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Article

Controlled Release of TBH Herbicide Encapsulated on Ca-ALG Microparticles: Leaching and Phytointoxication Plants

Liberação Controlada do Herbicida TBH Encapsulado em Micropartículas de ALG-Ca: Lixiviação e Fitointoxicação de plantas

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ABSTRACT - The aim of this work was to demonstrate a detailed study of the controlled release of the herbicide Tebuthiuron (TBH) encapsulated in microparticles of calcium alginate (Ca-ALG), to evaluate the phytotoxicity in different concentrations of herbicide (4, 6 and 8 g L^{-1}), and their correlation with the depth of leaching using bioindicator plants. The Ca-ALG microparticles were prepared from the crosslinking of sodium alginate by Ca^{++} containing varied amounts of TBH supplied in calcium chloride ($CaCl_2$) aqueous solution. The results showed that TBH herbicide, when encapsulated, leached to shallow depths relative to the conventional, non-encapsulated herbicide (which moved to a depth of 40-50 cm). The concentration of 4 g L^{-1} was the one that leached most in the PVC columns, but its mobility did not exceed 30 cm of depth. The results of the dry mass corroborated with the phytotoxicity results of the bioindicator plants, evidencing the mobility of the conventional herbicide in the soil at depths around 40 and 50 cm, while the herbicide encapsulated in the Ca-ALG microparticles did not show leaching beyond 20-30 cm deep. The encapsulation of TBH in Ca-ALG microparticles can thus be considered as a more eco-friendly technology, reducing the leaching process and consequently soil contamination.

Keywords: pesticides, biodegradable polymer, environmental impact, encapsulation, agriculture.

RESUMO - O objetivo deste trabalho foi demonstrar um estudo detalhado da liberação controlada do herbicida Tebuthiuron (TBH) encapsulado em micropartículas de alginato de cálcio (Ca-ALG), para avaliar a fitotoxicidade em diferentes concentrações do herbicida (4, 6 e 8 g L^{-1}) e sua correlação com a profundidade de lixiviação usando plantas bioindicadoras. As micropartículas de ALG-Ca foram preparadas a partir da reticulação de alginato de sódio em Ca^{++} contendo quantidades variadas de TBH fornecidas em solução aquosa de cloreto de cálcio ($CaCl_2$). Os resultados mostraram que o herbicida TBH, quando encapsulado, lixiviava a profundidades mais rasas quando comparado ao herbicida convencional não encapsulado (que se deslocou até uma profundidade de 40-50 cm). A concentração de 4 g L^{-1} foi a que mais lixiviou nas colunas de PVC, mas sua mobilidade não ultrapassou 30 cm de profundidade. Os resultados da massa seca corroboraram os resultados de fitotoxicidade das plantas bioindicadoras, evidenciando a mobilidade do herbicida convencional no solo em profundidades em torno de 40 e 50 cm, enquanto o herbicida encapsulado nas micropartículas de ALG-Ca não apresentou lixiviação além de 20-30 cm de

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profundidade. O encapsulamento do herbicida TBH nas micropartículas de ALG-Ca pode ser considerado uma tecnologia mais ecologicamente correta, reduzindo o processo de lixiviação e, consequentemente, a contaminação do solo.

Palavras-chave: pesticidas, polímero biodegradável, impacto ambiental, encapsulamento, agricultura.

INTRODUCTION

Agriculture uses large amounts of pesticides to reduce productivity losses caused by biotic agents. Among the phytosanitary products regularly used in agriculture are herbicides, which are regularly used for weed control. In recent years, especially 2013 was consumed 902.408 tons of commercial product, the total amount of pesticides sold in Brazil increased relative to consumption in 2012 (an increase of 9.6%), corresponding to 367.778 tons of active ingredient (Ferreira and Vegro, 2015) and although it has an important application in agricultural productivity, it is found in worrisome quantities in superficial water bodies and can generate serious environmental impacts (Grillo et al., 2012).

Agricultural pesticides presently used in agriculture have different physicochemical properties, which give them different degrees of environmental persistence, mobility and toxicity potential (Roowell, 1994). The TBH (N- {5- (1,1-dimethylethyl)-1,3,4-thiadiazol-2-il} -n, n'-dimethylurea), an inhibitor of photosystem II, has a long residual effect in the environment and a half-life of approximately 360 days. This herbicide is applied in pre-emergence in the cultivation of sugarcane, and used for control of mono and dicotyledonous species. As its final destination is the soil, according to Fontes et al. (2004), Wang et al. (2008), Tomco et al. (2010) and Faria et al. (2016), it is subject to processes such as leaching, volatilization, photodegradation, chemical and microbiological decomposition, surface runoff.

The two characteristics of the most essential agricultural pesticides related are leaching process and half-life time. Sorption generally determines the availability of an agricultural pesticide in the soil and the half-life reflects the persistence of this pesticide. (Katz and Mishael, 2014). Moreover, the leaching process occurs mainly with molecules of high solubility in water and low Kow (octanol-water partition coefficient). In this sense, the development of new formulations for controlled release of herbicides that are simultaneously effective in controlling weeds, safe for workers and the environment, is essential (Grillo, 2014). Studies on the encapsulation of herbicide molecules in microparticles, using biodegradable polymers aimed at their controlled release, have been the subject of research in recent years (Silva et al., 2010, 2011; Faria et al., 2016). Among those materials, polymeric microparticles (NPs) for the controlled release of pesticides has shown promising results like as verified by Grillo et al. (2012) and Kumar et al. (2014). Alginate (ALG) is a naturally occurring anionic polymer that may be used for the encapsulation of bioactive compounds due to its biocompatibility, low toxicity, low cost and biodegradability (Muzzarelli, 2010). Amongst the various methods developed to prepare alginate microparticles, ionic gelation appears as attractive once the process is relatively easy to control and does not require the use of organic solvents (Agnihotri et al., 2004; Fan et al., 2012).

However, the application of these formulations in the field is almost non-existent in Brazil (Dourado Júnior et al., 2017). Therefore, the overall objective of this work was to demonstrate a detailed study of the controlled release of the herbicide TBH encapsulated in microparticles of Ca-ALG, and to evaluate the phytotoxicity in different concentrations of herbicide and the relation with the depth of leaching, using bioindicator plants. To the best of our knowledge, this is the first study on a controlled release system using the herbicide TBH encapsulated in a biodegradable polymer with great prospects in the agricultural area.

MATERIALS AND METHODS

Obtaining the microparticles of Ca-ALG

All reagents used in the present study were of analytical grade. The sodium alginate (Na-ALG) biopolymer was purchased from Sigma-Aldrich (Brazil), with a molecular weight (M/W) of

100.000 g mol^{-1}, a viscosity of 15-20 cP, and 61% mannuronic acid and 39% guluronic acid. To obtain the Ca-ALG microparticles, calcium chloride ($CaCl_2$) (Sigma-Aldrich), sodium chloride, NaCl (Sigma-Aldrich) and deionized water (Milli-Q system (Millipore)) were also used. For the encapsulation of the TBH herbicide in the alginate microparticles, predetermined amounts of the commercial Combine® 500 SC formulation (4 g L^{-1}, 6 L^{-1} and 8 g L^{-1}) were prepared at 3% (m/v) of alginate solution. The microparticles of Ca-ALG containing the herbicide TBH were obtained by ionotropic gelation, according to the methodology described by Faria et al. (2016). The obtained microparticles were stored in deionized water and underwent a water exchange process for a period of 3 days. This procedure was adopted in order to remove as much NaCl as possible from the Ca-ALG microparticles. After this, the Ca-ALG microparticles were oven-dried at a temperature of 35 °C for a period of 24 h, according to Faria et al. (2016) and Dourado Júnior et al. (2017). Further information regarding the characterizations used in the microparticles of Ca-ALG and the kinetics of release containing the herbicide TBH can be obtained in the work of Faria et al. (2016).

Studies of the controlled release of the herbicide TBH encapsulated in Ca-ALG

After drying, the Ca-ALG microparticles were distributed into PVC columns 50 cm long and 15 cm in diameter. The PVC columns were filled with soil (Red Latosol) and demarcated every 10 cm. The lower edge of the PVC columns was supported to retain the soil with fine screens after collection, and then were transported to a greenhouse. The soil used to fill the PVC columns consisted of 56.5% clay, 6.5% silt, 37% sand and 46.9 g dm^{-3} of organic matter. The experimental design was completely randomized in a factorial scheme, consisting of 5 treatments with and without herbicide, and 5 levels of depth. (5 x 5).

The treatments were constituted by conventional application and microparticles containing the herbicide: 0.0 (control), 500 g L^{-1} of TBH (conventional herbicide), 4 g L^{-1}, 6 g L^{-1} and 8 g L^{-1}] and 5 levels of depth were evaluated in the soil profile (0-10, 10-20, 20-30, 30-40 and 40-50 cm) with three replicates. The conventionally applied herbicide was Combine 500 SC, sprayed on the top of PVC columns containing soil using a CO_2 pressurized knapsack sprayer, equipped with 4 Teejet 1103-BD fan tips, spaced 50 cm apart, at a working pressure of 2.5 bar, which provided a spray volume of 250 L ha^{-1}. At the time of application, the weather conditions were favorable to the operation, with air temperature around 28.6 °C, air humidity of 53.2% and wind speed of 5.6 km h^{-1}. The dried microparticles at different concentrations were distributed on the surface of the PVC columns manually. The amount of microparticles distributed was calculated by the total area (0.27 m^2) of the PVC columns, and calculated for the concentration of herbicide in each microparticle (4 g L^{-1}, 6 g L^{-1} and 8 g L^{-1}), according to the recommendation of the Combine 500 SC package insert, resulting in 12 microparticles for 4 g L^{-1}, 5 microparticles for 6 g L^{-1} and 4 microparticles for 8 g L^{-1}. Subsequently, the soil of the PVC columns was artificially irrigated to accumulate 100 mm of water, and then separated longitudinally in half, using an electric saw. Immediately, the two bioindicator species were sowed (*Cucumis sativus* – cucumber, *Lactuca sativa* – lettuce). These species were chosen because they showed high sensitivity to the herbicide of interest. Eight cucumber seeds and ten lettuce seeds were distributed to each, at a demarcation of 10 cm, in the PVC column.

Evaluation of phytotoxicity in bioindicator plants

Phytotoxicity evaluations of the bioindicator plants sown in the columns were performed at 14, 21, 28 and 35 days after sowing (DAS). As a criterion for the evaluation of plant phytotoxicity (injuries), the scale of grades from 0 to 100% was adopted (SBPD, 1995) where 0 corresponds to "no injury" and 100% is equivalent to "plant death," and these percentage results were related to the concepts of herbicide control efficiency of the Latin American Weed Association - ALAM (1974). To evaluate the dry mass of the cucumber and lettuce, plants were collected at 35 DAS, packaged in paper bags, and dried in a forced circulation oven at a temperature of 72 °C until reaching a constant mass. Subsequently, they were measured in an analytical balance to determine their dry mass.

Results were subject to analysis of variance, and the means of the treatments were compared by the Tukey test, at 5% of probability. The percentage data (%) was transformed into sine arc $\sqrt{x/100}$.

RESULTS AND DISCUSSION

Production and characterization of the Ca-ALG microparticles containing the herbicide TBH

As mentioned in the section of material and methods more information about the process of obtaining the Ca- ALG microparticles containing the herbicide TBH and their respective characterizations can be verified in the work developed by Faria et al. (2016). In the present work, we present only a representative SEM image used for determining the size distribution and morphology of the Ca-ALG microparticles with TBH herbicide as shown in Figure 1 for the 4 L^{-1} concentration. The SEM image shows a Ca-ALG microparticle with spherical shape and rough surface and a diameter of 1.152 and 1.172 mm in the horizontal and vertical directions, respectively. The herbicide TBH is distributed on the surface and at the center of the Ca-ALG microparticle explaining the different release mechanisms as reported by Faria et al. (2016). Understanding these release mechanisms is extremely important for field applications and leaching reduction.

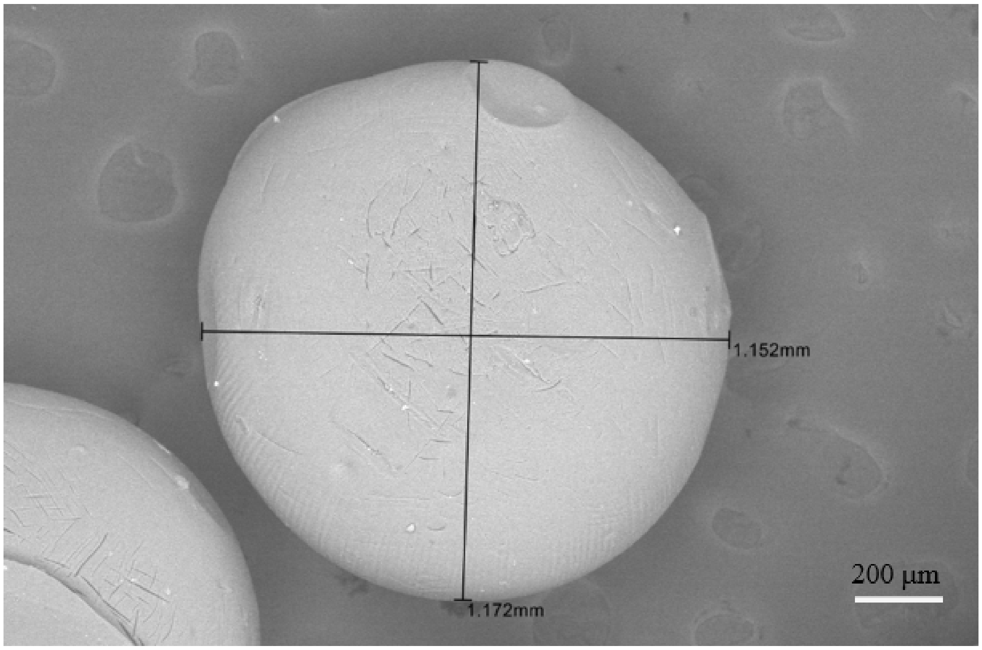

Figure 1 - SEM micrograph of Ca-ALG microparticle for 4 g L^{-1} herbicide concentration.

Kinetics of field release

When the bioindicator species were submitted to the recommended doses of TBH, growth was paralyzed due to chlorophyll photooxidation, which causes foliar chlorosis, followed by ruptures in the cellular cytoplasmic membrane, which was in response to the lipid peroxidation caused by the toxic radicals (triplet chlorophyll and singlet oxygen). Visually, chlorosis symptoms start from the edge of the leaves to the center and progress to the necrosis and death of the plant, as can be observed in Figure 2 for the 5 treatments used in this work.

In Figure 2A,B, the phytotoxicity symptoms are synthesized in the cucumber plants at 35 DAS treated with the herbicide in conventional form and chlorosis (Cl) are observed in the old leaves, later this chlorosis progresses to the total necrosis of the tissues culminating on plant death due to disintegration of cell membranes and lipid peroxidation. Figure 2C,D show the observed

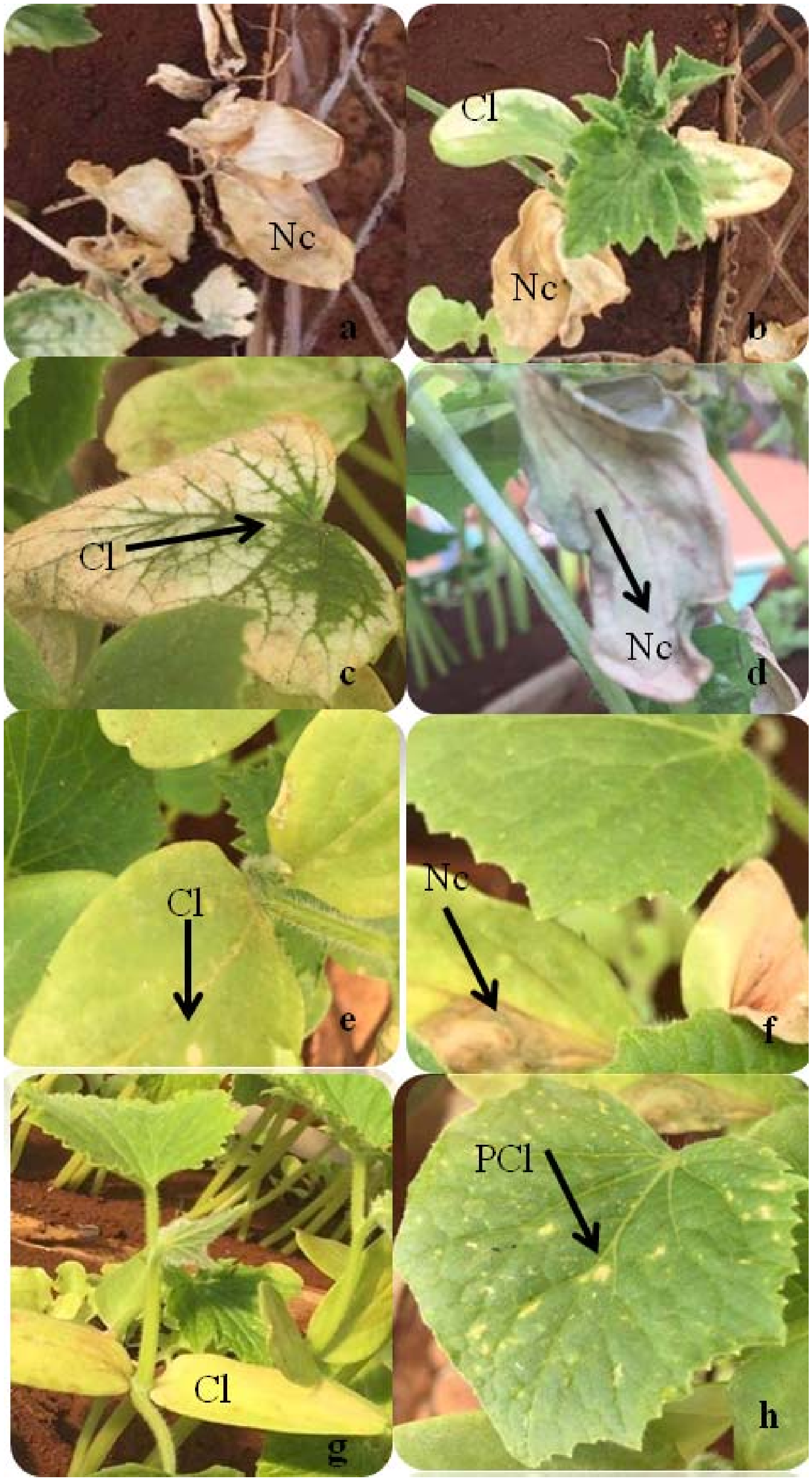

Figure 2 **-** Symptoms caused by the TBH herbicide in bioindicator plants.

symptoms for the 4 g L^{-1} concentration of TBH, which it is clearly notorious that the chlorosis began from the edges to the center of the leaf and that main ribs initially remained green and only later lost their coloration. That the entire sheet is completely necrotic. As regards the concentration of 6 g L^{-1} (Figure 2E,F) the leaves present total chlorosis and onset of necrosis by small scores, due to the rupture of the membranes of organelles and cells, causing extravasation of protoplasm and necrosis. In Figures 2G,H, chlorotic (PCl) scores are evident which spread systematically throughout the leaf, inhibiting the photosynthetic process by paralyzing the growth and causing the plant to die. The symptoms observed in the bioindicators plants used in this study corroborate with Oliveira Jr (2011) when sensitive plants are treated with the herbicide TBH.

Leaching is fundamental for the superficial incorporation of most herbicides, reaching seeds or plants in germination, and that directly interfere in the behavior of the herbicide in the soil, which can make it more or less efficient in the control of weeds. However, the ideal is to only reach the layer where the weed seeds are able to germinate (Inoue et al., 2014).

In the 0-10 cm depth layer, the conventional herbicide presented the highest percentage of phytointoxication of the plants, while the other treatments did not differ. This behavior of the conventional herbicide was maintained throughout the assessments of phytotoxicity, with 91.67 and 98.33%, at 28 and 35 DAS, respectively, indicating the vertical movement of TBH. For the other treatments at this depth, the concentration of 4 g L^{-1}, presented 40% of injuries as can be seen in Table 1.

The highest percentage of phytointoxication at 10-20 cm depth layer was observed for the conventional treatment (with control of 96.66% of the plants). It is important to note that the ALG-Ca microparticles containing the different concentrations of TBH were not sufficient to cause damage of the lettuce plants at that depth. This result shows that the mobility of the encapsulated herbicide is much lower than the herbicide applied in the conventional form (see Table 2). Thus, the chance of contamination of the subsurface layers of the soil is lower. Inoue et al. (2014) studied the leaching process and the degradation of diuron in two soils of contrasting texture. According to the authors, lighter soils with lower organic matter content have higher potential for herbicide leaching.

Table 1 **-** Percentage of phytotoxicity of *Lactuca sativa* (lettuce) plants submitted to treatments in the layer of 0-10 cm depth

Treatment	Days after sowing			
	14	21	28	35
Control	-	-	-	-
Conventional	-	70.00 b	91.67 c	98.33 c
4 g L^{-1}	-	1.67 a	13.33 b	40.00 b
6 g L^{-1}	-	0.00 a	9.00 ab	21.67 a
8 g L^{-1}	-	0.00 a	1.67 a	9.00 a
F Treatment	-	173.70**	272.80**	156.42**
MSD	-	11.94	11.55	14.31
CV%	-	25.48	15.27	12.95
Default Error	-	2.64	2.55	3.16

Averages of the treatments followed by *a*, *b* or *c* in the column differ from each other by the Tukey's test (P≤0.05). The percentage data (%) transformed sine arc $\sqrt{x/100}$. ** significant at 1% probability.

Table 2 **-** Percentage of phytotoxicity of *Lactuca sativa* (lettuce) plants submitted to treatments in the layer of 10-20 cm depth

Treatment	Days after sowing			
	14	21	28	35
Control	-	-	-	-
Conventional	-	73.33 b	91.66 b	96.66 c
4 g L^{-1}	-	0.00 a	8.33 a	10.00 b
6 g L^{-1}	-	0.00 a	4.66 a	7.66 a
8 g L^{-1}	-	0.00 a	0.00 a	7.33 a
F Treatment	-	21.275**	98.80**	509.24**
MSD	-	36.03	19.96	8.87
CV%	-	75.10	29.17	11.15
Default Error	-	7.94	4.40	1.95

Averages of the treatments followed by *a*, *b* or *c* in the column differ from each other by the Tukey's test (P≤0.05). The percentage data (%) transformed sine arc $\sqrt{x/100}$. ** significant at 1% probability.

At 21 DAS, highest percentage of phytointoxication was observed with the conventional treatment, while the other treatments did not present any injury. At the end of the evaluations at this depth, the percentage of phytointoxication was unsatisfactory even for the herbicide applied in the conventional way. Therefore, there was no mobility of the herbicide vertically (Table 3) The low-leaching at this depth may be related to the high organic matter content of the soil used in this study (46.9 g dm^{-3}), and even if the TBH has a high GUS index leaching potential of 5.4 (Gustafson, 1989; Gomes et al., 2001) it may have its mobility restricted in the most superficial layers of the ground, as observed by Gomes et al. (2006), who stated that lower levels of organic matter and clay of the Quartzarenic Neosol (sandy) seem to be the determinant parameters in the higher leaching of TBH in these soils, compared to the Dystrophic Red Latosol (loamy.)

In the 30-40 cm depth layer, the results of percentage of phytotoxicity indicate low percentage of phytointoxication of all treatments, in all the evaluation periods (Table 4). According to

Table 3 **-** Percentage of phytotoxicity of *Lactuca sativa* (lettuce) plants submitted to treatments in the layer of 20-30 cm depth

Treatment	Days after sowing			
	14	21	28	35
Control	-	-	-	-
Conventional	-	36.66 b	36.67 b	48.33 b
4 g L^{-1}	-	0.00 a	0.00 a	0.00 a
6 g L^{-1}	-	0.00 a	0.00 a	0.00 a
8 g L^{-1}	-	0.00 a	0.00 a	0.00 a
F Treatment	-	121.00**	69.14**	11.53**
MSD	-	7.56	9.99	32.27
CV%	-	31.49	41.66	102.06
Default Error	-	1.67	2.21	7.12

Averages of the treatments followed by *a*, *b* or *c* in the column differ from each other by the Tukey's test (P≤0.05). The percentage data (%) transformed sine arc √x/100. ** significant at 1% probability.

Table 4 **-** Percentage of phytotoxicity of *Lactuca sativa* (lettuce) plants submitted to treatments in the layer of 30-40 cm depth

Treatment	Days after sowing			
	14	21	28	35
Control	-	-	-	-
Conventional	-	21.67 b	20.00 a	16.67 b
4 g L^{-1}	-	0.00 a	0.00 a	0.00 a
6 g L^{-1}	-	0.00 a	0.00 a	0.00 a
8 g L^{-1}	-	0.00 a	0.00 a	0.00 a
F Treatment	-	8.89**	3.00^{ns}	5.26*
MSD	-	16.47	26.17	16.47
CV%	-	116.15	200.00	151
Default Error	-	3.64	5.78	3.64

Averages of the treatments followed by *a*, *b* or *c* in the column differ from each other by the Tukey's test (P≤0.05). The percentage data (%) transformed sine arc √x/100. ** significant at 1% probability. * significant at 5% probability. ^{ns} not significant.

Blanco (1979) and Buhler and Forcella (1997), the leaching process will only be effective when it reaches the weed seed bank (about 5 cm in the soil profile). However, when it occurs at greater depths, as in this case, the herbicide is taken to a region in which there is no presence of seeds capable of germinating, resulting in unavailability of the product to produce weed management, and which results in agronomic inefficiency of the molecule, or even contamination of the environment.

At the depth 40-50 cm, there was no significant percentage of phytointoxication for all treatments. Therefore, low leaching of the treatments tested (Table 5). However, in the work of Souza et al. (2008) using a modified drainage lysimeter, it was verified that about 24% of TBH reached 30 cm of depth, while only 5.4% reached 52 cm, in the period of 180 days after the application of 2.4 L ha^{-1} of the herbicide. This fact evidenced that more than 70% of TBH was retained at less than 30 cm depth and/or degraded by factors, mainly biotic environmental factors, since, according to Rodrigues and Almeida (2011), degradation by abiotic factors is insignificant. In addition, the results of this work also confirm those verified by Souza et al. (2008), in which the movement of this herbicide did not exceed 20 and 10 cm depth, respectively, in soils of medium and loamy texture.

According to the results at 0-10 cm depth layer, the highest percentage of phytotoxicity of cucumber plants was observed for treatment 4 g L^{-1} at 14 days, while the conventional and 6 g L^{-1} did not differ from each other. At 21 DAS, the highest percentage of phytointoxication was observed for the conventional treatment, while the other treatments did not differ from each other. At 28 DAS, conventional treatment reached 90%, followed by the treatment 4 g L^{-1}, while the others did not exceed 20% of injury. At 35 DAS, conventional treatment resulted in 98.33% phytotoxicity. For the other treatments at this depth, the best results were presented by the treatment of 4 g L^{-1}, reaching 58.33% of plant phytotoxicity (Table 6). According to the concept of ALAM (1974), the results for the conventional treatment were excellent, and this would efficiently control the weed seeds that remain on the soil surface forming the seed bank, which would be those in the first 20 cm, where 90% of the seeds are found (Monquero and Christoffoleti, 2005). However, only those of the first 5 cm are the target of pre-emergence herbicides (Blanco, 1979; Buhler and Forcella, 1997).

Regarding depths of 10-20 cm at 14 DAS, a significant difference between treatments was observed, and the highest percentage of injuries were caused by conventional treatment, whereas the other treatments did not present phytotoxicity. At 21 DAS, the conventional treatment obtained 70.67% of phytotoxicity; For the other treatments, no differences were observed, and the percentage of phytotoxicity exceeded 9% of phytotoxicity. At 28 DAS, conventional treatment showed 90% de injury. At the end of the evaluations, at 35 DAS, it was observed that the conventional treatment remained the best result. However, the treatment with 4 g L^{-1} presented injury of 70%, indicating

Table 5 **-** Percentage of phytotoxicity of *Lactuca sativa* (lettuce) plants submitted to treatments in the layer of 40-50 cm depth

Treatment	Days after sowing			
	14	21	28	35
Control	-	-	-	-
Conventional	-	5.00 a	6.67 a	8.33 a
4 g L^{-1}	-	0.00 a	0.00 a	0.00 a
6 g L^{-1}	-	0.00 a	0.00 a	0.00 a
8 g L^{-1}	-	0.00 a	0.00 a	0.00 a
F Treatment	-	1.00^{ns}	1.00^{ns}	8.33^{ns}
MSD	-	11.33	15.11	18.89
CV%	-	346.41	346.41	346.41
Default Error	-	2.5	3.34	4.17

Averages of the treatments followed by *a*, *b* or *c* in the column differ from each other by the Tukey's test (P≤0.05). The percentage data (%) transformed sine arc √x/100. ^{ns} not significant.

Table 6 **-** Percentage of phytotoxicity of *Cucumis sativus* (cucumber) plants submitted to treatments in the layer of 0-10 cm depth

Treatment	Days after sowing			
	14	21	28	35
Control	-	-	-	-
Conventional	-	51.67 b	90.00 c	98.33 c
4 g L^{-1}	10.00 a	10.00 a	20.00 b	58.33 b
6 g L^{-1}	15.00 b	12.00 a	15.00 ab	35.00 a
8 g L^{-1}	20.00 c	15.00 a	9.00 a	28.33 a
F Treatment	1.009**	23.53**	325.82**	120.00**
MSD	4.67	18.48	9.53	13.08
CV%	0.00	31.85	10.87	9.09
Default Error	0.00	4.07	2.11	2.89

Averages of the treatments followed by *a*, *b* or *c* in the column differ from each other by the Tukey's test (P≤0.05). The percentage data (%) transformed sine arc √x/100. ** significant at 1% probability.

its potential to control weeds, while also reducing environmental impacts. The other treatments were not efficient because they presented 30%injuries, indicating low, vertical mobility of the herbicide molecule, when encapsulated in Ca-ALG (Table 7). It is noteworthy that the studies with herbicides encapsulated in microparticles, and tested in weeds or bioindicators, with the aim of reducing environmental contamination, are scarce. It is possible to only cite the ones performed by Grillo et al. (2014) and Dourado Júnior et al. (2017), and further related research is needed.

At 20-30 cm, the percentage of phytotoxicity was relatively low, even for conventional treatment. However, the results show the movement of the conventional molecule to undesired depths (Table 8). Although the herbicide TBH presents good solubility in water that facilitates its movement in the soil, the results of this work corroborate with Santana (2012); that is, the movement of TBH cannot be diagnosed, since it depends on the bioindicator species.

Table 7 **-** Percentage of phytotoxicity of *Cucumis sativus* (cucumber) plants submitted to treatments in the layer of 10-20 cm depth

Treatment	Days after sowing			
	14	21	28	35
Control	-	-	-	-
Conventional	-	70.67 b	90.00 c	95.00 c
4 g L^{-1}	0.00 a	8.33 a	30.00 b	70.00 b
6 g L^{-1}	0.00 a	7.33 a	15.00 ab	26.66 a
8 g L^{-1}	0.00 a	1.67 a	0.00 a	13.33 a
F Treatment	16.621**	18.539**	35.571**	59.076**
MSD	9.82	33.94	29.98	22.35
CV%	84.97	59.41	33.95	16.66
Default Error	2.17	7.49	6.62	4.94

Averages of the treatments followed by *a*, *b* or *c* in the column differ from each other by the Tukey's test (P≤0.05). The percentage data (%) transformed sine arc √x/100. ** significant at 1% probability. ^{ns} not significant.

Table 8 **-** Percentage of phytotoxicity of *Cucumis sativus* (cucumber) plants submitted to treatments in the layer of 20-30 cm depth

Treatment	Days after sowing			
	14	21	28	35
Control	-	-	-	-
Conventional	-	31.67 b	48.33 b	68.33 b
4 g L^{-1}	0.00 a	0.00 a	2.33 a	0.00 a
6 g L^{-1}	0.00 a	0.00 a	0.00 a	0.00 a
8 g L^{-1}	0.00 a	0.00 a	0.00 a	0.00 a
F Treatment	1.009^{ns}	19.00**	30.263**	60.036**
MSD	0.00	16.47	19.60	19.99
CV%	0.00	79.47	59.17	44.71
Default Error	0.00	3.64	4.33	4.40

Averages of the treatments followed by *a*, *b* or *c* in the column differ from each other by the Tukey's test (P≤0.05). The percentage data (%) transformed sine arc √x/100. ** significant at 1% probability. ^{ns} not significant.

At the depth of 30-40 cm, the highest phytotoxicity occurred with conventional treatment, while the other treatments did not differ from each other. These results indicate that at this depth, the leaching process for TBH applied in the conventional form still occurs (Table 9). The depth at which the bioindicator presented phytotoxicity with the conventional herbicide corroborates the results obtained by Santana (2012) and Inoue et al (2014) so that all authors point out that in these cases, the risks of groundwater contamination are imminent.

At depth of 40-50 cm there was no difference in the percentage of phytotoxicity for treatments at 14, 21 and 28 DAS. At 35 DAS, the highest percentage of lesions was for conventional treatment, while the others did not present injuries (Table 10). At this depth reach the bioindicator is not desirable, since it shows greater leaching in the soil where weed control is not necessary; That is, part of the product is being lost, resulting in greater environmental contamination and greater economic loss. In this case, TBH alginate encapsulation has shown great potential for leaching reduction, and may even reduce it in areas with sandy soil and with lower organic matter content, which are common in areas of sugarcane production in Brazil, which according to Inoue et al. (2008, 2014), soils with these physico-chemical characteristics are more susceptible to the undesired occurrence of leaching.

Table 9 **-** Percentage of phytotoxicity of *Cucumis sativus* (cucumber) plants submitted to treatments in the layer of 30-40 cm depth

Treatment	Days after sowing			
	14	21	28	35
Control	-	-	-	-
Conventional	-	15.00 b	20.00 b	46.66 b
4 g L^{-1}	0.00 a	0.00 a	0.00 a	0.00 a
6 g L^{-1}	0.00 a	0.00 a	0.00 a	0.00 a
8 g L^{-1}	0.00 a	0.00 a	0.00 a	0.00 a
F Treatment	1.009^{ns}	27.00**	16.00**	21.189**
MSD	0.00	6.55	11.33	22.98
CV%	0.00	66.67	86.60	75.25
Default Error	0.00	1.45	2.5	5.06

Averages of the treatments followed by *a*, *b* or *c* in the column differ from each other by the Tukey's test (P≤0.05). The percentage data (%) transformed sine arc √x/100. ** significant at 1% probability. ^{ns} not significant.

Table 10 **-** Percentage of phytotoxicity of *Cucumis sativus* (cucumber) plants submitted to treatments in the layer of 40-50 cm depth

Treatment	Days after sowing			
	14	21	28	35
Control	-	-	-	-
Conventional	-	8.34 a	8.34 a	35.00 b
4 g L^{-1}	0.00 a	8.34 a	8.34 a	35.00 b
6 g L^{-1}	0.00 a	0.00 a	0.00 a	0.00 a
8 g L^{-1}	0.00 a	0.00 a	0.00 a	0.00 a
F Treatment	1.009^{ns}	0.00 a	0.00 a	0.00 a
MSD	0.00	3.571^{ns}	1.000^{ns}	7.00**
CV%	0.00	9.98	18.89	29.98
Default Error	0.00	183.3	346.41	130.93
		2.20	4.16	6.62

Averages of the treatments followed by *a*, *b* or *c* in the column differ from each other by the Tukey's test (P≤0.05). The percentage data (%) transformed sine arc √x/100. ** significant at 1% probability. ^{ns} not significant.

As for the dry mass of the lettuce plants when comparing the various treatments at each depth (line) in the 10 cm deep layer, the lower dry mass was presented by the conventional treatment and 4 g L^{-1}, and in the layer of 20 cm, the lower dry mass was presented by the conventional treatment. In the other depths, there was no difference between treatments. On the other hand, when comparing each treatment in its different depths (column), for the conventional, the smaller dry masses occurred in the layers of 10 and 20 cm of depth; In the treatments 4 g L^{-1} and 6 g L^{-1}, no differences were observed within the depths studied in each treatment; When the lettuce plants were submitted to 8 g L^{-1}, a lower mass was recorded in the 30, 40 and 50 cm depth layers (Table 11).

The results obtained by dry mass for the cucumber when comparing the different treatments at each depth, verified that at the 10, 20 and 30 cm depth (line), the lowest dry mass was obtained by conventional treatment, and at these 3 depths, which was different from the dry mass presented by the other treatments, including the control treatment. At 40 cm, there was no difference in the dry mass between the treatments. However, at 50 cm, the lower dry mass was obtained by the plants treated with the herbicide in the conventional form and 6 g L^{-1}. However, when comparing each treatment at its various depths (column), the conventional and 4 g L^{-1} presented lower mass in the layers of 10 and 20 cm depth; For the concentration of 6 g L^{-1}, there was no difference between the depths, whereas for 8 g L^{-1}, it occurred in the 30 cm depth layer, presenting the lowest mass (Table 12).

The results of the dry mass corroborated with the phytotoxicity results of the bioindicator plants, evidencing the mobility of the conventional herbicide in the soil at depths around 40 and 50 cm, while the herbicide encapsulated in the Ca-ALG microparticles did not show leaching from 20-30 cm deep. The results presented in this work will allow future implementation of the technique in a large scale, advocating correct and safe TBH recommendations that allow for

Table 11 **-** Dry mass (g) of the *Lactuca sativa* (lettuce) plant submitted to treatments in the layers 0-10, 10-20, 20-30, 30-40 and 40-50 cm

Depth (cm)	Control	Conventional	4 g L^{-1}	6 g L^{-1}	8 g L^{-1}
	(g)				
10	0.18 Aa	0.00 Bb	0.05 Ab	0.14 Aa	0.17 Aa
20	0.09 Bab	0.00 Bc	0.33 Abc	0.07 Aabc	0.12 Aba
30	0.08 Ba	0.04 Aba	0.05 Aa	0.95 Aa	0.07 Ba
40	0.12 Ba	0.09 Aa	0.11 Aa	0.08 Aa	0.08 Ba
50	0.13 Ba	0.99 Aa	0.11 Aa	0.08 Aa	0.09 Ba
Blocks	2	0.00	0.00		
Depth	4	0.05	0.013	3.18	
Treatment	4	0.025	0.01	1.52	
Depth x Treatment	16	0.06	0.00	3.17	
Residue	48	0.00	0.00		
CV%	40.95				

Averages followed by uppercase letters *A* or *B* in the column and *a*, *b* or *c* lowercase in the row differ statiscally from the each other by Tukeys test (P≤0.05).

Table 12 **-** Dry mass (g) 0f the *Cucumis sativus* (cucumber) plant submitted to treatments in the layers 0-10, 10-20, 20-30, 30-40 and 40-50 cm

Depth (cm)	Control	Conventional	4 g L^{-1}	6 g L^{-1}	8 g L^{-1}
	(g)				
10	2.34 Aa	0.20 Bc	0.97 CDb	2.08 Aa	2.26 Aba
20	1.91 Aba	0.23 Bb	0.62 Db	1.76 Aa	2.38 Aa
30	1.60 Bab	1.15 Ab	1.41 BCab	1.91 Aa	1.59 Bab
40	2.02 ABa	1.57 Aa	1.75 Ba	1.85 Aa	1.98 Aba
50	2.17 Bab	1.61 Ab	2.55 Aa	1.78 Ab	2.13 ABab
Blocks	2	0.96	0.48		
Depth	4	13.14	3.28	4.99	
Treatment	4	4.19	1.05	1.59	
Depth x Treatment	16	10.53	0.66	6.60	
Residue	48	4.78	0.10		
CV%	18.87				

Averages followed by uppercase letters *A* or *B* in the column and *a*, *b* or *c* lowercase in the row differ statiscally from the each other by Tukeys test (P≤0.05).

weed control efficiency in different soils, with low environmental impact. It is worth mentioning that the encapsulation of herbicides in microparticles of alginate is highly innovative, and there are still gaps in the literature about its behavior in soil, the environment and its efficiency in weed control. The only works available in the literature are the works conducted by Faria et al. (2016), using the herbicide TBH, and by Grillo et al. (2014) and Dourado Júnior et al. (2017), using the herbicide sulfentrazone and paraquat, respectively. It is important to point out that all the works were very promising in the reduction of leaching along the soil profile, and efficient in the control of weeds, when compared to the conventional way.

The herbicide TBH, when encapsulated in Ca-ALG microparticles, leached shallow depths when compared to conventional herbicide (which shifted to a depth of 40-50 cm). The results obtained in this work demonstrate the potential of the technique of encapsulation of herbicides as a green technology, because leaching losses were smaller when compared to the conventional form of application of TBH and, consequently, will reduce soil contamination.

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