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Efficiency of electrostatic application of imazamox with and without adjuvants for the control of aquatic plants and acute toxicity to aquatic bioindicator organisms

Eficiência da aplicação eletrostática de imazamox com e sem adjuvantes para controle de plantas aquáticas e toxicidade aguda para organismos bioindicadores aquáticos

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Abstract

Macrophytes cause damage to water bodies, requiring ecotoxicological studies to understand their effects on the aquatic environment for chemical control purposes. This study aimed to assess the efficiency of electrostatic spraying of imazamox with and without adjuvants for controlling Eichhornia crassipes, Myriophyllum aquaticum, and Salvinia molesta, as well as the acute toxicity (LC50;7d) for the bioindicators Lemna minor, Azolla caroliniana, and Wolffia brasiliensis. For efficacy, 800, 900, and 1000 g a.i. ha-1 of imazamox without and with 0.5% Dash® HC and 0.5% Aterbane® BR were used with one control and ten replicates. For LC50;7d, six concentrations ranging from 0.1 mg L-1 to 383.5 mg L-1 of imazamox with and without adjuvants and the adjuvants alone were used. For E. crassipes, 100% control was achieved 30 days after application (DAA) at 900 and 1000 g a.i. ha-1. For Myriophyllum aquaticum, 90% control was achieved at 1000 g a.i. ha-1 + 0.5% Aterbane®BR at 60 DAA. For Salvinia molesta, no control was achieved at any dose tested. L. minor was most sensitive to imazamox+0.5% Dash with an LC50;7d of 1.66 mg L-1, W. brasiliensis to imazamox+0.5% Dash with an LC50;7d of 5.52 mg L-1, and A. caroliniana was most sensitive to imazamox+0.5% Aterbane with an LC50;7d of 5.99 mg L-1. The electrostatic application of imazamox with and without adjuvants was effective in controlling the aquatic plants water hyacinth and water pine, but ineffective in controlling giant salvinia (S. molesta). The test plant, duckweed, was more sensitive to the herbicide, adjuvants, and their addition to the spray mixture, and could be applied as a bioindicator for environmental biomonitoring.

Keywords: Chemical control, aquatic macrophytes, bioindication, sensitivity, herbicide.

Resumo

As macrófitas causam prejuízos aos corpos hídricos e seu controle químico exige estudos ecotoxicológicos para compreender seus efeitos no ambiente aquático. O objetivo deste estudo foi avaliar a eficiência da pulverização eletrostática de imazamox com e sem adjuvantes para controle de *Eichhornia crassipes, Myriophyllum aquaticum* e *Salvinia molesta* e a toxicidade aguda (CL50;7d) para os bioindicadores *Lemna minor*, *Azolla caroliniana* e *Wolffia brasiliensis*. Para eficácia foram utilizados 800, 900 e 1000 g i.a. ha^{-1} de imazamox sem e com 0,5% de Dash® HC e 0,5% Aterbane® BR com um controle e dez repetições. Para CL50;7d foram utilizadas seis concentrações de 0,1 mg L^{-1} a 383,5 mg L^{-1} de imazamox sem e com adjuvantes e os adjuvantes isolados. Para *E. crassipes* ocorreu 100% de controle em 30 dias após a aplicação (DAA) em 900 e 1000 g i.a ha^{-1}. Para *Myriophyllum aquaticum* ocorreu 90% em 1000 g i.a ha^{-1} + 0,5% de Aterbane®BR em 60 DAA. Para *Salvinia molesta* não ocorreu controle em nenhuma dose testada. A *L. minor* foi mais sensível ao imazamox+0,5% de Dash com CL50;7d de 1,66 mg L^{-1}, *W. brasiliensis* ao imazamox+0,5% de Dash com CL50;7d de 5,52 mg L^{-1} e *A. caroliniana* mais sensível ao imazamox+0,5% Aterbane com CL50;7d de 5,99 mg L^{-1}. A aplicação eletrostática de imazamox com e sem adjuvantes foi eficaz no controle das plantas aquáticas aguapé e pinheirinho d'água, mas ineficaz no controle da salvinia gigante (*S. molesta*). A planta de teste, lentilha-d'água, foi mais sensível ao herbicida, aos adjuvantes e à sua adição à calda de pulverização, podendo ser utilizada como bioindicador para biomonitoramento ambiental.

Palavras-chave: Controle químico, macrófitas aquáticas, bioindicação, sensibilidade, herbicida

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Introduction

Aquatic plants have phenotypic differences and vegetative reproduction capacity, allowing them to adapt to a wide variety of aquatic environments (Henry-Silva et al., 2008). Notable among these are *Eichhornia crassipes, Salvinia molesta, and Myriophyllum aquaticum*. These plants cause serious damage to aquatic environments due to their rapid proliferation and are favored by the absence of natural enemies, resulting in eutrophication of water bodies and hindering the multiple uses of water (Gettys et al., 2014; Pitelli et al., 2018).

Chemical control of aquatic plants has been evaluated due to its high efficacy, good benefit/cost ratio, and historical success in other countries (Gettys et al., 2014). In the United States, the herbicides bispyribac-sodium, carfentrazone-ethyl, and flumioxazin are registered for floating, submerged, and emergent aquatic plants by the United States Environmental Protection Agency (USEPA) (Glomski & Mudge, 2013), as are copper ethylenediamine, diquat, florpyrauxifen-benzyl, fluridone, glyphosate, imazapyr, triclopry, tropamezone, and 2,4-D (Sepro Corporation, 2024). In Brazil, only the herbicide fluridone is registered for the control of the submerged aquatic plants *Egeria densa* and *E. najas*, but it is not commercially available (Instituto Brasileiro do Meio Ambiente Recursos Naturais Renováveis, 2024).

In Brazil, CONAMA resolution n. 467 of July 16, 2015, was published, which allows the use of chemical products to control aquatic plants and algae. Therefore, it is necessary to develop studies on application technology and herbicides to control aquatic plants in Brazilian water bodies. The herbicide diquat was used to control water hyacinth (*E. crassipes)* (Almeida et al., 2016), imazapyr for *E. crassipes, Pistia stratiotes, and Salvinia molesta* (Cruz et al., 2015a), glyphosate for *E. crassipes, P. stratiotes, S. molesta, S. herzogii, and Urochloa subquadripara* (Cruz et al., 2015b), for *S. molesta* mixtures of glyphosate with carfentrazone, diquat, endothall, flumioxazine in combinations with adjuvants (Mudge et al., 2016) imazapyr and mixtures of imazapyr + carfentrazone and 2,4-D + carfentrazone for M. aquaticum (Kuehne et al., 2018), the 2,4-D, florpyrauxifen-benzyl, flumioxazin, glyphosate, imazamox, imazapyr and triclopyr for *Limnobium spongia* (Turnage et al., 2023) and glyphosate, imazamox and florpyrauxifen-benzyl for American lotus (*Nelumbo lutea*), water lily (*Nymphaea odorata*) and water shield (*Brasenia schreberi*) (Lázaro-Lobo et al., 2024).

Imazamox ((RS)-2-(4-isopropyl-4-methyl-5-oxo-2-imidazolin-2-yl)-5-methoxymethylnicotinic acid) is a systemic herbicide of the imidazolinone group that results in the reduction of the synthesis of three branched-chain aliphatic amino acids: valine, leucine, and isoleucine. This inhibition inhibits the synthesis of acetohydroxy acid (AHA), a common enzyme in the biosynthetic pathway of these amino acids. This inhibition interrupts protein synthesis, which interferes with DNA synthesis and cell growth in plants. This herbicide can be used to control aquatic plants and is used to control annual, perennial grasses, and eudicotyledonous. An important characteristic for use in aquatic plant control is its slow target control rate, which favors the slow release of nutrients from the degradation of dead plants into the water, limiting the eutrophication effect. The application of 900 g a.i. ha^{-1} of imazamox was effective in controlling 94% of *S. molesta*, with a 95% reduction in fresh biomass and 92% in dry weight. It caused an increase in biochemical oxygen demand (BOD) but did not change water temperature, pH, dissolved oxygen, or electrical conductivity (Garlich et al., 2021).

Electrostatic spraying is a technological innovation that can be used in weed control. It is a system that electrically charges the droplets generated in the spray nozzles, causing them to be attracted to the oppositely charged target (Schroder & Loeck, 2006; Campos et al., 2020). This spraying provides better target coverage compared to uncharged spraying, minimizing waste and environmental impact due to excess product application and off-target spray drift (Patel et al., 2017).

To improve herbicide performance, the use of adjuvants is recommended: substances that improve leaf surface moisture, retention, and product penetration into the plant (Mirgorodskaya et al., 2020). Furthermore, the use of electrostatic spraying combined with adjuvants can reduce droplet diameter (Sasaki et al., 2015). Spraying with electrostatic equipment can reduce the spray volume applied, increase product deposition on the target, decrease drift losses, and consequently reduce the negative impacts these molecules can have on the aquatic environment and non-target organisms.

Therefore, environmental monitoring of herbicides in aquatic environments using bioindicator organisms from different levels of the food chain is necessary to understand the environmental dynamics of these molecules. Among the bioindicators that can be used are the aquatic plants *Lemna minor* for copper and diquat sources (Garlich et al., 2016), *Azolla caroliniana* for 2,4-D, glyphosate, clomazone, oxyfluorfen, and imazapyr (Silva et al., 2012; Cruz et al., 2015b), and *Wolffia brasiliensis* for glyphosate, diquat, paraquat, imazapyr, 2,4-D, saflufenacil, imazamox, penoxsulam, metsulfuron-methyl, and atrazine (Pereira et al., 2019).

Thus, the objective of the study was to evaluate the efficiency of electrostatic spraying in the application of imazamox without and with the addition of adjuvant for the control of *Eichhornia crassipes, Salvinia molesta and Myriophyllum aquaticum* under greenhouse conditions and to determine the acute toxicity LC50;7d of imazamox and adjuvants for the aquatic plants *A. caroliniana, L. minor and W. brasiliensis.*

Material and Methods

The herbicide used was imazamox, in the Raptor® formulation, at 700.0 g L^{-1}, and the adjuvants were a mixture of methyl esters, aromatic hydrocarbon, unsaturated fatty acid, and surfactant at 933.0 g L^{-1} (Dash® HC) and nonylphenol ethoxylate at 309.0 g L^{-1} (Aterbane® BR).

Aquatic weed control efficacy

For the efficacy tests, 2.5-L plastic containers containing water and a substrate composed of coarse sand, organic substrate, and red latosoil (1:1:1 vv^{-1}) were used. Three young plants were used for the experiments with *S. molesta*, four shoots of 20 cm long for *M. aquaticum*, and one plant per experimental unit for *E. crassipes*. After growth and 80% surface area coverage of the experimental units, applications were made using doses of 800, 900, and 1000 g a.i. ha^{-1} of imazamox, equivalent to 1.14; 1.28; and 1.42 kg p.c. ha^{-1}, without or with the addition of the adjuvants Dash® HC 0.5% and Aterbane® BR 0.5%. All experiments were conducted in a completely randomized experimental design (CRD) with ten replicates and a control (no application).

The applications were carried out with an electrostatic sprayer, containing an induction device for electrifying jets of drops from hydraulic nozzles adapted to a manual backpack sprayer equipped with a spray bar and a BD80.005 fan nozzle developed by Magnojet Ltda., with a pressure of 200 KPa, application rate of 200 L ha^{-1} at a speed of 1.0 m s^{-1}.

Efficacy assessments were performed at 7, 15, 21, 30, 45, and 60 days after application (DAA), according to control scores, which were assigned based on visual signs of toxicity, growth inhibition, and plant regrowth, according to the scale proposed by Velini et al. (1995).

At the end of the experimental period, fresh biomass (g) and dry mass (g) of the plants were evaluated. To obtain dry mass, the plants were placed in a forced-air circulation oven at 65°C until they reached constant mass.

The dry and fresh mass results were subjected to log10 for normal distribution, and after this transformation, they were subjected to analysis of variance (ANOVA) using the AgroEstat® statistical software (Barbosa & Maldonado Júnior, 2014). After this verification, the means were compared using the Tukey 5% test.

Acute toxicity to aquatic plants

For acute toxicity tests, the bioindicator plants were acclimated in a bioassay room with constant lighting of 1,000 lx for 7 days. After the acclimation period, four colonies with three *L. minor* fronds, five *A. caroliniana* plants, and the test plant *W. brasiliensis* were collected using a syringe and plunger, measuring 19 mm in size. The selected plants were transferred to a glass container with a maximum capacity of 100 mL containing 50 mL of Hoagland's culture medium for an additional 24 h. After this period, 50 mL of Hoagland's culture medium was added with the test products.

Concentrations of 0.1; 1.07; 3.5; 11.2; 36.5 and 118.0 mg L^{-1} of imazamox alone, of the adjuvant Dash® HC and of the mixtures of imazamox with 0.5% Dash® HC and 0.5% Aterbane® BR were applied to *L. minor* and *A. caroliniana*. Of Aterbane® BR alone, the concentrations 21.16; 43.73; 66.30; 96.40; 202.15; 293.10 mg L^{-1} were applied to *L. minor* and 1.15; 1.61; 5.19; 16.82; 55.04 and 177.94 mg L^{-1} to *A. caroliniana*.

In the trials with *W. brasiliensis*, the applied concentrations of imazamox alone were 3.5; 7.7; 16.94; 37.27; 81.99 and 180.38 mg L^{-1}, the applied concentrations of the adjuvant Dash®

HC were 20.28; 36.5; 65.7; 118.26; 212.87 and 383.16 mg L^{-1} and the applied concentrations of the adjuvant Aterbane® BR alone and the mixtures of imazamox with 0.5% Dash® HC and 0.5% Aterbane® BR were 0.1; 1.07; 3.5; 11.2; 36.5 and 118.0 mg L^{-1}.

After seven days of exposure, *L. minor* mortality was assessed by counting the number of fronds, chlorosis, and total frond necrosis (Organization for Economic Co-operation and Development, 2002). For *A. caroliniana* and *W. brasiliensis*, the assessment was based on the percentage of plant mortality using a grading scale (E to A), with the grades converted to mortality percentages as recommended by Silva et al. (2012).

Using the percentage mortality data for the test plants, the LC50;7d was estimated using the Trimmed Spearman-Karber statistical method (Hamilton et al., 1977), and the toxicity classification was performed according to the USEPA ecotoxicological classification (United States Environmental Protection Agency, 2023).

Results and Discussion

Efficacy for water hyacinth (E. crassipes)

Imazamox without and with the addition of adjuvants showed unsatisfactory control 7 and 15 days after application (DAA) for water hyacinth (Table 1). At 21 DAA, moderate control occurred at all tested concentrations of the herbicide alone and in mixture with adjuvants, with 58 to 75% control (Table 1), differing from the application of 1250 g i.a. ha-1 of imazapyr with 95% control of water hyacinth at 28 DAA (Foloni & Pitelli, 2005).

After 30 DAA, excellent control of water hyacinth was observed at all tested doses (100%, Table 1), except for the 800 g a.i. ha-1 dose, which achieved 90%. At 45 and 60 DAA, the same control pattern was observed, similar to that achieved by imazapyr for E. crassipes, with 875 and 1000 g a.i. ha^{-1}, achieving 90% to 100% control (Cruz et al., 2015b). The use of imazamox for the control of aquatic plants is interesting because, in addition to its excellent efficacy, its mechanism of action promotes slower death of the control targets, avoiding the effect of rapid nutrient release into the water body through plant degradation. The application of 0.1 to 0.4 kg ha^{-1} of diquat showed water hyacinth control between 66.06% and 84.88% in just 5 days after application (Pitelli et al., 2011).

In the reduction of *E. crassipes* biomass, there was a significant difference between the control and all tested treatments (Table 2). At doses of 900 and 1000 g a.i. ha^{-1}, there was a 100% reduction in fresh and dry mass at all tested doses. With 70.1 g a.i. ha^{-1} of imazamox alone and 70.1 g a.i. ha^{-1} + 1.0% methylated and organosilicone vegetable oil, there was a 30 to 69% reduction in the dry mass of *E. crassipes* plants at 60 DAA (Mudge & Netherland, 2014). With the dose of 560 g a.i. ha^{-1} of imazamox + 0.25% surfactant, there was a 61.10% reduction in the biomass of *E. crassipes* plants (Emerine et al., 2010).

Table 1. Percentage of control of the herbicide imazamox alone and in mixture with adjuvants for the control of *Eichhornia crassipes*.

Doses (g i.a. ha^{-1})	Days after application (DAA)					
	7	15	21	30	45	60
Imazamox 800	17a	25cd	58b	90b	95b	95b
Imazamox 900	10b	42a	75a	100a	100a	100a
Imazamox 1000	10b	40ab	75a	100a	100a	100a
Imazamox 800 +Dash	10b	37ab	70ab	100a	100a	100a
Imazamox 900 +Dash	7b	32bc	72ab	100a	100a	100a
Imazamox 1000+Dash	7b	32bc	75a	100a	100a	100a
Imazamox 800 + At	10b	28cd	75a	100a	100a	100a
Imazamox 900 + At	7b	22d	70ab	100a	100a	100a
Imazamox 1000 + At	8b	22d	70ab	100a	100a	100a
F value	**25,10****	**56,78****	**50,55****	**1185,33****	**1189,67****	**29741,67****
CV	**16,66**	**10,11**	**8,78**	**1,78**	**1,77**	**0,35**
DMS	**4,19**	**8,13**	**16,33**	**4,57**	**4,57**	**0,91**

**Significant at 5% probability. Means followed by the same letter in the column do not differ statistically from each other by Tukey's test ($p < 0.05$). CV = coefficient of variation; DMS = least significant difference. At = Aterbane® BR

Efficacy for watermilfoil control (M. aquaticum)

Imazamox alone and with adjuvants showed unsatisfactory control of watermilfoil at 7 and 15 DAA. At 21 DAA, significant control occurred only with the addition of adjuvants, with 40% to 55% control. At 30 DAA, imazamox with and without adjuvant did not show satisfactory efficacy at any of the doses tested, except at 900 g a.i. ha^{-1} de imazamox + Dash®, with 70% efficacy (Table 3), differing from 1250 g a.i. ha^{-1} of imazapyr, at 28 DAA, which controlled 100% of watermilfoil (Foloni & Pitelli, 2005) and 29.4 to 58.8% of florpyrauxifen-benzyl with control between 92.3% and 100%, within 30 days after application (Howell et al., 2022).

At 60 DAA, the efficacy was satisfactory only in the treatments with imazamox plus adjuvants, with control from 72.5% to 90%, with the best results occurring at doses of 900 and 1000 g a.i. ha^{-1} of imazamox plus Aterbane® BR, with 85% and 90%, respectively (Table 3). The Eurasian watermilfoil (*Myriophyllum spicatum*) and hybrid watermilfoil (*Myriophyllum spicatum* × *Myriophyllum sibiricum*) are plants considered difficult to control, as they

Table 2. Average values and percentage of reduction in fresh mass and dry mass of *E. crassipes*.

Treataments	Dose (g i.a. ha^{-1})	Fresh mass (g)	Reduction (%)	Dry mass (g)	Reduction (%)
Control	**0**	184,0a	0	14,5a	0
Imazamox	**800**	6,5b	96,4	0,5b	96,5
	900	0,0b	100	0,0b	100
	1000	0,0b	100	0,0b	100
Imazamox +Dash®	**800**	0,0b	100	0,0b	100
	900	0,0b	100	0,0b	100
	1000	0,0b	100	0,0b	100
Imazamox + Aterbane®	**800**	0,0b	100	0,0b	100
	900	0,0b	100	0,0b	100
	1000	0,0b	100	0,0b	100
Valor F	**-**	**148,31****	**-**	**132,82****	**-**
CV	**-**	**75,56**	**-**	**81,26**	**-**
DMS	**-**	**0,26**	**-**	**0,14**	**-**

**Significant at 5% probability. Means followed by the same letter in the column do not differ statistically from each other by Tukey's test ($p < 0.05$). CV = coefficient of variation; DMS = least significant difference.

Table 3. Percentage of control of the herbicide imazamox alone and with adjuvants for the control of *Myriophyllum aquaticum*.

Treataments	Dose (g i.a. ha^{-1})	Days after application (DAA)					
		7	15	21	30	45	60
Imazamox	**800**	5b	7b	18a	20d	30c	35e
	900	5b	7b	18a	22d	35c	45de
	1000	5b	7b	18a	22d	38c	50d
Imazamox + Dash® HC	**800**	18a	30a	50a	65ab	70ab	80abc
	900	15a	30a	55a	70a	72ab	80abc
	1000	12a	28a	48a	65ab	70ab	75bc
Imazamox + Aterbane® BR	**800**	12a	28a	45ab	55c	65b	72c
	900	12a	30a	50[a]	60bc	68b	85ab
	1000	12a	32a	40b	68ab	78a	90a
Valor F	**-**	**17,19****	**70,42****	**101,56****	**229,35****	**179,58****	**136,92****
CV	**-**	**24,21**	**13,29**	**9,52**	**6,60**	**6,25**	**6,85**
DMS	**-**	**6,72**	**7,65**	**9,41**	**8,53**	**9,50**	**12,13**

**Significant at 5% probability. Means followed by the same letter in the column do not differ statistically from each other by Tukey's test ($p < 0.05$). CV = coefficient of variation; DMS = least significant difference.

present genotypes with differential sensitivity to herbicides, such as 2,4-D (Chorak et al. 2023). At a dose of 800 g a.i. ha^{-1} + 0.5% of Aterbane® BR, in 60 DAA, lateral shoots of plants appeared, similar to Wersal & Madsen (2007), in 42 DAA the herbicide imazamox at a concentration of 561 g a.i. ha^{-1} presented 76.7% control, with the emergence of lateral shoots for *Myriophyllum aquaticum.*

For the fresh biomass and dry mass of *M. aquaticum*, there was a significant difference between the control and the tested doses (Table 4). The reductions in dry mass occurred at all tested doses of imazamox + Aterbane® BR from 91.63% to 92.44% and imazamox + Dash® HC with a reduction of 88.08% to 90.54%. Imazamox alone caused reductions of 77.97% to 87.72% (Table 4), these values differed from 561 g a.i. ha^{-1} of imazamox with 39% reduction in dry mass for *M. aquaticum*, while imazapyr showed a 100% reduction in biomass with 584 g a.i. ha^{-1}.

Efficacy for Giant salvinia

For Giant salvinia (*S. molesta*), imazamox alone and with the addition of adjuvants showed unsatisfactory or fair control throughout the evaluation period, ranging from 2 to 48% (Table 5), similar to the 560 g a.i. ha^{-1} imazamox dose at 35 DAA, with 18% (Emerine et al., 2010).

Table 4. Average values and percentage of reduction in fresh biomass and dry mass of *M. aquaticum*.

Treataments	Dose (g i.a. ha^{-1})	Fresh mass (g)	Reduction (%)	Dry mass (g)	Reduction (%)
Control	**0**	39,99a	0	10,99a	0
Imazamox	**800**	8,45b	78,87	2,24b	77,97
	900	7,35b	81,62	1,48bc	86,53
	1000	6,83b	82,92	1,35bc	87,72
Imazamox+Dash	**800**	6,57b	83,57	1,31bc	88,08
	900	6,17b	84,57	1,23bc	88,81
	1000	6,16b	84,60	1,04bc	90,54
Imazamox+Aterbane	**800**	5,85b	85,37	0,97bc	91,17
	900	4,80b	88,00	0,92c	91,63
	1000	4,79b	88,02	0,83c	92,44
Valor F	-	**8,40****	-	**14,53****	-
CV	-	**25,48**	-	**49,18**	-
DMS	-	**0,47**	-	**0,43**	-

**Significant at 5% probability. Means followed by the same letter in the column do not differ statistically from each other by Tukey's test ($p < 0.05$). CV = coefficient of variation; DMS = least significant difference.

Table 5. Percentage of control of the herbicide imazamox alone and with adjuvants for the control of *Salvinia molesta*.

Treatments	Doses (g i.a. ha^{-1})	Days after application (DAA)					
		7	15	21	30	45	60
Imazamox	**800**	2a	3a	10a	15a	30ab	40ab
	900	2a	3a	10a	15a	25ab	25bc
	1000	2a	3a	8a	20[a]	20b	25bc
Imazamox + Dash® HC	**800**	2a	2a	10a	18a	20b	23c
	900	2a	5a	10a	18a	28ab	38abc
	1000	2a	5a	10a	18a	32ab	48a
Imazamox + Aterbane® BR	**800**	2a	5a	15a	20a	25ab	25bc
	900	2a	5a	12a	25a	25ab	25bc
	1000	2a	5a	12a	25a	25ab	25bc
Valor F	-	**2,00NS**	**9,22****	**2,68***	**11,39****	**16,74****	**16,17****
CV	-	**43,03**	**24,32**	**41,27**	**20,72**	**16,44**	**20,25**
DMS	-	**2,24**	**2,74**	**6,75**	**10,42**	**10,93**	**16,04**

**Significant at 5% probability. Means followed by the same letter in the column do not differ statistically from each other by Tukey's test ($p < 0.05$). CV = coefficient of variation; DMS = least significant difference.

The imazapyr was more effective than imazamox for controlling *S. molesta* at doses of 875 and 1000 g a.i. ha^{-1}, with 70 and 90% control after 60 days of application (Cruz et al., 2015a). Glyphosate for S. *molesta* and *S. herzogii* showed control effectiveness of 90 to 95% in 3840 g ha^{-1}, respectively (Cruz et al., 2015b).

For the reduction in fresh mass of *S. molesta*, there was a significant difference between the control and all treatments tested, but no difference between treatments (Table 6). For dry matter, there was a significant difference between the control and 800 g a.i. ha-1, with a reduction of 54.52%. Imazamox + Dash® HC showed a difference between the concentrations and the control, and for imazamox + Aterbane® BR, there was a difference at the doses of 800 and 1000 g a.i. ha-1, with reductions of 45% and 48%, respectively (Table 6). For Nelson et al. (2001), imazapyr at a dose of 1680 g a.i. ha^{-1} did not significantly reduce the dry mass of *S. molesta*, while the mixture of imazapyr + glyphosate (1680 + 987 g a.i. ha^{-1}) reduced the biomass by 98.7% compared to untreated plants.

Acute ecotoxicity of imazamox and adjuvants

In the evaluation of the acute toxicity of imazamox for *L. minor*, the 50% lethal concentration (LC50;7d) was 1.89 mg L^{-1} (Table 7). Based on the USEPA classification (United States Environmental Protection Agency, 2023), imazamox was considered moderately effective for this bioindicator plant, with a coefficient of determination of 92% (Table 7). The 50% lethal concentration (LC50;7d) of imazamox for water fern (*A. caroliniana*) was >118.0 mg L^{-1}, and the LC50;7d for *W. brasiliensis* was 27.63 mg L^{-1} (Table 7). The bioindicator plant of acute toxicity for imazamox was *L. minor*, being less sensitive than *Azolla caroliniana* to a mixture of 50% atrazine and 35% isoproturon with an EC50;21d of 0.03 mg L^{-1} (Coutris et al., 2011), that the alga *Rhodomonas salina* with EC50 of 6.27 µg L^{-1} for diuron, 13.40 µg L^{-1} for metribuzin, 8.50 µg L^{-1} for hexazinone, 19.30 µg L^{-1} for bromacil, 112.0 µg L^{-1} for tebuthiuron, 118.0 µg L^{-1} propazine, 790.0 µg L^{-1} for imazapic, > 3.70 µg L^{-1} for haloxyfop and > 279.0 µg L^{-1} for 2,4-D (Thomas et al., 2020).

The 50% lethal concentration (LC50;7d) of the Dash® HC adjuvant for duckweed (*L. minor*) was 6.17 mg L^{-1}, for water fern (*A. caroliniana*) it was 383.5 mg L^{-1} and for water flour (*W. brasiliensis*) it was 164.60 mg L^{-1}. The toxicity of the Aterbane® BR adjuvant for *L. minor* was 7.36 mg L^{-1} (Table 7) and considered moderately toxic according to USEPA (United States Environmental Protection Agency, 2023). For *A. caroliniana* the LC50;7d was 56.18 mg L^{-1} and for *W. brasiliensis* it was 32.39 mg L^{-1} (Table 7) considered little toxic according to USEPA (United States Environmental Protection Agency, 2023). Duckweed (*L. minor*)

Table 6. Average values and percentage of reduction in fresh biomass and dry mass of *S. molesta.*

Treatment	Dose (g i.a. ha^{-1})	Fresh mass (g)	Reduction (%)	Dry mass (g)	Reduction (%)
Controle	0	103,27a	0	9,72a	0
Imazamox	800	49,20b	52,36	4,42b	54,52
	900	65,27b	36,80	5,50ab	43,42
	1000	59,69b	42,20	6,10ab	37,24
Imazamox+Dash HC®	800	58,94b	42,93	5,00b	48,56
	900	55,14b	46,61	4,74b	51,23
	1000	59,16b	42,71	4,78b	50,82
Imazamox+Aterbane® BR	800	68,49b	33,68	5,28b	45,67
	900	63,57b	38,44	5,66ab	41,77
	1000	66,34b	35,76	4,97b	48,87
Valor F	-	**1,0**	-	**3,56****	-
CV	**-**	**17,58**	**-**	**23,20**	**-**
DMS	**-**	**0,47**	**-**	**0,24**	**-**

**Significant at 5% probability. Means followed by the same letter in the column do not differ statistically from each other by Tukey's test ($p < 0.05$). CV = coefficient of variation; DMS = least significant difference.

Table 7. Acute ecotoxicity of the herbicide and adjuvants for bioindicator plants.

Duckweed (*L. minor*)					
Concentration (mg L^{-1})	**LI**	**CL50;7d**	**LS**	**R^2**	**Linear equation**
Imazamox	1,22	1,89	2,94	92	y = 13,874x + 11,307
Dash® HC	5,17	6,17	7,32	88	y = 23,571x – 33,667
Imazamox + Dash® HC	1,01	1,66	2,73	93	y = 16,543x + 10,6
Aterbane® BR	5,60	7,36	9,67	95	y = 18,057x – 17,2
Imazamox + Aterbane® BR	1,28	2,32	4,20	93	y = 15,457x + 9,7333
Water fern *(A. caroliniana*)					
Imazamox	-	>118,0	-	68	y = 3,8286x – 7,4
Dash® HC	-	383,5	-	50	y = 9,8x – 18,4
Imazamox + Dash® HC	8,49	9,82	11,37	89	y = 23,771x – 39,867
Aterbane® BR	52,88	56,18	59,69	87	y = 22,171x – 13,933
Imazamox + Aterbane® BR	5,30	5,99	6,77	89	y = 24,8x – 36,467
Water flour (*W. brasiliensis*)					
Imazamox	25,47	27,63	29,97	82	y = 22,086x – 25,8
Dash® HC	148,65	164,60	182,28	79	y = 17,914x – 28,2
Imazamox + Dash® HC	4,88	5,52	6,23	84	y = 24,686x – 35,067
Aterbane® BR	27,88	32,39	37,63	72	y = 18,229x – 35,467
Imazamox + Aterbane® BR	13,83	15,47	17,30	76	y = 22,886x – 43,267

LC50;7d = 50% lethal concentration (mg L^{-1}); LI = lower limit; UL = upper limit.

was the most sensitive bioindicator to the adjuvant compared to the two aquatic plants evaluated, similar to LC(50;96h) of the adjuvants Agral® with 3.29 mg L^{-1}, Aterbane® BR with 8.21 mg L^{-1}; Energic® with 2.34 mg L^{-1}, Gotafix® with 4.37 mg L^{-1} and Fixade® with 3.38 mg L^{-1} for the mato-grosso fish (*H. eques*) (Carraschi et al., 2011) and for Aterbane® BR for the pacu, mato-grosso, guaru and zebrafish fish with LC(50;96h) ranging from 5.81 to 9.45 mg L^{-1} (Cruz et al., 2016).

Para duckweed the LC50;7d of imazamox + 0.5% Dash® HC was 1.66 mg L^{-1} and imazamox + Aterbane® BR foi de 2.32 mg L^{-1} (Table 7), differing from the acute toxicity for 4 bioindicator fish (pacu, mato-grosso, guaru and zebrafish) with LC50;96h with values higher than 900 mg L^{-1} for glyphosate + 0.5% Aterbane® BR and higher than 400.0 mg L^{-1} for glyphosate + 1.0% Aterbane® BR (Cruz et al., 2016).

The LC50;7d of imazamox+ Dash® HC was 9.82 mg L^{-1} (Table 7), which indicates that the addition of the adjuvant increases the acute toxicity of the herbicide for *A. caroliniana*, and with the addition of the adjuvants the classification was changed to moderately toxic according to USEPA (United States Environmental Protection Agency, 2023).

Conclusion

The electrostatic application of imazamox with and without adjuvants was effective in controlling the aquatic plants water hyacinth and watermilfoil, but was ineffective in controlling Giant Salvinia (*S. molesta*). The duckweed test plant was more sensitive to the herbicide, adjuvants, and their addition to the spray solution and can be used as a bioindicator for environmental biomonitoring.

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