EFEITOS TÓXICOS DE PESTICIDAS COMERCIAIS E BOTÂNICOS SOBRE OS PARÂMETROS COMPORTAMENTAIS DE *ZEBRAFISH* (*Danio rerio*)

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RESUMO

O objetivo deste trabalho foi avaliar o efeito tóxico dos pesticidas comerciais, óleos essência e suas misturas, que apresentaram atividade pesticida contra *Plutella xylostella*, nos parâmetros comportamentais atividade locomotora e comportamentos sociais do *zebrafish* (*Danio rerio*). Os animais adultos foram expostos a pesticidas comerciais deltametrina, azadiractina, clorantraniliprole, óleos essenciais *Eugenia caryophyllus*, *Melaleuca alternifolia*, *Citrus aurantium var. dulcis*, *Citrus aurantiifolia*, *Eucalyptus globulus*, *Melaleuca leucadendra* e suas misturas. Foram avaliados mortalidade e os parâmetros comportamentais em exposição crônica, pelo método de varredura instantânea, atividade locomotora e comportamento social, de acordo com seguintes parâmetros: distância percorrida, velocidade média e tempo inativo. Houve mortalidade de 100% nos animais expostos a deltametrina sendo este o pesticida mais tóxico. Foram observadas modificações comportamentais ($p < 0,05$) em forragear, descansar e ataque nos diferentes pesticidas. Não foram observadas alterações na atividade locomotora e comportamento social. Pode-se concluir que a exposição aos pesticidas comerciais, óleos essenciais e suas misturas foram tóxicos para *zebrafish*. A exposição as baixas concentrações destes pesticidas comprometeram a sobrevivência e o comportamento do *zebrafish*. Mesmo o biopesticida sendo considerado seguro, pode trazer riscos ao ambiente aquático e seus efeitos tóxicos devem ser estudados para determinar concentrações seguras de uso na atividade agrícola.

Palavras-chave: Toxicidade, Óleo essencial, Comportamento animal, Atividade locomotora.

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TOXIC EFFECTS OF COMMERCIAL AND BOTANICAL PESTICIDES ON THE BEHAVIORAL PARAMETERS OF ZEBRAFISH (*Danio rerio*)

ABSTRACT

The objective of this work was to evaluate the toxic effect of commercial pesticides, essential oils, and their mixtures, which present pesticide activity against *Plutella xylostella*, on the behavioral parameters, locomotor activity, and social behavior of *zebrafish* (*Danio rerio*). Adult animals were exposed to commercial pesticides deltamethrin, azadirachtin, chlorantraniliprole, essential oils *Eugenia caryophyllus*, *Melaleuca alternifolia*, *Citrus aurantium var. dulcis*, *Citrus aurantiifolia*, *Eucalyptus globulus*, and *Melaleuca leucadendra,* and mixtures of the substances. Mortality and behavioral parameters during chronic exposure were evaluated using the instantaneous scan sampling method, and locomotor activity and social behavior were assessed, according to the following parameters: distance covered, average speed, and inactive time. 100% mortality in animals exposed to deltamethrin was observed. Behavioral changes ($p < 0.05$) were found in foraging, resting, and attacking behaviors. Locomotor activity and social behavior were not affected by exposure. It can be concluded that exposure to commercial pesticides, essential oils, and their mixtures was toxic to zebrafish. Exposure to low concentrations of these pesticides affected the survival and behavior of zebrafish. Although biopesticides are considered safe, they can cause risks to the aquatic environment, and their toxic effects need to be studied to determine their safe use in agricultural activities.

Keywords: Toxicity, Essential oil, Animal behavior, Locomotor activity.

EFECTOS TÓXICOS DE PESTICIDAS COMERCIALES Y BOTÁNICOS EN LOS PARÁMETROS DE COMPORTAMIENTO DEL PEZ CEBRA (*Danio rerio*)

RESUMEN

El objetivo de este trabajo fue evaluar el efecto tóxico de plaguicidas comerciales, aceites esenciales y sus mezclas, que mostraron actividad plaguicida contra Plutella xylostella, sobre parámetros de comportamiento, actividad locomotora y conductas sociales del pez cebra (*Danio rerio*). Los animales adultos fueron expuestos a pesticidas comerciales deltametrina, azadiractina, clorantraniliprol, aceites esenciales *Eugenia caryophyllus*, *Melaleuca alternifolia*, *Citrus aurantium var. dulcis*, *Citrus aurantiifolia*, *Eucalyptus globulus*, *Melaleuca leucadendra* y mezclas de los mismos. Se evaluaron parámetros de mortalidad y comportamiento en exposición crónica por el método de escaneo instantáneo, actividad locomotora y comportamiento social, según los siguientes parámetros: distancia recorrida, velocidad promedio y tiempo de inactividad. Hubo 100% de mortalidad en animales expuestos a deltametrina, que es el pesticida más tóxico. Se observaron cambios de comportamiento ($p < 0,05$) en el forrajeo, reposo y ataque a los diferentes plaguicidas. No se observaron cambios en la actividad locomotora y el comportamiento social. Se puede concluir que la exposición a pesticidas comerciales, aceites esenciales y sus mezclas fue tóxica para el pez cebra. La exposición a bajas concentraciones de estos pesticidas comprometió la supervivencia y el comportamiento del pez cebra. Incluso el biopesticida se considera seguro, puede traer riesgos al medio acuático y sus efectos tóxicos deben ser estudiados para determinar concentraciones seguras para su uso en la actividad agrícola.

Palabras llave: Toxicidad, Aceite esencial, Comportamiento animal, Actividad locomotora.

INTRODUCTION

The diamondback moth (*Plutella xylostella*) is an insect of the order Lepidoptera (Plutellidae) that attacks several species of the Brassicaceae family. The most common method of controlling *P. xylostella* is through the application of pesticides, which are often used intensively, due to the high reproductive rate of this insect (1). The biology of this insect contributes to increased resistance to commercial pesticides, including deltamethrin (2), chlorantraniliprole (3), and azadirachtin (4). Deltamethrin is used for various purposes, such as controlling arthropods in agriculture and domestic pets (5). The high consumption of deltamethrin has made it available in the environment, contaminating surface water, and bringing risks to aquatic animals (6). Due to its broad spectrum of action, deltamethrin is found in veterinary products as an emulsifiable concentrate (7), and in shampoos, lotions, collars, spot-ons, and sprays, being a source of indirect contamination to water bodies after washing animals, through owners, and residential environments (6). This compound has a half-life of 17.9 days in an aquatic environment and 45.2 days in sediment (8).

Chlorantraniliprole is used to control pests in coffee, apple, rice, sugar cane, and peach crops (9). Lahm et al (10) consider this pesticide safe for mammals because of the difference between insect and mammal ryanodine receptors. Excessive use of this pesticide can contaminate the soil and can be toxic to non-target organisms, for example, earthworm reproduction was affected after exposure to 5.0 mg/kg of chlorantraniliprole (11). For aquatic animals such as *jundiá* fish (*Rhamdia quelen*), changes were found in the patterns of cortisol, glucose, and plasma protein (9).

Azadirachtin is a tetranortriterpenoid compound extracted from the neem tree, with pesticide activity, widely used in insect control, and is considered a less toxic pesticide (12). In addition, the same authors reported that azadirachtin has antibacterial and antifungal properties and is also used in the treatment of various diseases as it has pharmacological properties. Azadirachtin is a biopesticide, and the literature reports that this pesticide is toxic to zebrafish (*Danio rerio*) (13) and common carp (*Cyprinus carpio* L) (12).

As an alternative to commercial pesticides, essential oils (EOs) are used, aiming to reduce toxicity to the environment (14). EOs are secondary metabolites of plants, with volatile and aromatic characteristics, obtained from non-woody parts, which contain monoterpenes, diterpenes, sesquiterpenes, and terpenoids in their composition (15), serving as a defense for plants against fungi, arthropods, and herbivores (16). Due to these characteristics, properties such as flavoring, fragrance, antioxidant, antimicrobial, antiviral, antimycotic, antitoxic, antiparasitic, and insecticide have been reported (15). Promising results were found using EOs against *P. xylostella* with biopesticides obtained from *Eugenia caryophyllus*, *Melaleuca alternifólia*, *Citrus aurantium var. dulcis*, *Citrus aurantiifolia*, *Eucalyptus globulus*, and *Melaleuca leucadendra* EOs (17). However, the literature lacks research on the possible toxic effects of these biopesticides in animal models such as fish. As an example, toxicity tests were assayed with tambaqui (*Colossoma macropomum*) exposed to eugenol, which is known to have anesthetic activity in fish. The results revealed an intense neural excitability response with seizure patterns (18). In view of this, it is necessary to evaluate the toxicity of biopesticides from *Eugenia*, *Melaleuca*, *Citrus*, and *Eucalyptus* EOs as although they demonstrate good activity against *P. xylostella* (17), this does not guarantee that biopesticides from EOs are safe for other animals, such as fish.

Zebrafish are used as a model in neurological research for behavioral monitoring in response to different environmental stimuli, such as social interaction, and reactions to individuals, and predators in order to understand neurological disorders (19). Behavioral analysis can provide quantification of the functional capacity of zebrafish in larval and adult stages, as these animals are a useful tool in the assessment of neurobehavioral toxicology when exposed to environmental pollutants (20). Video monitoring is a good tool for zebrafish behavioral assessment (19). It is already known that exposure to environmental pollutants can cause changes in psychomotor speed, dexterity, and memory (21). In exploratory testing of new environments, reductions in diving, freezing, and exploration may be related to anxiety patterns (19). This test can also be used for the evaluation of pesticides that have neurotoxic effects. In this scenario, the current study aims to comparatively evaluate the toxic effects of commercial pesticides, essential oils, and their mixtures that showed good pesticidal activity against *P. xylostella* by collecting behavioral data using the scan sampling method, locomotor activity, and social behavior, with *Danio rerio* as an animal model.

MATERIAL AND METHODS

The experiments were carried out at the *Laboratório de Ecofisiologia e Comportamento Animal* (LECA), *Universidade Federal Rural de Pernambuco* with the protocols previously approved by the Ethics Committee in the Use of Animals (CEUA/UFRPE), license nº 019/2019. The pesticides deltamethrin (Decis®, lot # 001-18-9960, CAS number 52918-63-5, Bayer, São Paulo, SP, Brazil), chlorantraniliprole (Premio®, lot # 005-18-1998, CAS number 500008-45-7, Dupont, Barueri, SP, Brazil), azadirachtin (Azamax®, lot # 001-13-3024, CAS number 11141-17-6, UPL, Ituverava, SP, Brazil), and the solvent dimethylsulfoxide (lot # 85713, CAS number 67-68-5, Dinâmica, Indaiatuba, SP, Brazil) were commercially obtained. Essential oils and their mixtures *Eugenia caryophyllus, Melaleuca alternifólia*, *Citrus aurantium var. dulcis, Citrus aurantiifolia*, *Eucalyptus globulus*, and *Melaleuca leucadendra* were produced in the *Laboratório de Produtos Naturais Bioativos* of the same university, which provided their respective chemical characterizations. The major compounds of the EOs are shown in Table 1 (17). The other reagents used in the research were of analytical grade.

Table 1. Main compounds found in essential oils characterized by GC-MS/MS (Gas Chromatography Coupled to Mass Spectrometry) (17).

Compound %	*Eugenia caryophyllus*	*Melaleuca alternifolia*	*C. aurantium var. dulcis*	*Citrus aurantiifolia*	*Eucalyptus globulus*	*Melaleuca leucadendra*
Eugenol	72.80 ± 0.80					
Eugenol acetate	12.51 ± 0.04					
Terpinen-4-ol		45.92 ± 0.68				
α-terpinene		12.78 ± 0.73				
1,8–cineol					77.91 ± 1.14	
Limonene			75.15 ± 1.34	57.65 ± 0.95		4.26 ± 0.03
Menthol			7.44 ± 0.48			
Z-β-ocimene				15.53 ± 0.33		
ρ-cimene					10.19 ± 0.37	
(E)-nerolidol						85.44 ± 0.59

Adult animals of *Danio rerio* (≈ 6 months of age), wild type (WT), were obtained from a commercial supplier and housed in 80-liter glass aquariums with artificial aeration for 30 days, for a health assessment. The abiotic parameters adopted were; dissolved oxygen 11 mg/L, temperature 26 ± 1 °C, pH 7.5 ± 0.5, and photoperiod 14/10 h (light/dark), which are considered optimal. The fish were fed daily in the early morning with extruded commercial feed (45% crude protein) during the experiment.

Toxicological tests were carried out using 16 experimental groups, with 10 animals per group (total of 160 animals) housed in glass aquariums with a capacity of 8 L. The water was partially renewed twice a week and fully once a week with the maintenance of abiotic parameters as described above. The concentrations chosen were based on the methodology (22), which corresponded to 1% of the LC_{50} 48 h for *P. xylostella* (17). The mixtures that obtained the best synergism rates against *P. xylostella* were selected (17). The commercial pesticides, essential oils, and their mixtures used in the experiments were deltamethrin 400 µg/L, azadirachtin 330 µg/L, chlorantraniliprole 30 µg/L, *E. caryophyllus* 410 µg/L, *M. alternifolia* 2940 µg/L, *C. aurantium var. dulcis* 2990 µg/L, *C. aurantiifolia* 1900 µg/L, *E. globulus* 3010 µg/L, *M. leucadendra* 1990 µg/L, *M. leucadendra* + *E. caryophyllus* (1:1) 1550 µg/L, *M. alternifólia* + *E. globulus* (9:1) 1470 µg/L, *E. caryophyllus* + *C. aurantiifolia* (9:1) 3140 µg/L, *E. globulus* + *C. aurantiifolia* (9:1) 1300 µg/L, azadirachtin + *C. aurantium var. dulcis* (9:1) 390 µg/L, and deltamethrin + *C. aurantiifolia* (9:1) 250 µg/L. Commercial pesticides, essential oils, and their mixtures were dissolved in dimethylsulfoxide (DMSO) to obtain a DMSO final concentration of 0.003% (v/v). This concentration is safe for conducting toxicity tests with zebrafish (23).

As response variables of the experiment, mortality and behavioral patterns exhibited by *D. rerio* were evaluated. Mortality was determined daily according (24). Behavioral data were collected according to an ethogram developed previously (25,26). The scan sampling method (25) was used with 30 min sessions intercalated with 1-min of observation and 1-min of interval, twice a week for 70 days. After 70 days of exposure, video capture was performed (patent BR 102019009007-3) for assessment of locomotor activity and social behavior using 10 animals. One animal at a time was placed in an acrylic aquarium measuring 28 x 18 x 25 cm with a 5 cm water depth and the animal's circulation region was divided into two areas (named area 1 and area 2) to assess the behavior of open field and shoal interaction tests. The test included 10 min for acclimatization, after which the animal was recorded for 5 min. (open field test). Next, the division was removed to show a zebrafish shoal in another aquarium placed near area 1. The same fish was recorded again for another 5 min. (shoal interaction test). Data are presented as mean ± SD. The results obtained were evaluated by one-way ANOVA when the difference was significant, and the means were compared by the Tukey test ($p < 0.05$). The software used was Origin Pro Academic 2015 (Origin Lab. Northampton, MA USA).

RESULTS

Data evaluation showed 100% mortality of animals exposed to deltamethrin in 24 h of exposure. In addition, 30% mortality of animals exposed to azadirachtin was observed after 6 days. In the experimental group exposed to *E. caryophyllus* EO, we observed 10% mortality after 43 days, remaining at the same level until the end of the experiment. Animals exposed to *C. aurantium var. dulcis* EO presented 10% mortality after 8 days and 20% after 43 days. In 50 days of exposure to *C. aurantiifolia* EO, we observed 10% mortality, while 60% mortality was observed after 8 days in animals exposed to the Del + Ca mixture, reaching 100% mortality in 43 days. Therefore, deltamethrin proved to be the most toxic commercial pesticide, *C. aurantium var. dulcis* the most toxic EO, and Del + Ca the most toxic mixture.

The results of the scan sampling method over 70 days of exposure to commercial pesticides, essential oils, and their mixtures are shown in Tab. 2. It was not possible to collect behavioral data for deltamethrin due to the high mortality that occurred at the beginning of the experiment. The commercial pesticides azadirachtin and chlorantraniliprole did not affect behavioral patterns. In general, the reduction in forage frequency was the only behavioral change observed in animals exposed to EOs. *E. caryophyllus*, *M. alternifólia*, and *C. aurantiifolia* EOs had low toxicity in this analyzed parameter. For Ma + Eg, and Del + Ca mixtures, a reduction in forage behavior was also observed. This reduction in food searching is indicative of a stress response, which may be related to chemical exposure (Table 2). The group exposed to the Del + Ca mixture also showed an increase in the frequency of rest behavior and a reduction in forage and attack behaviors. This inactivity may also be related to toxicity as this mixture was the most toxic among those studied. The presence of deltamethrin in the mixture was probably responsible for inducing the higher toxicity.

Regarding the assessment of locomotor activity after 70 days of exposure, no significant changes were observed when the animals were submitted to open field tests with distances covered from 13798.19 to 28604.23 cm ($F(13, 69) = 0.83$, $p < 0.63$), average speed from 45.94 to 95.22 cm/s ($F(13, 69) = 0.82$, $p < 0.63$), and inactive time from 1.10 to 112.93 s ($F(13, 69) = 1.06$, $p < 0.41$) during the 5 min of observation. In addition, no significant differences were found in the shoal interaction test with distances covered from 12621.55 to 24141.09 cm ($F(13, 69) = 1.20$, $p < 0.30$), average speed from 42.03 to 80.43 cm/s ($F(13, 69) = 1.20$, $p < 0.30$), and inactive time from 16.75 to 90.74 s ($F(13, 69) = 0.42$, $p < 0.96$). This demonstrates the low toxicity of these EOs and their mixtures, despite their good pesticidal activity against *P. xylostella*.

Table 2. The average number of events (n = 20) of behaviors exhibited by adult *Danio rerio* during 70 days of exposure to commercial pesticides, essential oils, and their mixtures by the instantaneous scan sampling method.

	NL	**NR**	**FL**	**DE**	**AS**	**NI**	**MC**
Control	13.6 ± 15.1	50.1 ± 26.1	3.2 ± 4.7	3.1 ± 3.9	0.0 ± 0.0	0.0 ± 0.0	0.2 ± 0.6
Deltamethrin	ND	ND	ND	ND	ND	ND	ND
Azadirachtin	13.9 ± 15.9	32.2 ± 18.1	2.1 ± 3.7	1.0 ± 2.2	0.0 ± 0.0	0.0 ± 0.0	0.2 ± 0.4
Chlorantraniliprole	19.7 ± 20.8	45.7 ± 28.0	5.3 ± 7.4	1.8 ± 2.9	0.0 ± 0.0	0.0 ± 0.0	0.2 ± 0.4
E. caryophyllus	25.2 ± 25.4	50.5 ± 27.1	3.3 ± 4.7	0.6 ± 1.8	0.0 ± 0.0	0.0 ± 0.0	0.2 ± 0.4
M. alternifólia	16.0 ± 18.1	46.0 ± 23.4	4.3 ± 5.3	2.3 ± 2.8	0.0 ± 0.0	0.0 ± 0.0	0.3 ± 0.6
C. aurantium var. dulcis	16.4 ± 17.9	40.1 ± 20.9	4.9 ± 6.3	2.3 ± 4.5	0.0 ± 0.0	0.0 ± 0.0	0.3 ± 0.7
C. aurantiifolia	21.8 ± 23.0	43.9 ± 31.6	6.9 ± 5.8	3.4 ± 4.5	0.0 ± 0.0	0.0 ± 0.0	0.2 ± 0.6
E. globulus	24.1 ± 17.8	49.3 ± 24.8	4.7 ± 5.3	3.0 ± 6.2	0.0 ± 0.0	0.0 ± 0.0	0.5 ± 1.2
M. leucadendra	27.5 ± 21.0	45.0 ± 27.5	7.8 ± 9.0	5.3 ± 7.9	0.0 ± 0.0	0.0 ± 0.0	0.2 ± 0.7
Ml + Ec	19.8 ± 16.3	46.4 ± 21.5	4.8 ± 5.8	0.9 ± 2.1*	0.0 ± 0.0	0.0 ± 0.0	0.4 ± 0.7
Ma + Eg	15.7 ± 16.3	50.2 ± 22.6	1.8 ± 3.0	1.9 ± 5.5	0.0 ± 0.0	0.0 ± 0.0	0.4 ± 0.8
Ec + Ca	12.7 ± 13.6	47.3 ± 19.7	3.8 ± 3.7	3.2 ± 6.8	0.0 ± 0.0	0.0 ± 0.0	0.2 ± 0.5
Eg + Ca	24.6 ± 19.1	56.8 ± 28.2	3.9 ± 3.5	1.1 ± 2.0	0.0 ± 0.0	0.0 ± 0.0	0.2 ± 0.5
Aza + Cavd	17.4 ± 21.4	53.5 ± 25.9	4.3 ± 4.1	1.8 ± 4.3	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.3
Del + Ca	2.8 ± 2.9	23.0 ± 30.8	5.7 ± 6.0	**9.7 ± 12.6***	0.0 ± 0.0	0.0 ± 0.0	0.2 ± 0.4

to be continued.

Continuation.

	FO	CA	AG	PE	FU	AT	AF	RA
Control	27.4 ± 16.3	0.1 ± 0.2	5.3 ± 5.7	7.6 ± 6.7	7.6 ± 6.8	13.2 ± 7.2	16.7 ± 8.6	2.2 ± 3.0
Deltamethrin	ND	ND	ND	ND	ND	ND	ND	ND
Azadirachtin	16.1 ± 8.1	0.1 ± 0.2	4.3 ± 4.2	8.7 ± 5.0	7.6 ± 4.3	7.8 ± 3.8	10.4 ± 5.2	3.2 ± 4.1
Chlorantraniliprole	23.8 ± 11.9	0.1 ± 0.3	5.1 ± 6.3	7.7 ± 6.4	7.5 ± 6.4	14.5 ± 6.6	17.0 ± 7.6	2.0 ± 2.8
E. caryophyllus	**11.0 ± 9.0***	0.1 ± 0.3	6.6 ± 6.2	7.0 ± 6.4	7.0 ± 6.4	12.4 ± 6.3	16.8 ± 7.6	2.9 ± 3.3
M. alternifólia	**14.3 ± 9.4***	0.0 ± 0.0	6.2 ± 6.9	9.5 ± 6.4	9.5 ± 6.4	16.9 ± 7.6	21.3 ± 11.1	2.2 ± 3.0
C. aurantium var. dulcis	18.3 ± 11.2	0.1 ± 0.2	5.0 ± 5.4	6.6 ± 5.6	6.7 ± 5.5	11.8 ± 4.9	15.3 ± 8.4	2.8 ± 3.6
C. aurantiifolia	**11.9 ± 8.5***	0.1 ± 0.2	5.4 ± 6.0	7.7 ± 6.1	7.6 ± 6.2	16.0 ± 8.6	19.8 ± 12.0	1.5 ± 2.1
E. globulus	**14.4 ± 10.1***	0.1 ± 0.2	6.3 ± 7.1	7.1 ± 6.4	7.2 ± 6.5	13.6 ± 5.5	18.3 ± 10.0	1.8 ± 2.4
M. leucadendra	23.0 ± 13.1	0.0 ± 0.0	5.0 ± 5.2	5.5 ± 5.0	5.4 ± 4.9	9.8 ± 6.0	13.9 ± 9.8	2.0 ± 2.4
Ml + Ec	20.6 ± 12.0	0.5 ± 1.4	7.0 ± 6.9	6.7 ± 7.1	6.9 ± 7.1	13.8 ± 7.5	19.9 ± 11.0	2.4 ± 2.9
Ma + Eg	**13.7 ± 11.5***	0.0 ± 0.0	10.8 ± 8.6	8.1 ± 6.7	7.7 ± 6.7	15.4 ± 7.6	22.7 ± 11.9	1.9 ± 1.6
Ec + Ca	21.2 ± 8.5	0.0 ± 0.0	7.7 ± 6.9	7.3 ± 6.6	7.3 ± 6.6	16.4 ± 8.3	21.5 ± 12.0	1.7 ± 2.3
Eg + Ca	16.6 ± 12.1	0.1 ± 0.3	7.5 ± 7.1	5.1 ± 5.9	5.0 ± 5.9	10.6 ± 6.7	15.8 ± 10.2	2.8 ± 3.2
Aza + Cavd	21.2 ± 9.9	0.0 ± 0.0	8.0 ± 7.2	5.2 ± 5.0	5.3 ± 5.2	12.5 ± 7.8	19.2 ± 11.3	1.7 ± 1.5
Del + Ca	**3.5 ± 5.1***	0.0 ± 0.0	2.3 ± 3.1	4.0 ± 6.4	4.1 ± 6.5	**4.1 ± 5.6***	5.3 ± 5.6	0.6 ± 1.5

Legend: SS – Slow swimming; FS – Fast swimming; FL – Float; RE – Rest; JU – Jump; IS – Inclined swimming; CS - Circular swimming; FO – Forage; EA – Eat; CC– Continuous chase; CH – Chase; ES– Escape; AT – Attack; CE – Continuous escape; AB – Aerial breath. Ml + Ec - *M. leucadendra* + *E. caryophyllus*; Ma + Eg - *M. alternifolia* + *E. globulus*; Ec + Ca - *E. caryophyllus* + *C. aurantiifolia*; Eg + Ca - *E. globulus* + *C. aurantiifolia*; Aza + Cavd - Azadirachtin + *C. aurantium var. dulcis*; Del + Ca - Deltamethrin + *C. aurantiifolia*. * Statistically significant difference from control ($p < 0.05$) by Tukey's test. The significant results in the one-way ANOVA were DE ($F(14, 290) = 2.88$, $p < 0.001$), FO ($F(14, 290) = 5.09$, $p < 0.001$), AT ($F(14, 290) = 4.06$, $p < 0.001$).

DISCUSSION

Among the commercial pesticides tested, deltamethrin and azadirachtin were toxic to zebrafish. Regarding mortality, deltamethrin (400 µg/L) was the most toxic compound, causing 100% mortality in animals in 24 h, a concentration equivalent to 1% of the LC_{50} 48h for the insect *Plutella xylostella*. Deltamethrin is highly toxic at concentrations even lower than those adopted in our study. Other authors found a 24-hour LC_{50} for *D. rerio* of 14.43 µg/L (22). According to the authors, deltamethrin accumulates in the tissues of the liver and gills, causing respiratory problems, leading to hypoxia and death. On the other hand, azadirachtin (330 µg/L) caused 30% lethality in zebrafish 144 hours after exposure. Other authors (13) also found a 96 h LC_{50} of 0.22 mL/L (≈ 33 µg/L) in adult *Danio rerio*. According to Akinwande *et al.* (2006), azadirachtin caused epithelial changes in the gills of the fish *Heterobranchus bidorsalis*, causing an increase in the opercular beat, to compensate for the inability of the gills to carry out the gas exchange, leading to mortality. This could justify the mortality found in our study.

E. caryophyllus EO (410 µg/L), whose major component is eugenol, caused 10% mortality throughout the experiment. It was suggested that exposure to eugenol may lead to muscle immobilization by blocking sodium channels (18). This may have caused the interruption in opercular beats, reducing oxygen uptake, which could explain the mortality of some animals and the reduction in forage behavior found in our study. For the *M. alternifolia* EO, was observed that exposure to this EO (500 µL/L) caused an anesthetic effect in the fish *Rhamdia quelen (jundiá) (27)*. The same authors observed that the primary component terpinen-4-ol (200 µL/L) present in the *M. alternifolia* EO induced a high anesthetic effect. The other components present (α-terpinene and γ-terpinene) in this EO had no anesthetic action in fish, and α-terpinene when combined with terpinen-4-ol promoted an antagonistic effect, increasing the anesthetic induction time, which would explain the higher concentration of *M. alternifolia*

EO to induce anesthesia. This anesthetic effect of *M. auternifolia* EO could induce lethargy in the animals, reducing the forage behavior of zebrafish as observed in our study.

The major compound in *C. aurantium var. dulcis* and *C. aurantiifolia* EOs is limonene. Animals exposed to *C. aurantium var. dulcis* EO presented 20% mortality, although no behavioral changes were observed. On the other hand, exposure to *C. aurantiifolia* EO caused 10% mortality in animals and reduced forage behavior. Was observed 37% mortality in *R. quelen* exposed for 8 h to 200 µL/L of *C. aurantium* EO (28). The same authors observed an increase in opercular ventilation in the experimental groups that did not present mortality. They also observed an anesthetic effect in animals when exposed to *C. aurantium* EO, suggesting that this effect may be a result of limonene binding to adenosine A_{2A} receptors. This anesthetic action in chronic exposure may be related to the reduction in forage behavior and mortality observed in our study.

E. globulus EO (3010 µg/l), whose major component is 1,8–cineole, also caused a reduction in forage behavior. It was observed an anesthetic effect in an acute toxicity test where 1,8–cineole induces inhibition of AChE in *Poecilia reticulata (29)*. This inhibition induced paralysis and erratic swimming. The same authors determined a 24-h LC_{50} of 3997.07 mg/L for females and 1701.93 mg/L for males. We can suppose that 1,8–cineole may be responsible for the change in the frequency of forage behavior observed in our study since we worked with concentrations lower than those that induced mortality, paralysis, and erratic swimming used by (29).

The Ma + Eg mixture (1470 µg/L), which was combined in a 9:1 ratio, also caused a reduction in forage behavior. As previously reported, the major compounds terpinen-4-ol (27) and 1,8–cineole (29ccccc) are present in *M. alternifolia* and *E. globulus* EOs, and are responsible for causing anesthesia in fish. The combination of these compounds can reduce or increase this toxicity (5). However, in the Ma + Eg mixture, we did not observe any significant difference ($p > 0.05$) compared to the isolated compounds, indicating that there was no change in the toxicity of the mixture regarding the parameters studied.

In the current study, the highest toxicity was observed for the Del + Ca mixture, which caused mortality of 60% in animals after 8 days, prolonged survival for a longer period compared to deltamethrin alone, which can be explained by the lower concentration of this pesticide used in the mixture. Other authors (22) observed changes in zebrafish swimming at low concentrations of deltamethrin, a behavior similar to the escape reaction. Thus, behavioral patterns could be the main point to be observed, as they are visible before the animal's death. Increased time of exposure to deltamethrin can damage key organs such as gills, liver, and kidney (22) which could justify the late mortality observed in the mixture in our study. Damage to the gills may be related to the high mortality observed in groups exposed to deltamethrin, as well as the increase in rest behavior due to respiratory failure. Reductions in the frequency of forage behavior and attack behavior were also observed, which may be related to stress arising from a long period of exposure to the deltamethrin.

In the evaluation of locomotor activity and social behavior, after 70 days of exposure, we did not observe significant differences in the evaluated parameters of distance covered, speed, and inactive time. Jijie *et al.* (5) suggested an adaptive response of zebrafish after 48 h of exposure to deltamethrin, as the authors did not observe significant changes in the behavioral response after this period. It could thus be assumed that the lack of changes in locomotor activity and social behavior occurred due to the animals' adaptation to the concentrations used chronically.

CONCLUSION

Commercial pesticides, essential oils, and their mixtures caused toxic effects in adult *Danio rerio*. However, among the substances tested, the most toxic were deltamethrin and a mixture of deltamethrin and *Citrus aurantiifolia*. The less toxic substances and the most promising for the control of *Plutella xylostella*, regarding bioinsecticide activity, were the commercial pesticides azadirachtin, chlorantraniliprole, the *M. leucadendra* essential oil, and Ml + Ec, Ec + Ca, Eg + Ca, and Aza + Cavd mixtures. These can be used for biological control as they did not show toxic effects regarding the studied parameters. It can thus be concluded that exposure to low concentrations of commercial pesticides, essential oils, and their mixtures can compromise fish survival and behavior. Even though biopesticides are considered safe, they can present risks to the aquatic environment. Therefore, their toxic effects must be studied to determine safe concentrations before large-scale use in agricultural activities.

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