

# Toxicity Studies of Ametryne to Land and Aquatic Organisms

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Received: August 2, 2016    Accepted: August 20, 2016    Published: December 6, 2017

doi:10.5296/ast.v6i1.9828    URL: <http://doi.org/10.5296/ast.v6i1.9828>

## Abstract

The ametryne herbicide is largely used on sugar cane plantation in Brazil. It is persistent in the environment and can be found in bodies of water, impacting the aquatic and terrestrial ecosystems. Generally, in crops are applied mixtures of herbicides in order to obtain a higher success in combating weeds. This study evaluated the toxicity only of ametryne herbicide, without mixture with other herbicides, in order to quantify only the degree of dangerousness. This work evaluated the toxicity of ametryne to one aquatic test organism (*Daphnia similis*) and two land test organism (*Eruca sativa* and *Lactuca sativa*). Immobility of *D. similis* was evaluated in the presence of ametryne. Influences of ametryne on seed germination and root growth of *E. sativa* and *L. sativa* were evaluated. Even at low concentrations (5.00 mg/L), ametryne caused toxic effects on the mobility of *D. similis*, and 0.25 g/L caused toxic effects on the seeds. Root growth and the percentage of inhibition showed greater sensitivity to ametryne compared with seed germination. Thus, ametryne resulted in toxic effects to the analyzed organisms, which may bring damage to both aquatic and terrestrial ecosystems.

**Keywords:** ametryne, ecotoxicity, soil, contamination

## 1. Introduction

Pesticides, used to control pests and weeds, are rarely found alone in the environment. Typically, they present a diversity on commercial formulations (Faust et al., 2003). Therefore, the organisms are not exposed to a single contaminant, but usually mixtures of contaminants (Kurth et al., 2015). Pesticides may reach non-target organisms through runoff or leaching, resulting in adverse effects to terrestrial ecosystem (Velki & Ečimović, 2015), and influencing aquatic ecosystem by changing its structure biotic (Faust et al., 2003).

When applied to the environment, the pesticides achieve the molecular portion of the body, resulting in a common toxicological pattern between different reactions that can affect it (Faust et al., 2003).

**One of the adverse effects of pesticides is their environmental persistence. According to Bruzzoniti et al. (2006), the persistence in the environment depends on the applied dose, the chemical nature of the compound, soil characteristics, and hydro-geological characteristics of the contaminated area.** The concentration of a compound at a certain place depends on its rate of degradation and transportation in the environment (Schwarzenbach et al., 2003). Malinowska & Jankowski (2015) detected the presence of fungicides and insecticides in *Achillea millefolium* samples, an alarming fact as such plants are used for medicinal purposes.

Brazil is one of the largest food producers in the world, due to its large acreage and high productivity, and may be in the next decade the largest food exporter according to OCDE-FAO (2015). **However, the large scale use of ametryne results in negative effects on the environment. Ametryne is difficult to be degraded by soil microorganisms due to an aromatic ring structure in its molecule, and can undergo leaching affecting aquatic organisms (Farré et al., 2002; Gao et al., 2009; Kasozi et al., 2012).** Pfeuffer & Rand (2004) detected ametryne in surface water and sediment samples in South Florida, United States. Koskine & Harper (1990) detected the presence of triazine herbicide residues in Mécarchez waters on the island of Martinique, France. The presence of ametryne was detected in Brazilian waters (Botelho et al., 2015; Laabs et al., 2002).

The effect of toxic substances to the organisms depends on the applied dose and time of exposure to the products (Sánchez-Bayo, 2006). One of the representative organisms for aquatic ecotoxicological tests is *Daphnia similis*, which are a kind of crustaceans, the Cladocera order, commonly known as water fleas. They are ideal organisms for the evaluation of compounds in their physiology. They also have short life cycle and are easily handled (Herrera et al., 2014). Novelli et al. (2012) assess the potential toxicity of abamectin herbicide to *D. similis*, concluding that the herbicide was highly toxic to daphnids.

In addition, bioassays with vegetable seeds, such as *Eruca sativa* and *Lactuca sativa*, can be used to evaluate the phytotoxicity effects of pure compound or mixtures, during both germination and root development. (Sobrero & Ronco, 2004). Studies with seeds of *E. sativa* have been evaluated for Malathion pesticide, being sensitive to the presence of the contaminant (Gafar et al., 2013).

This study evaluated the toxicity of ametryne only herbicide without mixing with other herbicides. This work evaluated the toxicity of ametryne to aquatic organism *D. similis* and the phytotoxicity to *E. sativa* and *L. sativa* seeds.

## 2. Materials and Methods

The assays were conducted with commercial ametryne herbicide (500.00 g/L), from GESAPAX. The solutions were prepared with deionized water in laboratory scale, and the concentration gradients were set based on sugar cane field data. Concentrations of ametryne

added in each test were shown in Table 1.

Table 1. Concentrations used in ecotoxicological tests for each test organism.

Test organisms	Ametryne concentrations
<i>D. similis</i>	5.00/ 10.00/ 15.00/ 25.00/ 50.00/ 75.00/ 100.00 mg/L
<i>E. sativa</i>	0.25/ 0.50/ 1.00/ 5.00/ 10.00 g/L
<i>L. sativa</i>	0.25/ 0.50/ 1.00/ 5.00/ 10.00 g/L

### 2.1 Toxicity test with *D. similis*

Sighted toxicity test with *D. similis* was performed according to the technical standard ABNT (2004). Neonates of *D. similis* were used to evaluate the immobility of the organism. After 48 h exposure to ametryne, Median Effective Concentration (EC50) was calculated.

The tests were run in four replicates in containers with 10.0 ml of sample and 5 neonates of *D. similis*. The containers were placed in trays, in the dark, at 20 °C, for 48 h. The immotility of neonates in each container was observed. Farming water without ametryne was used as a negative control. The assay was performed in triplicate.

The percentage of each of immobility calculated using Eq. 1:

$$\% \text{ Immobility} = \frac{\text{average sample}}{\text{average negative control}} \times 100 \quad (1)$$

### 2.2 Ametryne Phytotoxicity

The seeds of the *L. sativa* and *E. sativa* were placed in sterile Petri plate. Each plate contained a filter paper with 10 cm diameter, 20 seeds equidistant organized on the filter surface. Each plate was added 3.00 ml of ametryne concentrations. The plates were incubated in a BOD incubator (Marconi model MA 403) in absence of light at 22 °C for 120 h. The phytotoxicity test was adapted from the study Sobrero & Ronco (2004). The assay was performed in triplicate. For the sensitivity tests, 3.00 ml of 0.05 M zinc sulphate (Synth) solution were used as positive control, and 3.00 mL of deionized water as negative control.

At the end of each test, the length of the germinated roots was measured. The percentage of root growth inhibition rate was evaluated using Eq. 2:

$$\% \text{ Inhibition} = \frac{(\text{average negative control} - \text{average treatment})}{\text{average negative control}} \times 100 \quad (2)$$

### 2.2 Statistical Analysis

For *D. similis* toxicity tests, Trimmed Spearman-Kärber method (Hamilton et al., 1977) was used for statistical analysis, expressing the results in a concentration that causes 50% effect on organisms, CE50. For the tests with seed *E. sativa* and *L. sativa*, Tukey's test was used at 5% probability.

Differences were considered statistically significant at  $p$  value  $< 0.05$ , using OriginPro 8.0 software developed by Origin Lab Corporation, for the Bartha and Pramer respirometric method.

### 3. Results

#### 3.1 Assessment of the ametryne's toxicity to *D. similis*

All tested concentrations caused inhibitory effects on *D. similis* after 48 h exposure. After this period, the immobility percentages in the presence of ametryne was calculated. The results were shown in Table 2.

Table 2. Details of the concentrations used and obtained immobility percentages.

<b>Ametryne concentrations (mg/L)</b>	<b>Organism immobility (%)</b>
Control	0
5.00	5
10.00	15
15.00	30
25.00	45
50.00	100
75.00	100
100.00	100

Through statistical test Trimmed Spearman-Kärber, it was observed that the EC<sub>50</sub> of ametryne for *D. similis* was 25.73 mg/L.

It can be seen that with the ametryne concentration increasing, the amount of living organisms after 48 h exposure decreased. Negative effects were not observed in the control group, with 0% immobility. Thus, the effect that *D. similis* suffered was due to the presence of ametryne in water.

In the 10.00 mg/L concentration of herbicide, 15% organisms suffered toxic effects assessed by lack of mobility. In the 50.00 mg/L concentration all organisms presented immobility after 48 h exposure. In groups from 50.00 mg/L to 100.00 mg/L, all the *D. similis* suffered toxic effects of ametryne molecule.

There was an increase in immobility of *D. similis* between 5.00 mg/L and 10.00 mg/L concentrations. From the concentrations of 5.00 mg/L to 25.00 mg/L, there was a gradual and significant increase ( $p = 0.0253$ ) at percentage of immobilized organisms.

#### 3.2. Toxicological effects of ametryne to *E. sativa* and *L. sativa* seeds

In order to evaluate the influence of ametryne on the land community, the effects of the herbicide on *E. sativa* and *L. sativa* seeds were analyzed at different concentrations (Table 3

and Table 4).

Table 3. Phytotoxicity with ametryne for *E. sativa* seeds.

Concentration of ametryne (g/L)	Germination of seeds (%)	Growth of root (cm)	Inhibition of growth of root (%)
Negative control	100	31.75 <sup>a</sup> ± 0.12	0.00
0.25	70	20.90 <sup>b</sup> ± 0.21	34.17
0.50	70	17.80 <sup>c</sup> ± 0.66	43.93
1.00	65	16.55 <sup>d</sup> ± 0.25	47.87
5.00	50	14.20 <sup>e</sup> ± 0.48	55.27
10.00	45	10.10 <sup>f</sup> ± 0.40	68.18
Positive control	100	0.00 <sup>g</sup> ± 0.00	100.00

The means followed by the same letter, do not differ by Tukey's test at 5% probability

It was observed an increased in growth inhibition of *E. sativa* root related to the increase of ametryne concentrations. Even in small concentrations, such as 0.25 g/L, the ametryne caused inhibitory about 35% in root growth.

*E. sativa* had a sensitivity to the herbicide, even in small amounts. At three variables evaluated the germination, growth of root and inhibition of growth of root, the seed was suffering from negative.

The concentration of 5.00 g/L and 10.00 g/L caused more than 55% inhibitory effect on root growth. The germination variable showed less sensitivity to the presence of ametryne, however, the concentration 10.00 g/L, interfered in 45% germination capacity of *E. sativa*.

At 0.25 g/L concentration, 34.64% seeds of *E. sativa* suffered toxic effects on root growth. However, for *L. sativa* seed, only 1.40% suffered adverse effects on root growth. The Table 4 shows the results for toxicity using seeds of *L. sativa*.

Table 4. Phytotoxicity with ametryne for *L. sativa* seeds.

Concentration of ametryne (g/L)	Germination of seeds (%)	Growth of root (cm)	Inhibition of growth of root (%)
Negative control	100	31.35 <sup>a</sup> ± 0.14	0.00
0.25	75	20.7 <sup>b</sup> ± 0.19	33.97
0.50	70	19.4 <sup>b</sup> ± 0.17	38.11
1.00	60	16.5 <sup>c</sup> ± 0.14	47.36
5.00	55	15.9 <sup>c</sup> ± 0.16	49.28
10.00	55	11.7 <sup>d</sup> ± 0.13	62.67
Positive control	0.00	0.00 <sup>e</sup> ± 0.00	100.00

The means followed by the same letter, do not differ by Tukey's test at 5% probability

It was observed that with the increase in ametryne concentration there was an increase in phytotoxicity for *L. sativa* seeds. Concentrations from 0.25 g/L to 0.50 g/L had no significant

differences in root growth. There was a root inhibition growth about 20% in concentrations between 1.00 g/L and 5.00 g/L. The ametryne phytotoxicity for *L. sativa* results showed significant effect on seeds even at low herbicide concentrations.

With the increase in concentrations, little difference occurred between the results of germination. However, at concentrations of 5.00 g/L and 10.00 g/L the ametryne prevented the germination of seeds over half of the samples *L. sativa*.

The inhibition percentage on root growth seed, was the most sensitive among tests. The increase in growth inhibition concentrations of 0.25 g/L and 0.50 g/L was significant ( $p < 0.05$ ).

The concentration of 10.00 g/L caused major negative effects on the development of *L. sativa*, reducing about 60% compared to negative control, in the root growth.

#### **4. Discussion**

Even at low concentration (Table 2), ametryne caused toxic effects on *D. similis*. Gutierrez et al. (2013) beheld that atrazine herbicide were highly toxic to aquatic organisms as *Mesocyclops longisetus*, even at low concentrations.

The concentration that caused mobility 50% of organisms was 25.73 mg/L for *D. similis*. For *Daphnia magna*, Farr éet al. (2002) observed that ametryne caused 28 mg/L toxic effect.

From the concentration 50.00 mg/L organisms not present mobility. This was observed by Sánchez-Bayo (2006), concluding that the ametryne is very toxic to crustaceans as brine shrimp, resulting in LC 50 of 20-50 mg/L, once that, in average, the toxicity of triazine herbicides to Cladocera order is about 43 mg/L.

The *D. similis* is a representative organism for ecotoxicity tests. With increasing concentrations ametryne added in its growing water showed sensitivity interfering with the functions of their bodies, such as immobility and death.

The seeds *L. sativa* and *E. sativa*, they suffered toxic effects of ametryne even in low concentrations. The same was observed by R égo et al. (2014), that even in low concentrations in soil, ametryne caused reduced growth of the root *L. sativa*.

However, the seed *E. sativa* showed higher sensitivity compared to seed *L. sativa*. The ametryne interfered in the germination and root growth of seeds evaluated. All variables were negatively affected by the presence of the herbicide.

The ametryne is classified as a FSII herbicide, that is, extremely phytotoxic, inhibiting the electron transport chain of chloroplasts, even in low concentrations, becoming a threat to the aquatic environment (Sandoval-Carrasco et al., 2013; USEPA, 2005).

#### **5. Conclusion**

The ametryne result in toxic effects to the assessed organisms. The organisms *D. similis* suffered interference in its immobility, even at low concentrations of herbicide.

*L. sativa* and *E. sativa* seeds were sensitive to the presence of ametryne, because both suffered interference in its development during germination, root growth and inhibition of their growth.

Thus, it is necessary remedial measures for contaminated sites with ametryne in order to reduce the negative effects on aquatic and terrestrial ecosystems.

### Acknowledgment

Coordination Personnel Perfectioning Higher Education – CAPES- CnPQ.

### Conflicts of interest

There are no conflicts of interest in this research.

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