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Ecotoxicity of Malathion® 500 CE before and after UVC radiation and UV/H_2O_2 treatment

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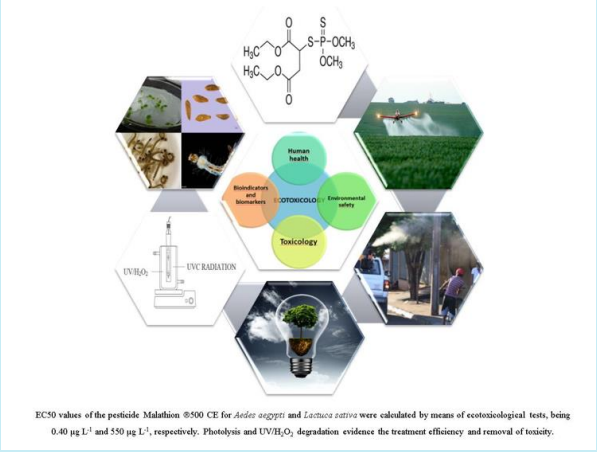
ABSTRACT: Since 2008, Brazil has been the largest consumer of agrochemicals in the world, using pesticides to combat pests and vectors, impacting both microbiota and human health. Aiming at the degradation of the contaminants present in the environment, treatments by advanced oxidative processes are based on the synthesis of free radicals that allow the degradation of the pollutant. Among these processes are included UV/H_2O_2. By evaluating the ecotoxicity and phytotoxicity of the commercial Malathion® 500 CE product, using *Aedes aegypti* larvae and *Lactuca sativa* seed, the high toxicity of this formulation was observed. EC_{50} values for *A. aegypti* being equal to 0.4 µg L^{-1} and for *L. sativa* equal to 550 µg L^{-1}. The commercial agrochemical degradation was carried out by UVC radiation and UV/H_2O_2, and the toxicity was evaluated after 30 and 120 min. After both treatments, the percentage of immobility was zero for *A. aegypti*, inferring the efficiency of the processes. However, for *L. sativa*, no treatment was able to remove or reduce initial toxicity.



EC50 values of the pesticide Malathion ®500 CE for *Aedes aegypti* and *Lactuca sativa* were calculated by means of ecotoxicological tests, being 0.40 µg L^{-1} and 550 µg L^{-1}, respectively. Photolysis and UV/H_2O_2 degradation evidence the treatment efficiency and removal of toxicity.

1. Introduction

Recently, epidemics of dengue, chikungunya, yellow fever and zika caused by the vector *Aedes aegypti* affected a large part of the Brazilian population. From this, government campaigns and control measures were adopted, providing large quantities of malathion insecticide to the environment[1-3]. Malathion is an organophosphate type C widely used in rural and urban areas to control of aphids, flies. It is formulated as household sprays and being sprayed in areas with high proliferation rate of *Aedes aegypti*[2, 4]. It presents the advantage of not being persistent in the environment and does not bioaccumulate. However, it presents high toxicity in animals due to its action mechanism, which inhibits the enzyme acetylcholinesterase, forming a complex with the esterase center of the enzyme. This results in accumulation of the neurotransmitter acetylcholine, interrupting the breakage of this substance in choline and acetic acid, causing several symptoms, such as nervous hyperactivity[5-9].

Aiming at the degradation of this pesticide as an alternative to make it less available in the environment, it is possible resort to simple and effective treatments, which includes advanced oxidative processes (AOPs). These processes are based on the generation of highly reactive and

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oxidizing species such as hydroxyl radicals, it can be obtained in various forms, including heterogeneous and homogeneous systems, with or without radiation. The main objective is to generate at the end of the process species less toxic such as water and carbon dioxide[10-12]. Recent works have shown the efficiency of AOPs, as photo-Fenton process[13], UVC/H_2O_2[14] and others[15], in the degradation of malathion in aqueous solutions. However, for the degradation of malathion, it is possible to obtain several by-products of the oxidation reaction, including substances like phorate sulfoxide, diethyl phosphate, pharatoxon sulfone and malaoxon, the latter being very toxic, which inhibition power under the enzyme acetylcholinesterase is 40 times higher than the parent compound[16, 17].

Like pesticides, their treatment products can also cause effects on the environment, which highlights the need for ecotoxicological assessments that can demonstrate and quantify possible adverse effects to organisms exposed to these substances[18, 19]. In this sense, the present work evaluated the acute ecotoxicity of malathion before and after degradation by UVC radiation and UV/H_2O_2 using *Lactuca sativa* seeds and *Aedes aegypti* larve as bioindicators. Lettuce seed (*Lactuca sativa*) was chosen as the indicator organism, using the observation of interferences in seed germination and root growth[20-22]. The species *Aedes aegypti* was also chosen for toxicity and larvicide evaluation because it is the target organism of malathion[23].

2. Experimental

For ecotoxicity evaluation, samples were collected at initial (0 min), intermediate (30 min), and final treatment times (120 min) in previously washed glass flasks. The pH was adjusted to 6~7, and when necessary, bovine catalase (Sigma-Aldrich) was added for residual H_2O_2 removal. Samples were analyzed immediately or, when this was not possible, they were kept frozen at −20 °C for a maximum of 20 days until the tests were performed. Two tests were selected: *Aedes aegypti* (acute toxicity) and *Lactuca sativa* seeds (phytotoxicity).

2.1 Ecotoxicological tests with Aedes aegypti

Aedes aegypti tests were performed according to methodology described by the World Health Organization[24]. Healthy eggs (Rockfeller variety) were used, provided by the Laboratory of Physiology and Control of Arthropod Vectors of the Oswaldo Cruz Foundation – RJ. Egg hatching was performed in a 1 L Becker containing 500 mL of mineral water with the addition of a small amount of fish feed. Packing was carried out in a B.O.D. incubator, with photoperiod of 16 h clear and 8 h dark, maintained at a temperature of 28 °C.

Commercial product Malathion® 500 CE were used for the test solutions preparation, which were diluted with ultrapure water. In all of them, there were three preliminary tests (data not show). The concentration in focus refers to that recommended by the manufacturer, which indicates the dilution of 30 mL of the pesticide in 10 L of water[25].

Preliminary tests were performed in order to determine the concentration range necessary to determine the effective concentration at 50% of the organisms (EC_{50}), being the range of 0.05; 0.1; 0.2; 0.4; 0.8 and 1 μg L^{-1} [16]. Tests were performed in triplicate, the definitive ones and the treated samples were done in quadruplicate, in each replicate, containing 25 mL of solution and 20 organisms, whose life stage is between the 3rd and 4th larval stage, approximately 96 h of life. The assays were maintained in B.O.D. at 23 °C, without photoperiod, for 24 h. After that, the number of immobile organisms was counted, thus obtaining the percentage of immobility.

2.2 Phytotoxicity with Lactuca sativa

In order to obtain the EC_{50}, solutions of the commercial product Malathion® 500 CE were prepared for the realization of the bioassay with *L. sativa*. Preliminary tests were performed with concentrations ranging from 1 μg L^{-1} to 400 μg L^{-1}, and the concentration range selected for the final test was 250, 350, 450, 550, 600 and 700 μg L^{-1}, where adverse effects were observed.

The seed germination/root elongation phytotoxicity assays were performed according to the methodology described by Sobrero and Ronco[22] and Young *et al.*[26]. Tests were carried out in Petri dishes lined with filter paper (Unifil, weight 80 g m^{-2}) and with 15 seeds each (cv. Boston), containing 4 mL of sample dilution or negative/positive control, with osmosis water and commercial glyphosate 3% (Dipil), respectively. The assay was done in triplicate. Seeds were incubated at 22 ± 2 °C, in the dark, for 120 h. At the end of the test, the number of germinated seeds

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and the root elongation data were used to calculate the germination index (GI)[20] and the relative growth index (RGI)[26]. The RGI values were divided into three categories according to the observed toxicity effects: (a) inhibition of the root elongation: $0 < RGI < 0.8$; (b) no significant effects: $0.8 \leq RGI \leq 1.2$; and (c) stimulation of the root elongation: $RGI > 1.2$[26].

2.3 Statistical analysis

Statistical data evaluation was performed with the BioEstat 5.3 software (BioEstat Software, Belém, Brazil). The effective concentrations 50% (EC_{50}) were calculated using the Probit method. When the EC_{50} was not reached by the tested effluent samples, toxicity was expressed as percentage of toxic effect. For *L. sativa* tests, data were subject to Kolmogorov-Smirnov normality test. As data were normally distributed, they were submitted to one-way analysis of variance (ANOVA) and Tukey test ($p < 0.05$). Significance values are indicated as follows:

*$p < 0.05$ and **$p < 0.01$.

2.4 Treatment of Malathion® 500 CE by UVC radiation and UV/H_2O_2

Photolysis (UVC radiation) and UV/H_2O_2 treatments of the commercial product Malathion® 500 CE were performed using the concentrations that gave rise to 50% of test organisms (EC_{50}), which were 550 µg L^{-1} for *L. sativa* and 0.4 µg L^{-1} for *A. aegypti*. The experiments were conducted in a borosilicate bench photoreactor with 300 mL capacity, equipped with a water-cooled system and a magnetic stirrer. Artificial radiation was provided by a high-pressure mercury vapor lamp (125 W, Philips) placed in the solution through a quartz jacket. The initial concentration of H_2O_2 was 1000 mg L^{-1} and the residual hydrogen peroxide was determined by UV-Vis spectroscopy, using method based on ammonium metavanadate[27].

3. Results and discussion

3.1 Determination of EC_{50} of Malathion® 500 CE to A. aegypti *and* L. sativa

When it is intended to determine EC_{50} of one (or more than one) substance to organisms that have not been reported yet, preliminary tests are of great value to explore and determine it correctly. Preliminary concentrations to *A. aegypt* were based on EC_{50} of Malathion 500 CE to *Daphnia magna*, which ranges between 0.36-3.8 ng L^{-1} [28-30].

After preliminary results, new limits of concentration were settled down and this new range of values (0.05; 0.1; 0.2; 0.4; 0.8 and 1 µg L^{-1}) allowed determine a reliable EC_{50} to *A. aegypti* larvae. In the definitive assays, EC_{50} value obtained was 0.4 µg L^{-1}.

From data obtained at definitive assay (250, 350, 450, 550, 600 and 700 µg L^{-1}) with *Lactuca sativa*, it was possible to calculate the EC_{50} (550 µg L^{-1}).

Comparing both organisms, it is possible to recognize their sensibility difference. *L.sativa* is about one hundred times more resistant than *A.aegypti*. This result was already expected once *A. aegypti* is a target organism, but comparing with literature, species like *D.magna* are two times more sensible than the target larvae showing the importance to treat this compound before achieve aquatic ecosystems[19].

Brazilian and American legislation indicates maximum permissible values of pesticides in drinking water, but malathion is not contemplated[31, 32]. In Brazil, resolution nº. 357/2005 stipulates the maximum concentration range of organophosphates in water, which ranging from 0.1 µg L^{-1} (freshwater, saline and brackish class 1) to 100 µg L^{-1} (freshwater class 3). This means that the higher values would bring acute toxicity effects, according to the value of EC_{50} determined in this work[33].

Preliminary tests are of great value when it is intended to determine the EC_{50} of organisms when exposed to one or more substances, therefore, preliminary tests with *A. aegypti* have been carried out using the EC_{50} value of known organisms, such as *Daphnia magna*, which effective concentration varies between 0.36-3.8 ng L^{-1} for Malathion 500 CE[28-30].

3.2 Ecotoxicity of Malathion®500 CE to A. aegypti *and* L. sativa *after treatment by photolysis and UV/H_2O_2*

It was observed in both treatments that there was no immobility for *A. aegypti* (Table 1), inferring that the degradation of Malathion 500 CE was efficient, losing its larvicidal property, besides that; the byproducts generated were not toxic to the organisms[17].

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Table 1. Evaluation of the ecotoxicity of Malathion®500 CE (0.4 μg L^{-1}) using *Aedes aegypti* before and after treatment with UVC radiation and UV/H_2O_2

	Negative control	T0	UVC 30 min	UVC 120 min	UV/H_2O_2 30 min	UV/H_2O_2 120 min
Average*	0	10	0	0	0	0
Immobility (%)*	**0**	**50**	**0**	**0**	**0**	**0**

*Three replicates with 20 organisms.

Comparing the results obtained before and after treatment, the toxicity of the commercial product reduced 100% when tested on *A. aegypti*, demonstrating that it is a viable alternative on the treatment of this organophosphate. This process has the advantage of no needing high pressures, temperatures, elevated times of exposure and the oxidizing agent (hydrogen peroxide) can be removed through catalase abatement[34-36].

The values of p (probability of significance) for ANOVA followed by Tukey's test, obtained from *L. sativa* bioassays (Table 2), indicate that in all samples there was statistical significance and RGI (Relative Growth Index) less than 0.80, it means that all treatments inhibited root growth. The Germination Index (GI) was greater than 90% in all samples, indicating that there was no inhibition of germination.

Table 2. Evaluation of the phytotoxicity of Malathion®500 CE (550 μg L^{-1}) using *Lactuca sativa* seeds before and after treatment with UVC radiation and UV/H_2O_2

Sample	Mean root length (cm)	RGI	GI%	Effect
Control	2.50 ± 0.7^{a}	---	100	------
T0	$1.25\pm0.1^{b**}$	0.5	97.8	I
UVC 30 min	$1.98\pm0.3^{c*}$	0.7	100	I
UVC 120 min	$1.62\pm0.1^{c**}$	0.6	100	I
UV/H_2O_2 30 min	$1.39\pm0.3^{d**}$	0.5	100	I
UV/H_2O_2 120 min	$1.92\pm0.5^{c*}$	0.7	100	I

Three replicates with 15 seeds. Abbreviations: GI%, germination index; I, inhibition; RGI, relative growth index. Mean values with different letters (a, b, c, d) are significantly different (Tukey's test, $p < 0.05$). *$p < 0.05$; **$p < 0.01$.

It can be verified that the treatments showed an efficiency in reduction of effects on root growth of lettuce seedlings in comparison to pre-treatment sample (T0 = 550 μg L^{-1}, with RGI = 0.50), since after UVC and UV/H_2O_2 processes all samples had RGI higher than 0.50.

However, UVC 120 min photolysis likely generated toxic degradation byproducts for lettuce, once in 30 min of radiation exposure there was 21% of inhibition in root growth (RGI = 0.79), while at 120 min the percentage of inhibition growth increased to 36% (RGI = 0.64). Even though there were no significant differences between them, this increase of the toxicity can be associated to the formation of inorganic anions such as sulfates and phosphates due to cleavage of the P-S bonds[17, 18].

For UV/H_2O_2, rootlets growth had high inhibition at 30 min of treatment (RGI = 0.55) and in 120 min was observed decrease of toxic effects (RGI = 0.76). It is possible that at the initial 30 min were formed intermediates by sulfur oxidation and generation of molecules from the combination of phosphorus, sulfur, carbon, hydrogen and oxygen,

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for example, phorate sulfoxide and phoratoxon sulfone[17, 18].

The influence of concentration of treated compound on processes efficiency is evident when comparing treatments carried out based on ecotoxicity of Malathion to *A. aegypti*, since concentration used was ten times lower in relation to *L. sativa* and after both treatments no toxic effects were obtained, it shows that, despite the objective of the treatment was to degrade the pesticide and eliminate sources of toxicity, the concentration of treated compound may interfere in the efficiency of the process and even increase toxic effects[17, 36].

4. Conclusions

As an insecticide, Malathion® 500 CE was shown to be more toxic to *Aedes aegypti* larva, evidenced by EC_{50}, which is much lower than that obtained for *L. sativa*. As regards phytotoxicity, it was found that the *L. sativa* seed presented moderate resistance to the pesticide studied, with an EC_{50} value of 536.11 μg L^{-1}, however; it was a low concentration, inferring that lower concentrations of Malathion® 500 CE can cause damage to terrestrial plants.

Degradation of the commercial product in both treatments was efficient and the byproducts generated were not harmful to *A. aegypti* larvae. However, in the phytotoxicity assays, it was evidenced that there was inhibition of root growth.

This compound is present in agriculture for biological control and in urban areas being used in the fight against vectors of several diseases like dengue requiring several ecotoxicological and toxicological studies of this insecticide. In the present work, it is possible to see that low concentrations can cause harmful effects to target and non-target organisms.

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