.7074	0.9416	1.1648
Londrina (PR)	0.0786	0.1179	0.9416	0.9416	1.1648
Machado (MG)	0.0786	0.1556	0.9416	1.1648	1.4557
Plácido de Castro (AC)	0.0786	0.1556	0.6052	0.9416	1.1648
Picos (PI)	0.0786	0.1179	0.6052	0.9416	1.4557
Recife (PE)	0.0393	0.0786	0.6052	0.9416	1.1648
Rio Branco (AC)	0.0393	0.0786	0.6052	0.7074	0.9416
Tunápolis (SC)	0.0786	0.1179	0.6052	0.9416	1.1648
Uirapuru (GO)	0.0786	0.1556	0.6052	0.9416	1.1648
Viçosa (MG)	0.0786	0.1556	0.6052	0.9416	1.1648

movements. Subsequently, the dishes were sealed using rubber bands. Then, the dishes were kept in the laboratory under constant conditions of temperature (27 ± 2 ºC) and relative humidity (70 ± 5 %).

The concentrations that caused 50 and 95 % of insect mortality (LC_{50} and LC_{95}) were determined using toxicity and synergism bioassays, and the fiducial intervals and toxicity ratios among deltamethrin, EOPA and their binary mixture were calculated according to Robertson & Preisler (1992). Insect mortality was considered achieved when the individuals no longer moved when touched with a brush or when they were unable to move.

Concentration-mortality data from the toxicity and synergism bioassays were subjected to probit analysis (SAS Institute 2011), in order to generate concentration-mortality curves. Confidence intervals for toxicity ratios (TR) were calculated according to Robertson & Preisler (1992).

A correlation analysis ($p < 0.05$) was also carried out between the toxicity ratio (TR_{50} and TR_{95}) of OEPA and deltamethrin, in addition to the correlation of the binary mixture (OEPA + deltamethrin), in order to infer whether or not there was a synergism relationship, using the PROC CORR procedure of the SAS software (SAS Institute 2011).

RESULTS AND DISCUSSION

The probit model was adequate to analyze the mortality data, as low values of χ^2 (chi-square) and high values of p (probability) were observed for each concentration-mortality curve for toxicity values ($\chi^2 < 5.98$; $p > 0.05$), deltamethrin ($\chi^2 < 6.40$; $p > 0.05$), EOPA and EOPA + deltamethrin combination ($\chi^2 < 5.95$; $p > 0.05$).

The Viçosa and Jacarezinho populations had lower and higher LC_{50} values (0.003 and 0.013 L cm^{-2}, respectively), with toxicity ratios of 1 to 4.13 times the LC_{50}. Similarly, the populations of Crixás and Plácido de Castro had lower and higher LC_{95} values (0.019 to 0.066 L cm^{-2}) and toxicity ratios of 1 and 3.44 times the LC_{95}, respectively. These findings support the existence of response variation among populations of *S. zeamais* exposed to deltamethrin (Table 2).

Resistance to pesticides has been recognized as the primary limiting factor in the control of *S. zeamais* populations (Guedes et al. 1995, Oliveira et al. 2005, Fragoso et al. 2007, Guedes et al. 2017). Resistant populations from five Brazilian areas, including Jacarezinho, have shown resistance to DDT and pyrethroids since the mid-1990s (Guedes et al. 1995). The resistance mechanisms of the Jacarezinho population to insecticides, mainly from the pyrethroid group, are related to their greater detoxification capacity and the insensitivity of the action site (Fragoso et al. 2003, Ribeiro et al. 2003).

The slopes of the concentration-mortality curves varied among populations, with Viçosa showing the lowest value (1.67) and Crixás having the highest one (3.92). Greater slopes of the concentration-mortality curves indicate that lower deltamethrin concentrations might cause significant mortality, resulting in mortality variability in these groups (Carvalho et al. 2017).

Table 2. Relative residual toxicity of deltamethrin in adults from Brazilian populations of *Sitophilus zeamais*. The deltamethrin concentration range was 0.003-0.08 µL cm^{-2} (24 h of exposure).

Population (city/state)	Slope ± MSE	LC_{50} (FI 95 %) µL cm^{-2}	TR (IC 95 %) LC_{50}	LC_{95} (FI 95 %) µL cm^{-2}	TR (IC 95 %) LC_{95}	χ^2	P
Viçosa (MG)	1.67 ± 0.18	0.003 (0.001-0.003)	-	0.031 (0.02-0.05)	1.59 (0.99-2.56)	5.08	0.17
Recife (PE)	2.03 ± 0.18	0.005 (0.005-0.006)	1.52 (1.14-2.04)	0.031 (0.02-0.05)	1.61 (0.98-2.66)	4.55	0.20
Picos (PI)	2.29 ± 0.19	0.006 (0.005-0.006)	1.74 (1.40-2.16)	0.029 (0.02-0.04)	1.49 (1.02-2.18)	5.36	0.15
Barbacena (MG)	2.29 ± 0.19	0.006 (0.005-0.006)	1.79 (1.47-2.19)	0.030 (0.02-0.04)	1.55 (1.04-2.83)	5.38	0.14
Machado (MG)	2.65 ± 0.22	0.006 (0.005-0.006)	1.82 (1.51-2.20)	0.024 (0.03-0.05)	1.25 (0.92-1.50)	5.98	0.11
Rio Branco (AC)	2.09 ± 0.18	0.006 (0.003-0.006)	1.87 (1.54-2.27)	0.037 (0.03-0.05)	1.88 (1.20-2.93)	4.85	0.18
Juiz de Fora (MG)	2.38 ± 0.20	0.006 (0.005-0.006)	1.97 (1.62-2.41)	0.031 (0.02-0.04)	1.58 (1.06-2.35)	4.65	0.20
Tunápolis (SC)	2.18 ± 0.18	0.007 (0.006-0.007)	2.09 (1.73-2.54)	0.038 (0.03-0.05)	1.96 (1.26-3.03)	5.03	0.16
Crixás (GO)	3.92 ± 0.30	0.007 (0.007-0.008)	2.32 (1.97-2.73)	0.019 (0.01-0.02)	-	5.74	0.12
Londrina (PR)	2.44 ± 0.19	0.008 (0.008-0.009)	2.57 (2.21-2.99)	0.038 (0.03-0.05)	1.99 (1.31-3.01)	4.89	0.18
Uirapuru (GO)	2.25 ± 0.18	0.010 (0.010-0.011)	3.10 (2.57-3.73)	0.053 (0.04-0.08)	2.74 (1.81-4.13)	4.96	0.17
Plácido de Castro (AC)	2.23 ± 0.17	0.012 (0.012-0.014)	3.83 (3,16-4.65)	0.067 (0.05-0.10)	3.44 (2.44-5.29)	5.00	0.17
Jacarezinho (PR)	2.46 ± 0.18	0.013 (0.013-0.014)	4.13 (3,41-5.00)	0.061 (0.05-0.09)	3.17 (2.09-4.83)	4.95	0.18

MSE: mean standard error; LC: lethal concentration; TR: toxicity ratio for LC_{50} and LC_{95}; FI 95 %: fiducial interval at 95 % of probability; χ^2: chi-square; P: probability.

The mechanisms of action of pyrethroids exert their toxicity primarily through the disruption of the target site of sodium channels, causing imbalance and osmoregulatory dysfunction, contributing to their activity (Soderlund 2012). However, the indiscriminate use of these synthetics has contributed to the selection of weevils and prominent mechanisms of resistance to pyrethroids (Ribeiro et al. 2003, Haddi et al. 2018). The Brazilian *S. zeamais* populations showed susceptibility to the essential oil of P. *aduncum* L. (EOPA) after exposure via contact. The results ranged from 0.2676 to 10.53 L cm^{-2}, with TR_{50} and TR_{95} values ranging from 1.0 to 3.72 times. Because the population from Barbacena had the lowest LC_{50} (0.2676 L cm^{-2}), it was regarded as the susceptibility standard among the populations. The Jacarezinho population, on the other hand, had the highest LC_{50} (0.819 L cm^{-2}) and LC_{95} (10.53 L cm^{-2}).

Except for Jacarezinho, which showed a higher LC_{50} for both the EOPA and deltamethrin, the concentration-mortality curves amongst populations exposed to EOPA appear to be distinct from the data provided in the bioassays with deltamethrin. The synthetic insecticide showed a greater toxicity for *Sitophilus zeamais* populations, as evidenced by the low LC_{50} and LC_{95} values.

There were no significant connections between the deltamethrin x EOPA toxicity ratios (TR_{50}: n = 13; r = 0.59; p = 0.31; TR_{95}: n = 13; r = 0.23; p = 0.43). Similarly, there were no changes in the slope of the population curve (1.43-1.87), strengthening the toxicological homogeneity among the tested populations and demonstrating that the populations showed reaction uniformity (Table 3).

Based on the obtained results, no significant correlation was observed between the toxicity ratios of *S. zeamais* populations for EOPA and deltamethrin, indicating the absence of cross-resistance between these compounds. These finding suggests that the defense mechanisms present in insects from pyrethroid-resistant populations (e.g., Jacarezinho) are not effective when exposed to EOPA.

The toxicity of the binary mixture of EOPA + deltamethrin varied from 0.003 to 0.272 μL cm^{-2}, with LC_{50} and LC_{95} ratios ranging from 1.0 to 1.68 times. There were no significant differences in the curve slope of the five analyzed populations (1.58-2.71), reaffirming the toxicological homogeneity. The populations from Jacarezinho had a greater LC_{50}, whereas the Plácido de Castro population had a higher LC_{95} (Table 4).

The combination of EOPA and deltamethrin was toxic to all the five groups studied, despite the little variation in the toxicity ratios calculated according to Robertson & Preisler (1992). These findings suggest that the EOPA has a synergistic impact in amplifying the deltamethrin's active component action. With this level of response consistency, any evidence of cross-resistance between EOPA and deltamethrin is ruled out.

The secondary metabolism of Piperaceae plants is one of the most adaptable of all known botanical families, because the EOPA and its constituents

Table 3. Relative residual toxicity of the essential oil of *Piper aduncum* L. in adults from *Sitophilus zeamais* Brazilian populations. The concentration range was 0.04-2.04 μL cm^{-2} (24 h of exposure).

Population (city/state)	Slope ± MSE	LC_{50} (FI 95 %) μL cm^{-2}	TR (IC 95 %) LC_{50}	LC_{95} (FI 95 %) μL cm^{-2}	TR (IC 95 %) LC_{95}	χ^2	P
Barbacena (MG)	1.43 ± 0.11	0.268 (0.22-0.33)	-	3.811 (1.03-6.27)	1.35 (0.74-2.45)	5.95	0.11
Rio Branco (AC)	1.47 ± 0.12	0.269 (0.22-0.33)	1.00 (0.75-1.34)	3.733 (2.51-6.33)	1.32 (0.80-2.18)	6.03	0.11
Recife (PE)	1.47 ± 0.12	0.316 (0.26-0.43)	1.18 (0.89-1.57)	4.176 (2.86-6.87)	1.48 (0.79-2.75)	5.70	0.13
Crixás (GO)	1.46 ± 0.12	0.350 (0.29-0.43)	1.31 (0.98-1.74)	4.684 (3.15-7.85)	1.66 (0.86-3.20)	6.12	0.11
Viçosa (MG)	1.52 ± 0.14	0.354 (0.29-0.43)	1.32 (1.00-1.74)	4.300 (2.89-7.51)	1.52 (0.79-2.92)	6.10	0.11
Juiz de Fora (MG)	1.67 ± 0.14	0.373 (0.31-0.44)	1.39 (1.12-1.73)	3.599 (2.57-5.63)	1.27 (0.83-1.94)	6.01	0.11
Uirapuru (GO)	1.87 ± 0.22	0.374 (0.25-0.54)	1.40 (1.08-1.80)	2.831 (1.54-9.54)	-	6.40	0.10
Plácido de Castro (AC)	1.65 ± 0.12	0.376 (0.31-0.45)	1.41 (1.05-1.89)	3.745 (2.73-5.65)	1.32 (0.73-2.39)	6.17	0.10
Londrina (PR)	1.57 ± 0.13	0.377 (0.31-0.45)	1.41 (1.09-1.82)	4.203 (2.88-7.04)	1.49 (0.80-2.78)	5.77	0.12
Tunápolis (SC)	1.69 ± 0.13	0.405 (0.34-0.48)	1.52 (2.00-1.95)	3.794 (2.69-6.02)	1.34 (0.78-2.31)	5.70	0.13
Picos (PI)	1.82 ± 0.15	0.418 (0.36-0.49)	1.56 (1.24-1.97)	3.350 (2.11-5.13)	1.18 (0,63-2.21)	5.26	0.15
Machado (MG)	1.42 ± 0.13	0.498 (0.41-0.61)	1.86 (1.44-2.40)	7.077 (4.62-12.77)	2.50 (1.27-4.94)	5.79	0.12
Jacarezinho (PR)	1.48 ± 0.14	0.819 (0.61-0.68)	3.06 (2.32-4.03)	10.525 (6.77-19.58)	3.72 (1.79-7.73)	4.86	0.18

MSE: mean standard error; LC: lethal concentration; TR: toxicity ratio for LC_{50} and LC_{95}; FI 95 %: fiducial interval at 95 % of probability; χ^2: chi-square; P: probability.

Table 4. Relative residual toxicity of the combined effect of the essential oil of *Piper aduncum* L. + deltamethrin in adults from *Sitophilus zeamais* Brazilian populations. The concentration range of the binary mixture was 0.0016-0.014 µL cm^{-2} (24 h of exposure).

Population (city/state)	Slope ± MSE	LC_{50} (FI 95 %) µL cm^{-2}	TR (IC 95 %) LC_{50}	LC_{95} (FI 95 %) µL cm^{-2}	TR (IC 95 %) LC_{95}	χ^2	P
Viçosa (MG)	1.72 ± 0.19	0.004 (0.003-0.005)	-	0.032 (0.02-0.05)	1.07 (0.62-1.86)	5.40	0.14
Uirapuru (GO)	1.71 ± 0.19	0.004 (0.003-0.005)	1.16 (0.90-1.49)	0.037 (0.02-0.07)	1.26 (0.67-2.35)	5.95	0.11
Recife (PE)	2.11 ± 0.20	0.005 (0.005-0.005)	1.40 (1.13-1.75)	0.030 (0.02-0.04)	-	5.06	0.17
Plácido de Castro (AC)	1.58 ± 0.19	0.005 (0.005-0.006)	1.45 (1.16-1.80)	0.056 (0.04-0.12)	1.89 (0.91-3.94)	4.70	0.19
Jacarezinho (PR)	1.90 ± 0.20	0.006 (0.005-0.006)	1.68 (1.34-2.11)	0.043 (0.03-0.07)	1.45 (0.70-2.99)	5.24	0.16

MSE: mean standard error; LC: lethal concentration; TR: toxicity ratio for LC_{50} and LC_{95}; FI 95 %: fiducial interval at 95 % of probability; χ^2: chi-square; P: probability.

contain complex chemicals (arylpropanoids and terpenoids) that are responsible for the majority of repellent and insecticidal actions (Souto et al. 2012, Durofil et al. 2021). Furthermore, the EOPA composition is characterized by the association of lignins with the methylenedioxyphenyl group, which are important inhibitors of enzymes such as cytochrome P450-dependent monooxygenases (Bernad et al. 1995). These enzymes act by detoxifying insecticidal molecules, mainly pyrethroids and organophosphates, what justifies their potential as synergists (Fazolin et al. 2016 and 2017).

The toxicity ratios were lower than 2. These ratios, however, were highly and positively linked to the binary mixture x deltamethrin TR_{50} (n = 13; r = 0.97; p = 0.01) and TR_{95} (n = 13; r = 0.90; p = 0.03). With a reduction in the amount of the product's final volume, this positive connection among the combined concentrations of the binary mixture demonstrated increased residual toxicity, when compared to the results of the EOPA and deltamethrin isolated effects (Table 4).

Several studies have already mentioned relevant combinations of natural and synthetic products. Silva et al. (2017) observed that the combination between the essential oil of *Occimum basilicum* L. and deltamethrin reduced the commercial product by 80 % in *Spodoptera frugiperda* (J. E. Smith, 1797) caterpillars. Fazolin et al. (2016) reported synergistic effects when mixing the essential oil of *P. aduncum* L. with pyrethroids in *S. frugiperda* caterpillars, similarly to the prior study. Faraone et al. (2015) observed that certain essential oils, in addition to acting as synergists, have also demonstrated antagonistic activity, impairing the effectiveness of the active ingredient of the synthetic product. Furthermore, other studies show that binary mixtures of essential oils and deltamethrin increased the mortality of bedbugs resistant to deltamethrin (Gaire et al. 2021) and contributed to the effectiveness of insecticide formulations in controlling multiple strains of mosquitoes (Norris et al. 2018).

Despite these combinations of natural and synthetic products, there are still challenges faced due to the non-regularization of these formulations registered in the country for the management of stored grain pests, what increases the exclusive dependence on synthetic products and the frequency of resistant genotypes. In order to mitigate selection pressure and preserve the effectiveness of insecticides, strategies such as adopting longer application intervals or incorporating supplementary active ingredients are suggested (Santos et al. 2009, Correa et al. 2015). The *S. zeamais* Brazilian populations evaluated in these studies show susceptibility to a response to EOPA exposure through contact. Although the Jacarezinho population showed higher CL_{50} to EOPA, the use of essential oils is a valid strategy to control stored grains, owing to the toxic effects discovered in experiments with *S. zeamais* individuals (Estrela et al. 2006, Oliveira et al. 2018). In this regard, the incorporation of alternative methods for the use of plant-based products is critical, as this technology can be passed on to small and medium farmers, while integrating it with older and more modern techniques and taking into account the cost and environmental compatibility (Stejskal et al. 2021).

In the present investigation, the EOPA effects induced a heightened response in conjunction with deltamethrin, in addition to its inherent toxicity. As a result, the use of EOPA in the control of *S. zeamais* through contact is promising, and more research should be conducted using other intoxication methods (e.g., fumigation or topical contact), in order to corroborate the use of natural and synthetic compound combinations for pest management.

CONCLUSIONS

1. The evaluated *Sitophilus zeamais* Brazilian populations showed non-uniformity in response to deltamethrin toxicity;
2. Among the thirteen *S. zeamais* Brazilian populations, Jacarezinho (Paraná state) and Plácido de Castro (Acre state) showed to be resistant to deltamethrin, whereas the population from Viçosa (Minas Gerais state) is susceptible with the lowest lethal concentration capable of causing 50 % of mortality;
3. The essential oil of *Piper aduncum* L. (EOPA) was toxic to the *S. zeamais* Brazilian populations, demonstrating response homogeneity, except for the population from Jacarezinho (Paraná state), which was resistant to both the EOPA and deltamethrin;
4. The binary mixture EOPA x deltamethrin inhibited the resistance mechanisms in five Brazilian *S. zeamais* populations (Viçosa, Uirapuru, Recife, Plácido de Castro and Jacarezinho), suggesting response consistency.

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