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Article

Herbicides for Rooted Aquatic Macrophytes

Herbicidas para Macrófitas Aquáticas Enraizadas

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ABSTRACT - The aim of this study was to evaluate the effectiveness of glyphosate, 2,4-D, and diquat to control the aquatic macrophytes *Brachiaria subquadripara*, *Hedychium coronarium*, and *Myriophyllum aquaticum* under greenhouse conditions. The following herbicides and doses were used: glyphosate at 240, 720, 1,680, 2,640, 3,600, and 4,320 g a.e. ha^{-1}, 2,4-D at 335, 1,005, 1,675, 2,345, and 3,015 g a.e. ha^{-1}, diquat at 100, 200, 300, 400, 500, and 600 g a.i. ha^{-1}, and a control with 10 replications. Sprayings were applied with a CO_2-pressurized backpack sprayer at a constant pressure of 172.36 KPa, tips DG 11002, and spray solution consumption of 200 L ha^{-1}. The effectiveness of control was evaluated visually at 3, 7, 15, 21, 30, 45, and 60 days after application (DAA) and dry matter accumulation at 60 DAA. Glyphosate promoted the best effectiveness, with 100% control of *B. subquadripara*, with no dry matter accumulation from 720 g a.e. ha^{-1}. The herbicide 2,4-D presented the best control for *M. aquaticum* with 100% control from 1,209 g a.e. ha^{-1}, and for *H. coronarium* with 99% control from 2.015 g a.e. ha^{-1}, both doses with no dry matter accumulation. The diquat was not effective in controlling the plants of *M. aquaticum* and *B. subquadripara*, besides allowing resprouts.

Keywords: chemical management, aquatic plant, weed, aquatic environment.

*RESUMO - O objetivo deste estudo foi avaliar a eficácia de glyphosate, 2,4-D e diquat no controle das macrófitas aquáticas **Brachiaria subquadripara**, **Hedychium coronarium** e **Myriophyllum aquaticum** em condições de casa de vegetação. Utilizaram-se os seguintes herbicidas e doses: glyphosate a 240, 720, 1.680, 2.640, 3.600 e 4.320 g e.a. ha^{-1}; 2,4-D a 335, 1.005, 1.675, 2.345 e 3.015 g e.a. ha^{-1}; e diquat a 100, 200, 300, 400, 500 e 600 g i.a. ha^{-1} e um controle, com dez repetições. As pulverizações foram efetuadas com um pulverizador costal à pressão constante de CO_2 de 172,36 KPa, ponta DG11002 e consumo de calda de 200 L ha^{-1}. A eficácia de controle foi avaliada visualmente aos 3, 7, 15, 21, 30, 45 e 60 dias após a aplicação (DAA), e o acúmulo de massa seca, aos 60 DAA. O glyphosate promoveu a melhor eficácia, com 100% de controle de **B. subquadripara** e sem acúmulo de massa seca, a partir de 720 g e.a. ha^{-1}. O 2,4-D apresentou o melhor controle para **M. aquaticum**, com 100% a partir 1.209 g e.a. ha^{-1}, e para **H. coronarium**, com 99% de controle a partir de 2.015 g e.a. ha^{-1}, ambas as doses sem o acúmulo de massa seca. O diquat não foi eficaz no controle das plantas de **M. aquaticum** e **B. subquadripara**, além de permitir rebrotas.*

Palavras-chave: manejo químico, planta aquática, planta daninha, ambiente aquático.

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INTRODUCTION

Aquatic plants play a key role in aquatic ecosystems as they perform basic functions of food chains, protection of banks, habitats and refuge for organisms, removal of organic material and toxic compounds, and spatial and temporal heterogeneity of them (Silva et al. 2012). However, anthropogenic activities have increased nitrogen and phosphorus, which facilitates eutrophication and biomass increase of primary producers (Souza et al., 2014). Therefore, these plants lead to a decrease in species richness, changes in quality standards, and reduced capacity and durability of irrigation channels, drainage, and catchment for public supply (Pieterse and Murphy, 1990), as well as water loss by evapotranspiration (Anda et al., 2015).

Aquatic plants exhibit rapid vegetative growth, ease of propagation, and support a wide variability of environmental conditions. Among these plants, stand out the species *Brachiaria subquadripara* (Trin.), a monocotyledonous belonging to the Poaceae family, perennial, and stoloniferous plant that reproduces vegetatively with a low seed production, originating in Africa, occupying large areas of floodplain and other flooded environments in Brazil, such as the Pantanal region of Mato Grosso, and at the beginning of its growth is anchored (Domingos et al., 2011); *Myriophyllum aquaticum* (Vell.), an eudicotyledonous plant of the order Myrtales, family Haloragaceae, originating in South America, which remains rooted in the mud at the bottom of lakes up to 2 m deep or in the margins (Cason and Roost, 2011); and *Hedychium coronarium* (J Koning), a monocotyledonous plant of the Zingiberaceae family, rhizomatous, perennial herbaceous habit, and sexual or asexual reproduction by rhizomes (Stone et al., 1992).

In order to reduce economic, environmental, and social impacts of aquatic plants, management measures, such as the mechanical control, which is used in Brazil (Pitelli et al., 2008), biological control (Tessmann, 2011) or use of herbicides, which have a good benefit/cost ratio and a historical basis of use in other countries (Gettys et al., 2014), and environmental safety for non-target organisms (Cruz et al., 2016), are necessary.

Among the herbicides used to control aquatic plants are the glyphosate, which acts on both monocotyledonous and eudicotyledonous plants (Amarante Jr et al., 2002); 2,4-D, which is recommended mainly for eudicotyledonous; and diquat, which is recommended for some monocotyledonous and eudicotyledonous (Negrisoli et al., 2003). According to Gettys et al. (2014), these herbicides have been used in the control of several aquatic macrophytes. Some studies have reported the use of diquat in *Pistia stratiotes* (L.), *Cyperus giganteus* (Vahl.), and *Eichhornia crassipes* (Mart.) (Mudge and Netherland et al., 2014). Other studies have been conducted with 2,4-D to control *M. aquaticum* (Negrisoli et al., 2003) and glyphosate in the control *Salvinia molesta* (Mitchell.), *Salvinia herzogii* (de la Sota.), *P. stratiotes*, *B. subquadripara*, and *E. crassipes* (Cruz et al., 2015).

Thus, the greater knowledge about the chemical control of rooted aquatic plants can contribute to the decision making on the management of them, which occupy the banks of water bodies, with a high potential of invasion and environmental dynamic change of biotic and abiotic components of aquatic ecosystems. For this, this study aimed to evaluate the effectiveness of the herbicides glyphosate, 2,4-D, and diquat to control *B. subquadripara*, *M. aquaticum*, and *H. coronarium* under greenhouse conditions.

MATERIAL AND METHODS

The herbicides used in this study were glyphosate in the commercial formulation Rodeo® (480 g a.e. L^{-1}), 2,4-D in the formulation DMA 806® (670 g a.e. L^{-1}), and diquat in the formulation Reglone® (200 g L^{-1}).

Primary cultivation of aquatic plants

For the primary cultivation, samples of the aerial part (pointers) of the plants *M. aquaticum* and *B. subquadripara* and tubers of *H. coronarium* were collected in a dam located in the municipality of Barretos, SP, Brazil (20°35'16.66" S and 48°32'21.24" W). Subsequently, plantations of the pointers were carried out in asbestos boxes with a capacity of 1,000 L filled with a substrate composed of coarse sand, organic matter, and soil (1:1:1; v/v) and continuous water flow. Five and three 7 cm pointers were selected from the plants *B. subquadripara* and *M. aquaticum*, respectively, which were transplanted to plastic pots with a capacity of 2.5 L filled with the same substrate of the primary cultivation and water depth of 5.0 cm above the substrate. For *H. coronarium*, the tubers were transplanted to a pot with a capacity of 5 L filled with substrate. *B. subquadripara* and *M. aquaticum* were maintained in a growth chamber for approximately 30 days, and *H. coronarium* for 60 days, also in a growth chamber.

Experimental procedure

An experiment was installed for each herbicide (glyphosate, 2,4-D, and diquat) and aquatic plants in order to evaluate the effectiveness. The experiments were conducted in a completely randomized design, with glyphosate being tested at doses of 240, 720, 1,680, 2,640, 3,600, and 4,320 g a.e. ha^{-1}, 2,4-D at doses of 335, 1,005, 1,675, 2,345, and 3,015 g a.e. ha^{-1}, and diquat at doses of 100, 200, 300, 400, 500, and 600 g a.i. ha^{-1}, in addition to a control, with ten replications.

Herbicide applications were performed with a CO_2-pressurized backpack sprayer at a constant pressure of 172.36 KPa, with a spray solution consumption of 200 L ha^{-1} and equipped with an application boom with four flat fan tips DG 11002VS spaced at 0.5 m. After spraying, the plants were taken to a greenhouse, where they remained until the end of the study, with a mean temperature of 26 ^{o}C and humidity of 55.5%.

The effectiveness of visual control was performed by three evaluators at 3, 7, 15, 21, 30, 45, and 60 days after application (DAA) through a visual score scale in percentage, where 0% corresponds to none injury and 100% to plant death (SBCPD, 1995). At the end of the study, the plants were submitted to drying at ambient temperature to obtain the fresh biomass and transferred to a forced air circulation oven at 70 ^{o}C for three days to quantify the dry matter in a semi-analytical scale, and calculation of the simple percentage in relation to the control. The results of injuries were submitted to analysis of variance (ANOVA) and the means were compared by the Tukey's test at 5% of probability. For this, the treatments of each herbicide were evaluated within each time of evaluation.

RESULTS AND DISCUSSION

For glyphosate, the first signs of toxicity in *B. subquadripara* (leaf yellowing) were observed at 7 DAA from the dose of 720 g ha^{-1}. These signs of toxicity evolved during the evaluations and good control was verified at 15 DAA from the dose of 1,680 g ha^{-1}. The dose of 240 g ha^{-1} provided, from the second evaluation, the first signs of intoxication in plants of *B. subquadripara* (Table 1).

At 21 DAA, the dose of 720 g ha^{-1} of glyphosate had effectiveness considered good, but lower than the higher doses, which provided excellent control, close to 100%. In the evaluations at 30, 45, and 60 DAA, the control of *B. subquadripara* reached 100% at all tested doses, except for the lowest dose (240 g ha^{-1}), which was ineffective (Table 1). According to Costa et al. (2012), glyphosate was also effective, with 98.8% control of *B. subquadripara* at a dose of 4,320 g ha^{-1} + 0.5% Aterbane® BR at 63 DAA. Cruz et al. (2015) also obtained a 100% control for *B. subquadripara* with a glyphosate dose of 3,360 g ha^{-1} + 0.5% v/v Aterbane® BR at 30 DAA, as observed. These results showed that the herbicide glyphosate is an efficient molecule to control this aquatic plant.

Up to 21 DAA, none of the tested glyphosate doses provided any sign of injury to plants of *M. aquaticum*. From 30 to 60 DAA, doses equal to and higher than 2,640 g ha^{-1} of glyphosate provided injuries to plants of *M. aquaticum* and only doses of 3,600 and 4,320 g ha^{-1} promoted its satisfactory control at the end of the study at 60 DAA (Table 1). Negrisoli et al. (2003) found an excellent result when using a dose of 3,300 g ha^{-1} + 0.5% Aterbane, with 97% control at 17 DAA. Emerine et al. (2010) also had excellent control of 94% when adding the adjuvant Agral® to the spray solution, which does not corroborate the results found in our study. This difference may probably be attributed to the use of adjuvants in the spray solution, which was not done in this study.

In the case of *H. coronarium*, the times of evaluation showed that the action of the herbicide glyphosate was slow, with few visual signs of toxicity up to 21 DAA (Table 1). The dose of 240 g ha^{-1}

Table 1 **-** Percentage of control of *Brachiaria* subquadripara, *Myriophyllum aquaticum* and *Hedychium coronarium* by the herbicide glyphosate at evaluation times

Dose (g a.e. ha^{-1})	*Brachiaria subquadripara*					
	Days after herbicide application					
	7	15	21	30	45	60
240	0.0 d	2.0 c	14.9 c	15.0 b	15.0 b	15.0 b
720	15.0 c	50.0 b	89.8 b	100.0 a	100.0 a	100.0 a
1,680	15.0 c	85.0 a	99.0 a	100.0 a	100.0 a	100.0 a
2,640	17.5 b	84.9 a	99.2 a	100.0 a	100.0 a	100.0 a
3,600	21.0 a	85.2 a	99.0 a	100.0 a	100.0 a	100.0 a
4,320	22.5 a	85.6 a	99.0 a	100.0 a	100.0 a	100.0 a
F treatment	282.430**	2403.613**	5178.714**	26791.210**	26791.210**	26791.210**
CV (%)	9.9	3.3	1.7	0.7	0.7	0.7
LSD	1.99	2.93	1.98	0.89	0.89	0.89
	Myriophyllum aquaticum					
240	0.0	0.0	0.0	0.0 b	0.0 b	0.0 c
720	0.0	0.0	0.0	0.0 b	0.0 b	0.0 c
1,680	0.0	0.0	0.0	0.0 b	0.0 b	0.0 c
2,640	0.0	0.0	0.0	40.0 a	45.0 a	50.0 b
3,600	0.0	0.0	0.0	40.0 a	45.0 a	80.0 a
4,320	0.0	0.0	0.0	40.0 a	45.0 a	80.0 a
F treatment	-	-	-	2069.017**	11082.771**	3547.934**
CV (%)	-	-	-	7.6	3.2	6.0
LSD	-	-	-	2.03	0.99	2.82
	Hedychium coronarium					
240	0.0 b	0.0 c	0.0 c	0.0 d	0.0 e	0.0 e
720	0.0 b	0.0 c	0.0 c	0.0 d	2.7 d	10.0 d
1,680	2.7 a	4.1 b	7.5 b	20.0 c	27.3 c	54.7 c
2,640	2.7 a	6.42 a	32.1 a	50.4 b	53.9 b	80.0 b
3,600	2.7 a	6.42 a	32.1 a	52.1 b	60.0 a	90.0 a
4,320	2.7 a	6.42 a	32.1 a	55.0 a	62.0 a	91.5 a
F treatment	117.161**	293.104**	3303.433**	1643.432**	1968.419**	5816.969**
CV (%)	22.6	14.9	5.2	6.8	5.9	3.0
LSD	0.54	0.77	1.21	2.72	2.69	2.24

Means followed by the same letter in the column do not differ statistically from each other by the Tukey's test (P>0.05). ** Significant at 1% probability.

did not provide any visual sign of apparent injury until the end of the study (60 DAA). In this last evaluation, the dose of 2,640 g ha^{-1} provided a satisfactory control of plants of *H. coronarium*, which was superior to that found in the lower doses, but lower than the two higher doses (3,600 and 4,320 g ha^{-1}), which provided controls considered excellent and were similar to each other (Table 1).

Regarding the dry matter accumulation in the three studied species, the dry matter production of *B. subquadripara* was only collected and evaluated in the treatment with a dose of 240 g ha^{-1}, which was 18.9% of that found in the control, because in the other doses of glyphosate, the control was 100% (Table 1).

For *M. aquaticum* (Figure 1A), dry matter production was reduced as glyphosate dose increased (from 720 g ha^{-1}), regardless of whether no visual control of the plants was observed at this dose and that of 1,680 g ha^{-1} (Table 1), showing reductions of 79.3 and 51.8%, respectively.

Regardless of the observed visual control, all the tested glyphosate doses affected dry matter accumulation in plants of *H. coronarium* (Figure 1B), being more effective from a dose of 1,680 g ha^{-1}, with a reduction of the order of 27.3%.

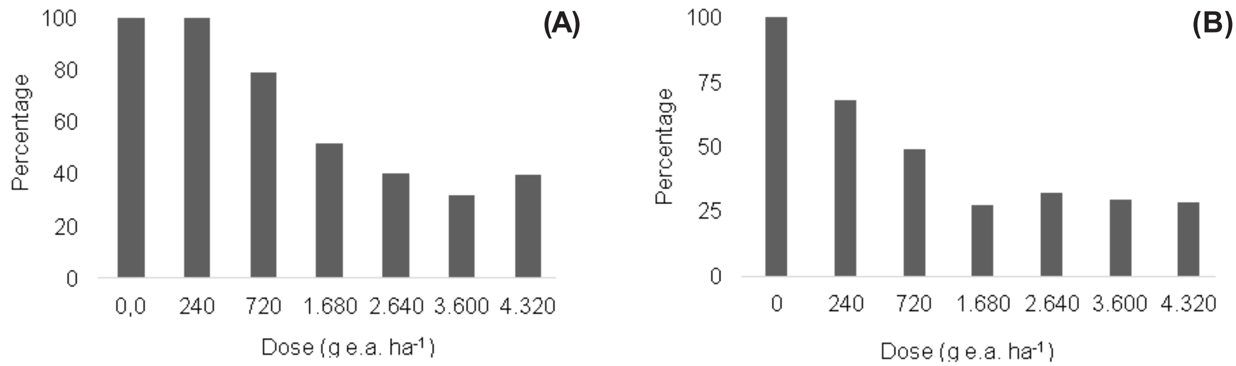

Figure 1 - Effect of the herbicide glyphosate on the total percentage accumulation of dry matter of the aerial part of *Myriophyllum aquaticum* (A) and *Hedychium coronarium* (B) in relation to the control.

In the experiment with 2,4-D, the macrophyte *B. subquadripara* showed no injuries. On the other hand, *M. aquaticum* showed signs of intoxication (necrosis of pointers and loss of stem support) at 3 DAA, regardless of the tested dose. Except for the lowest dose (335 g ha $^{-1}$), all other doses provided good and similar control of plants of *M. aquaticum* (Table 2).

Table 2 - Percentage of control of *Myriophyllum aquaticum* and *Hedychium coronarium* by the herbicide 2,4-D at evaluation times

Dose (g a.e. ha^{-1})	*Myriophyllum aquaticum*						
	Days after herbicide application						
	3	7	15	21	30	45	60
335	60.0 b	70.0 b	77.5 b	65.0 b	65.0 b	50.0 b	30.0 b
1,005	91.0 a	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a
1,675	91.0 a	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a
2,345	91.0 a	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a
3,015	91.0 a	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a
F treatment	2328.805**	2025.000**	1815.000**	8497.745**	8497.745**	12500.001**	31500.003**
CV (%)	1.0	1.0	0.7	0.5	0.5	0.7	0.6
LSD	1.16	1.21	0.94	0.69	0.69	0.81	0.72
	Hedychium coronarium						
335	0.0 b	2.5 c	30.0 d	35.0 c	50.8 c	52.2 c	52.2 c
1,005	0.0 b	3.1 c	57.5 c	72.5 b	85.0 b	95.0 b	95.0 b
1,675	0.0 b	15.0 b	87.5 b	99.0 a	99.0 a	99.0 a	99.0 a
2,345	7.5 a	25.0 a	98.5 a	99.0 a	99.0 a	99.0 a	99.0 a
3,015	7.5 a	25.0 a	98.5 a	99.0 a	99.0 a	99.0 a	99.0a
F treatment	484.99**	523.981**	2417.8**	3255.617**	3842.771**	1630.683**	1630.683**
CV (%)	19.6	10.8	2.5	1.9	1.2	1.8	1.8
LSD	0.74	1.97	2.43	2.00	1.37	2.07	2.07

Means followed by the same letter in the column do not differ statistically from each other by the Tukey's test ($P>0.05$). ** Significant at 1% probability.

From 7 DAA, except for the dose of 335 g ha^{-1}, all other 2,4-D doses provided the total control of plants of *M. aquaticum*. The lowest tested dose was inefficient to control this aquatic plant during the experimental period, including resprouts. In addition, Negrisoli et al. (2003) found excellent results of 2,4-D on *M. aquaticum*, with a 100% control for doses equal to or higher than 670 g ha^{-1}, which ratifies the results of the present study.

For *H. coronarium*, the herbicide 2,4-D provided small injuries at 3 DAA only from the dose of 2,345 g ha^{-1}, which progressively evolved at 7 DAA. However, in this period, the lower doses of 2,4-D (335 and 1,005 g ha^{-1}) also provided injuries to plants (Table 2).

At 15 DAA, plants of *H. coronarium* showed efficient control at the three highest doses (1,675, 2,345, and 3,015 g ha^{-1}), but still unsatisfactory at the two lower ones (335 and 1,005 g ha^{-1}). At

21 DAA, the three higher doses of 2,4-D provided excellent control of this aquatic plant, reaching 99% control, which was maintained until the end of the study, at 60 DAA. In addition, the lowest tested dose of 2,4-D (335 g ha^{-1}) provided poor control until the end of the study, but very good control of this aquatic macrophyte was observed from a dose of 1,005 g ha^{-1}, being statistically lower when compared to the three higher doses (Table 2).

Dry matter accumulation in *M. aquaticum* and *H. coronarium* was very low at the largest tested doses of 2,4-D. The biomass was decomposed until the end of the study, not allowing its evaluation. The material for analysis was only collected at plots that received the lowest dose (335 g ha^{-1}), being this accumulation of 3.07 and 32.08% in the plants *M. aquaticum* and *H. coronarium*, respectively, when compared to the control. Moreover, the reduction was 2.4% for *H. coronarium* at a dose of 1,005 g ha^{-1}.

Diquat was inefficient in the control of the studied plants. Injuries were not verified for *H. coronarium*. For *B. subquadripara* and *M. aquaticum*, regardless of the tested dose, control increased at a given time and later reduced during the experimental period. This was observed in both species due to the sprouts that occurred in the plants, leading to an unsatisfactory control at the end of the study (60 DAA) (Table 3).

Although visually the control of *B. subquadripara* and *M. aquaticum* provided by the herbicide diquat was unsatisfactory (Table 3), Figure 2 shows that diquat doses reduced dry matter accumulation in their plants. According to Costa et al. (2012), the occurrence of resprouts in *B. subquadripara* showed the importance of constant monitoring in the applied areas in order to evaluate the need for reapplication and avoid reinfestation. Because of plant resprouts, over time there would be a recomposition of accumulated dry matter, which would require new applications. This can be considered a management plan for mechanized harvests due to the reduction of dry biomass.

Thus, glyphosate was effective in controlling plants of *B. subquadripara* regardless of the tested dose and without dry matter accumulation, as well as providing good control of *M. aquaticum*

Table 3 **-** Percentage of control of *Brachiaria subquadripara* and *Myriophyllum aquaticum* by the herbicide diquat at evaluation times

Dose (g a.i. ha^{-1})	*Brachiaria subquadripara*						
	Days after herbicide application						
	3	7	15	21	30	45	60
100	0.0 e	15.0 e	20.0 f	27.5 e	20.0 f	15.0 d	15.0 d
200	5.0 d	30.0 d	40.0 e	45.0 d	40.0 e	35.0 c	35.0 c
300	10.0 c	55.0c	82.5 c	80.0 b	75.0 c	65.0 b	65.0 b
400	20.0 b	55.0 c	75.0 d	70.0 c	65.0 d	65.0 b	65.0 b
500	20.0 b	70.0 b	92.5b	90.0 a	80.0 b	70.0 a	70.0 a
600	25.0 a	75.0 a	96.5 a	92.0 a	85.0 a	70.0 a	70.0 a
F treatment	481.550**	975.904**	2738.681**	2453.910**	1491.765**	3780.251**	3780.251**
CV (%)	10.63	4.7	2.7	2.4	3.4	2.2	2.2
LSD	1.89	3.13	2.47	2.2	2.79	1.57	1.57
	Myriophyllum aquaticum						
100	28.0 d	47.5 c	50.0 c	40.0 e	35.0 d	30.0 d	30.0 d
200	50.5 c	65.0 b	70.0 b	60.0 d	50.0 c	35.0 c	35.0 c
300	67.5 b	88.0 a	98.5 a	70.0 c	60.0 a	50.0 b	50.0 b
400	67.5 b	88.0 a	98.5 a	75.0 b	60.0 a	50.0 b	50.0 b
500	67.5 b	88.0 a	98.5 a	80.0 a	55.0 b	55.0 a	55.0 a
600	72.5 a	88.0 a	98.5 a	80.0 a	60.0 a	55.0 a	55.0 a
F treatment	752.824**	746.187**	4946.897**	311.286**	123.697**	269.449**	269.449**
CV (%)	3.3	2.5	1.0	4.0	5.2	4.4	4.4
LSD	2.58	2.64	1.23	3.68	3.72	2.74	2.74

Means followed by the same letter do not differ statistically from each other by the Tukey's test (P>0.05). ** Significant at 1% probability.

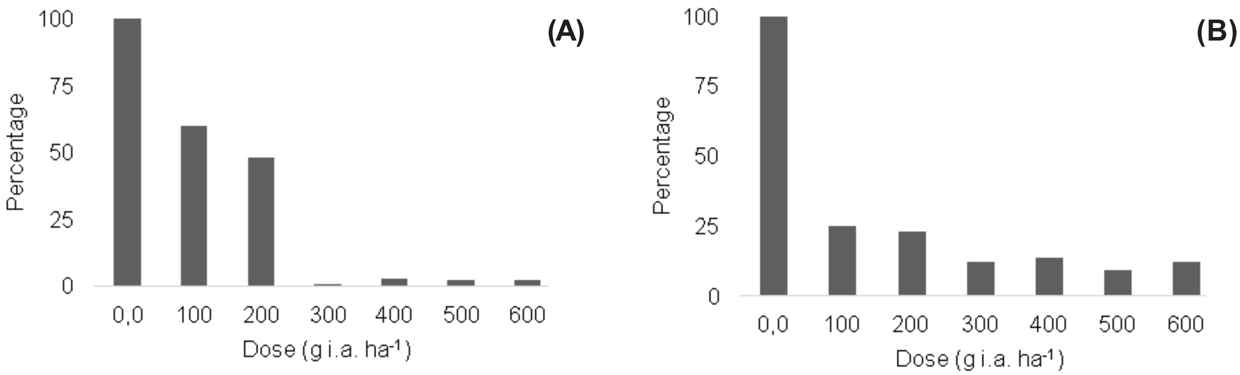

Figure 2 **-** Effect of the herbicide diquat on the total percentage accumulation of dry matter of the aerial part of *Brachiaria subquadripara* (A) and *Myriophyllum aquaticum* (B) in relation to the control.

only at the two higher doses, with a decrease in dry matter production. In addition, it was efficient for *H. coronarium*, depending on the analyzed dose.

The tested 2,4-D doses were effective in controlling plants of *M. aquaticum* and *H. coronarium*, without dry matter accumulation, except for the lowest dose. Visually, diquat provided unsatisfactory controls, but all doses reduced the dry matter accumulation of the aerial part, allowing drawing an integrated management plan with mechanical and chemical controls with this herbicide.

The most effective herbicides for the control of these three aquatic plants were glyphosate and 2,4-D, except for *B. subquadripara*, while diquat was the herbicide with the lowest control capacity, especially for *H. coronarium* and *M. aquaticum*.

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