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Effectiveness of Diquat, Copper Hydroxide, Copper Oxychloride and their Association in Control of Submerged Macrophytes *Ceratophyllum demersum*[1]

Eficácia do Diquat, Hidróxido de Cobre, Oxicloreto de Cobre e suas Associações no Controle da Macrófita Submersa ***Ceratophyllum demersum***

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ABSTRACT - The aims of this study were to evaluate the effectiveness of diquat, copper hydroxide, copper oxychloride and their associations diquat + 0.1% copper oxychloride and diquat + 0.1% copper hydroxide to control *Cerathophyllum demersum*. Therefore, the concentrations used were 0.1, 0.3, 0.5, 0.7, 1.0 and 1.5 mg L^{-1} oxychloride and copper hydroxide and 0.2, 0.4, 0.8 and 1.2 mg L^{-1} diquat and their associations with 0.1% copper oxychloride and 0.1% copper and a control hydroxide. The experimental design was completely randomized with ten replications for 45 days. For evaluation we used a scale of 0-100% control of notes and rated the weight (g) and length (cm) of pointers at the end of the trial period. Diquat showed 100% efficacy at 30 DAA, associations in 21 DAA and copper sources promoted regrowth of *C. demersum*. Diquat and its associations were more effective in controlling *C. demersum*. The use of herbicide in combination with a copper source is more efficient for the control of submerged weeds because it potentiates the effect of the herbicide in weed control

Keywords: aquatic plants, algaecide effect, chemical management.

RESUMO - O objetivo deste estudo foi avaliar a eficácia do diquat, hidróxido de cobre, oxicloreto de cobre e suas associações (diquat + 0,1% de oxicloreto de cobre e diquat + 0,1% de hidróxido de cobre) para o controle de ***Cerathophyllum demersum*** *em condições de laboratório. Para isso, foram utilizadas as concentrações de 0,1, 0,3, 0,5, 0,7, 1,0 e 1,5 mg L^{-1} do oxicloreto e hidróxido de cobre e de 0,2, 0,4, 0,8 e 1,2 mg L^{-1} do diquat e suas associações com 0,1% de oxicloreto de cobre e 0,1% de hidróxido de cobre e um controle. O delineamento experimental utilizado foi o inteiramente casualizado com dez repetições, ao longo de 45 dias de avaliação. Na avaliação foi empregada uma escala de notas de controle (0-100%) e avaliado o peso (g) e o comprimento (cm) dos ponteiros ao final do período experimental. O diquat apresentou 100% de eficácia aos 30 DAA, e as associações em 21 DAA; as fontes de cobre promoveram a rebrota de* ***C. demersum****. O diquat e suas associações foram mais eficazes no controle de* ***C. demersum****. A utilização de herbicida em associação com uma fonte de cobre é mais eficiente para controle de macrófitas submersas, pois potencializa o efeito do herbicida no controle das plantas.*

Palavras-chave: plantas aquáticas, efeito algicida, manejo químico.

INTRODUCTION

Macrophytes play a crucial role in the dynamics of aquatic ecosystems, as they are the main primary producers and also the base of the herbivory and detritivore food chain. However, due to human activities, water bodies become eutrophic, which promotes the increase of some species over others, forming settlements of single-species or poorly

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diversified plants, which negatively impact water bodies (Silva et al., 2012).

Of plants that colonize water bodies, the submerged macrophyte *Ceratophyllum demersum* stands out for its rapid invasion of new areas, for reducing water flow and causing imbalance in the water environment oxygenation, and there may be decrease in species richness and fish mortality (Gettys et al., 2009). This plant, belonging to the *Ceratophyllaceae* family, is native to tropical America and invasive in many environments, due to the formation of dense settlements. Its reproduction is by seed and stem fragmentation and develops in depth from 0.5 to 8.5 m (Gross et al., 2003).

Several procedures can be adopted to control submerged macrophytes, such as physical, which consists of pruning or manual collection; mechanical, with large machinery; biological, with natural enemies; and chemical, which uses products with herbicide action for weed control (Pompêo et al., 2008).

Chemical control is a viable alternative because of the convenience, control speed, effectiveness and high cost/benefit ratio (Henares et al., 2011). Studies on efficacy with diquat were carried out in *Hydrilla verticilata*, *Egeria densa* and *Egeria najas* (Henares et al., 2011) and *Ceratophyllum demersum* (Mudge, 2013); with fluridone, in *H. verticillata* (Hofstra & Clayton, 2001); and with copper sulphate associated to diquat, in *H. verticillata* (Blackbur & Weldon, 1970).

Diquat is a contact, non-selective herbicide, inhibiting photosynthesis I, belonging to the bipyridine (also known as bipyridyls, dipyridyls, and dipyridines) family of chemical compounds, has a low bioconcentration rate and offers environmental safety for aquatic organisms, especially fish (Henares et al., 2008). Its half-life is less than 48 hours, leaving a residue-free water as it binds to colloidal particles of the soil (Rodrigues & Almeida, 2011).

In addition to herbicides, copper sources may be used, which cause changes in the mitochondria, disorganization and changes in the composition of proteins and lipids, inhibiting cell division, and impairing photosynthesis (Kumar et al., 2014).

Copper, either alone or in combination with a herbicide, is described as effective in controlling macrophytes (Blackbur & Weldon, 1970; Gangstad, 1978; Gettys et al., 2009), in addition to acting as algaecide (Martins et al., 2008; Henares et al., 2011), which can inhibit the proliferation of algae after decomposition of the macrophytes.

Currently, there are several questions about the use of herbicides in the aquatic environment, especially on the effectiveness of environmental safety control and the effects of degradation and decay of macrophytes after their death, such as the release of nutrients in the environment, especially phosphorus and nitrogen (Mohr et al., 2007), which decisively contributes to eutrophication (or more precisely hypertrophication) and increased oxygen biological demand (Souza et al., 2014).

The use of herbicides that have side effects such as being algaecide or the application of algaecide products together with herbicides is an alternative, because it results in greater efficiency compared to single use, making it difficult to increase the population of algae due to the use of nutrients and release by decomposition of plants (Durborow et al., 2008; Gettys et al., 2009). To this end, the aim of this study was to evaluate the effectiveness of diquat, copper hydroxide, copper oxychloride and their associations to control *Cerathophyllum demersum* under laboratory conditions.

MATERIALS AND METHODS

To perform efficacy tests, plants of *C. demersum* from the laboratory cultivation sector were used, kept in containers with a capacity of 500 L with a substrate of Red Latosol, organic matter and sand (1:1:1 vv^{-1}).

After the occupation of 90%, pointers of *C. demersum* with good nutritional status (no leaf and stem chlorosis/necrosis) were collected and fresh weight (g) was obtained on a precision scale (Marte® model AS 2000C). To remove water excess, the pointers were lightly pressed against a filter paper.

Then, transplant of three 13 cm long pointers was performed in transparent plastic vials with a capacity of 1.3 L of water. The vials were kept for 24 hours in a bioassay room at a temperature of 25.0 ± 2.0 °C, with a photoperiod of 12 hours at 1,000 lux for acclimatization. Soon after, application of chemicals was done. Herbicide diquat (200 g L^{-1}), copper hydroxide (690 kg L^{-1}), and copper oxychloride (588 g L^{-1}) isolated, and associations diquat + 0.1% of copper hydroxide and diquat + 0.1% of copper oxychloride were used.

The concentrations tested were: 0.1, 0.3, 0.5, 0.7, 1.0 and 1.5 mg L^{-1} of copper oxychloride and copper hydroxide, and 0.2, 0.4, 0.8 and 1.2 mg L^{-1} of diquat and its associations with 0.1% of copper oxychloride and 0.1% of copper hydroxide and a control (control – without the addition of the testing products), with ten repetitions, in a completely randomized design (CRD).

Control efficacy was assessed by assigning scores according to visual signs of phytotoxicity, inhibition of growth and plant regrowth (Table 1), according to the scale proposed by Brazilian Society of Weed Science (*Sociedade Brasileira da Ciência das Plantas Daninhas*) (1995), where 0 (zero) correspond to no injury and 100 (one hundred) to plant death. Evaluations took place at 7, 14, 21, 30 and 45 days after application (DAA). At the end of the trial period, the pointers were evaluated for length (cm), fresh biomass (g) and growth and biomass rates (%).

Growth percentage (cm) and biomass (g) of the plants were assessed according to the formula by Sun-Shepard adapted from Püntener (1981), and the chemicals control effect by the equation proposed by Henderson & Tilton (1955).

Table 1 - Signs of phytotoxicity proposed for control evaluation of submersed macrophytes

Phytotoxicity signs	Percentage of effect
Chlorosis of the apex	0 – 40% Low control
Loss of chlorophyll pigment	
Loss of plant apex	
Stem chlorosis and total	41 – 70% Moderate control
Apex necrosis, stem and total	
Loss of leaves	71 – 100% Satisfactory control
Loss of plant structure	
Plant death	

RESULTS AND DISCUSSION

Diquat presented low control in concentrations of 0.2, 0.4, and 0.8 mg L^{-1} at 7, 14 and 21 DAA, respectively. In 1.2 mg L^{-1}, it presented a moderate control at 14 and 21 DAA. At 30 and 45 DAA, there was a satisfactory control of *C. demersum* with plants death in all concentrations tested (Figure 1A), differing from diquat with exposure of 30 min at 0.15 mg L^{-1} to obtain 100% of control of *C. demersum* at 14 days and of *Egeria najas* (0.15 mg L^{-1}) and *Egeria densa* (0.075 mg L^{-1}), with control at 7 and 21 days, respectively (Martins et al., 2008).

Diquat promoted 100% of mortality of *C. demersum* at the concentrations tested, interfering in photosynthetic activity. Bipyridine (also known as bipyridyls, dipyridyls, and dipyridines) family of chemical compounds radical oxidizes in the presence of water and oxygen, releasing electrons which, in the presence of oxygen, form a hydrogen peroxide molecule, which is enough of an oxidizer to destroy the chloroplast (Rodrigues & Almeida, 2011).

Copper hydroxide showed low control for *C. demersum* in 0.1 and 0.3 mg L^{-1}, respectively. In 0.5 mg L^{-1}, control was low at 7 and 14 DAA, with plant regrowth from 21 DAA. In 0.7, 1.0 and 1.5 mg L^{-1} control was moderate at 7 DAA and at 21 DAA control was satisfactory. At 21, 30 and 45 DAA the concentration of 0.7 mg L^{-1} presented plant regrowth. In 1.0 and 1.5 mg L^{-1} there was a satisfactory control (21 DAA). At 30 and 45 DAA control was satisfactory, but there was plant regrowth (Figure 1B).

Copper oxychloride presented low control in 0.1, 0.3, and 0.5 mg L^{-1} up to 45 DAA. With 0.7 mg L^{-1} control was moderate at 45 DAA. In 1.0 mg L^{-1} control was low at 7 and 14 DAA, and at 45 DAA control was satisfactory. In 1.5 mg L^{-1} control was low at 7 and 14 DAA; at 21 DAA it was satisfactory. At 30 DAA control was satisfactory and at 45 DAA control was satisfactory, but there was plant regrowth (Figure 1C).

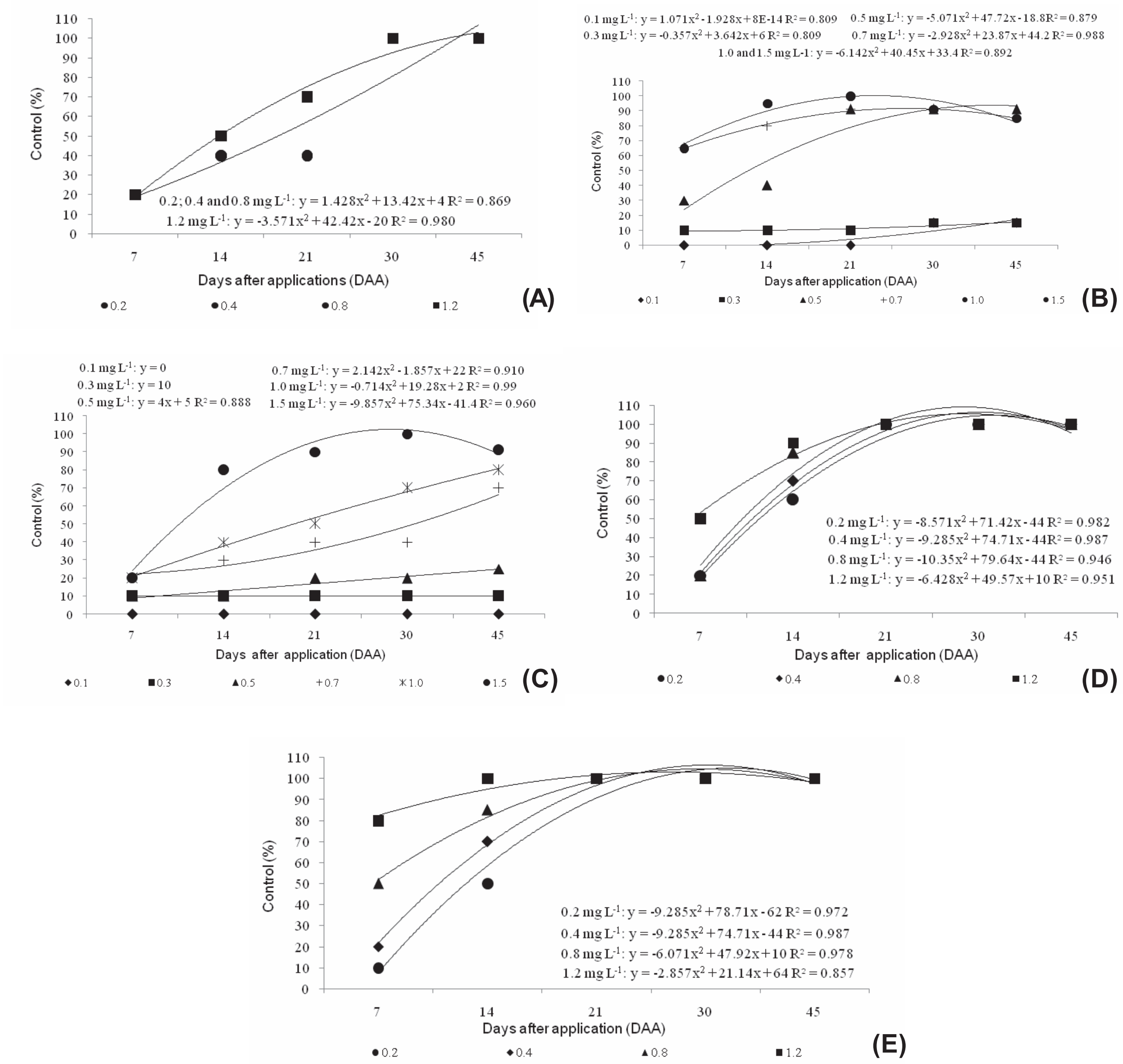

Figure 1 - Control efficacy percentage of diquat (A), copper hydroxide (B), copper oxychloride (C), diquat + 0.1% copper oxychloride (D) and diquat + 0.1% copper hydroxide (E) for *Ceratophyllum demersum*.

Copper hydroxide and copper oxychloride were not effective for *C. demersum* in concentrations up to 1.5 mg L^{-1}, but Blackbur & Weldon (1970) have described that copper sulphate in 20.0 mg L^{-1} was 100% effective to control *H. verticilata* at 30 DAA. Fluridone in 0.010, 0.020, 0.050 and 0.0150 mg L^{-1} was not effective either to control *H. verticillata* at 150 days, with reduction only in the plant biomass and signs of necrotic and chlorotic pointers (Hofstra & Clayton, 2001), similar to copper hydroxide and copper oxychloride for *C. demersum*. These results differ from those of 1.0 mg L^{-1} of endothall, which presented 95% of control for *H. verticillata* in exposure of 24 hours (Pennington et al., 2001).

Association of diquat + 0.1% of copper oxychloride in 0.2, 0.4 and 0.8 mg L^{-1} presented low control. In 1.2 mg L^{-1} there was moderate control at 7 DAA. At 14 DAA in 0.2 and 0.4 mg L^{-1} control was also moderate. In 0.8 and 1.2 mg L^{-1} control was satisfactory. From 21 DAA there was a satisfactory control with plant death in all concentrations (Figure 1D).

Diquat + 0.1% of copper hydroxide showed low control in 0.2 and 0.4 mg L^{-1}. In 0.8 mg L^{-1} control was moderate and in 1.2 mg L^{-1} it was satisfactory at 7 DAA. At 14 DAA, control was moderate in 0.2 and 0.4 mg L^{-1} and sufficient in 0.8 and 1.2 mg L^{-1}. From 21 DAA control was satisfactory, with plant death in all concentrations (Figure 1E).

The combination of herbicide and copper sources caused potentiation of the control effect of *C. demersum*, because more control speed occurred with the use of the associations (21 DAA) than for the isolated diquat (30 DAA), but the association of endothall + copper and endothall + diquat (1.0 + 0.5 mg L^{-1}) with exposure of 24 hours controlled from 85 to 100% of *H. verticillata*, only in 45 days (Pennington et al., 2001) and diquat (0.1 mg L^{-1}); diquat + flumioxazin (0.1 + 0.05 mg L^{-1}) and diquat + penoxsulam (0.1 + 0.005 mg L^{-1}) promoted 75% of reduction of dry matter of *C. demersum* after eight hours of exposure, in 60 days, regardless of the association of the herbicides or diquat isolated (Mudge, 2013). In a static system, diquat + copper (0.375 + 1.0 mg L^{-1}) in exposure of 24 to 144 hours caused reduction of the photosynthetic activity between 50 and 60% for *Myriophyllum spicatum*, *H. verticillata*, *Cabomba caroliniana* (Bultemeier et al., 2009).

For Gangstad (1978), the association of diquat + pentahydrate inorganic copper sulfate (1.0 + 0.86 mg L^{-1}) was of 93% at 82 DAA. The herbicide combination with copper was more effective to control submerged macrophytes and has the advantage of inhibiting algae growth (Durborow et al., 2008).

According to Sutton et al. (1972), the association of diquat with copper sources is more effective because of the better absorption of diquat by the plant, with increased permeability provided by copper, similar to that obtained with *C. demersum*.

The reduction in biomass and the length of the pointers at the end of the experimental period was 100% for diquat and its association with 0.1% copper hydroxide and 0.1% copper oxychloride in all concentrations tested (Figure 2A) because there was a total control of the test plant.

Copper hydroxide reduced the biomass and the pointers length in all concentrations tested, whereas the highest reduction average in the pointers length was at the concentration of 0.7 mg L^{-1} with an amount of 7.46% in relation to control, and the highest reduction average of the biomass was in 1.0 mg L^{-1} with an amount of -91.28% in relation to control (Figure 2B). Copper oxychloride only showed a reduction in the biomass of *C. demersum* in the concentration of 1.5 mg L^{-1} with an amount of -52.72% in relation to control (Figure 2B).

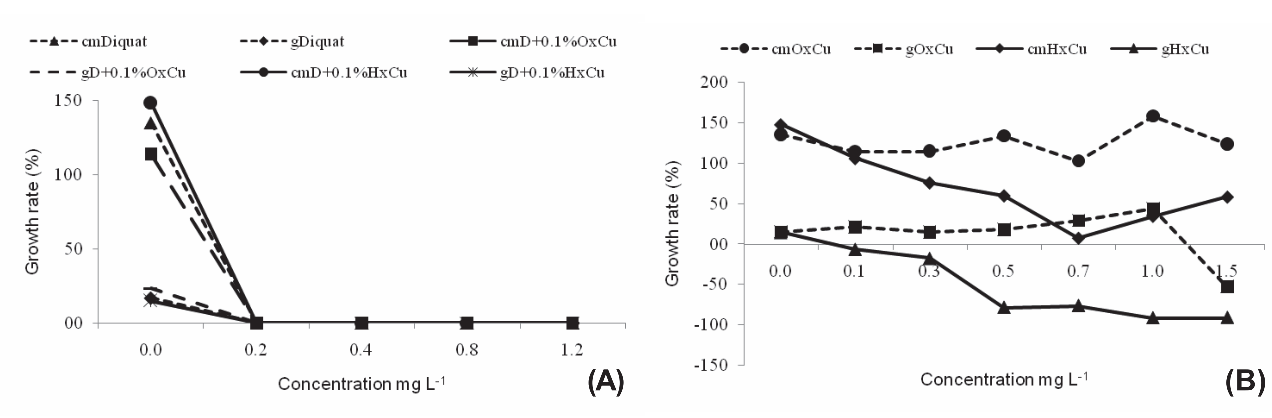

Figure 2 - Length growth percentage (cm) and biomass (g) of the pointers of *Ceratophyllum demersum* exposed to diquat, diquat + 0.1% of copper oxychloride (D + 0.1% OxCu) and diquat + 0.1% of copper hydroxide (D + 0.1% HxCu) (A) and copper hydroxide (HxCu) and copper oxychloride (OxCu) (B) during 45 days.

In a test conducted by Mudge et al. (2010), flumioxazin reduced 50% of the dry matter of *E. najas* with 3.285 mg L^{-1}; for *H. verticillata*, with 0.077 mg L^{-1}, and for *C. demersum*, with 0.034 mg L^{-1}, in waters with neutral pH, during 30 days. For Poovey & Getsinger (2002), the water physicochemical conditions can interfere with the control efficacy of macrophytes submerged by the herbicides.

The equation by Henderson & Tilton (1955) determines the efficacy percentage of chemicals in relation to plant growth in the control and treatments for a given period of time. Diquat and the copper sources were 100% effective in the reduction of the length and biomass of *C. demersum* pointers. Copper hydroxide presented efficacy from 70.21 to 97.23% for the fresh biomass and from 4.69 to 84.06% in length. Copper oxychloride was effective only at the concentration of 1.5 mg L^{-1}, with 63.58% for the fresh biomass. And for length, efficacy was only from 0.33 to 13.73% (Figure 3).

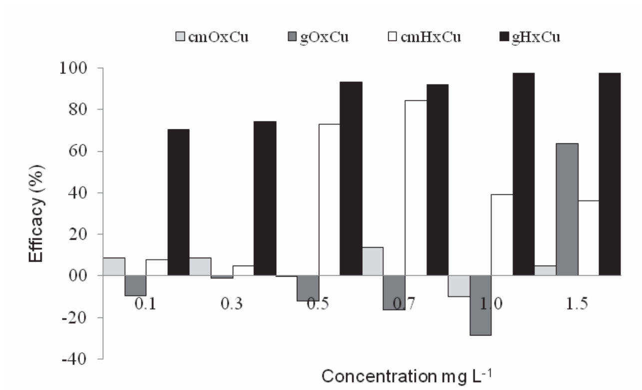

Figure 3 **-** Efficacy percentage of copper oxychloride (OxCu) and of copper hydroxide (HxCu), according to Henderson & Tilton (1955).

For Henares et al. (2011), diquat caused reduction of 71.20 to 78.6% of the biomass of *H. verticilata*, from 42.7 to 52% for *E. densa* and from 18.7 to 34.7% for *E. najas*. The reduction in length was from 89.5 to 93.5% for *H. verticilata*, from 28.8 to 69.6% for *E. densa* and from 28 to 37% for *E. najas*. Tanaka et al. (2002) classified the injury as mild or moderate of *E. densa* exposed to 0.1 mg L^{-1} and of *E. najas* at 0.1 and 1.0 mg L^{-1} of diquat, differing from the control obtained for *C. demersum*, as from 0.2 mg L^{-1} control was total.

Diquat herbicide associated with 0.1% copper oxychloride or copper hydroxide was more effective in controlling *C. demersum*, as there were more speed control or potentiation of the herbicide effect in weed control, which may interfere with the environmental dynamics and availability speed of nutrients for algae or even present an algaecide effect in controlling algae in the environment.

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