

# VOLATILITY OF DIFFERENT FORMULATIONS OF CLOMAZONE HERBICIDE<sup>1</sup>

## *Volatilidade de Diferentes Formulações do Herbicida Clomazone*

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**ABSTRACT** - Volatilization represents an important process in the displacement of pesticides for the environment. The physicochemical properties of the clomazone molecule indicate its relative volatility. Therefore, this study was carried out to assess the volatilization of different clomazone herbicide formulations using bioindicator species. To that end, airtight glass boxes were used with the presence of different clomazone formulations and plant species. The formulations used were Gamit 360 CS<sup>®</sup>, Gamit 500 EC<sup>®</sup> and Gamit Star<sup>®</sup>. The plant species assessed were maize, sorghum and rice. With the results obtained it is possible to conclude that, among the formulations, Gamit 360 CS<sup>®</sup> has caused less phytotoxicity to the bioindicator species in comparison to the formulations of Gamit 500 EC<sup>®</sup> and Gamit Star<sup>®</sup> formulations. In general, The Gamit 500 EC<sup>®</sup> and Gamit Star<sup>®</sup> have not differed in the phytotoxicity potential for the bioindicator species.

**Keywords:** phytotoxicity, Gamit®, volatilization.

**RESUMO** - A volatilização representa um processo importante no deslocamento de agrotóxicos para o ambiente. As características físico-químicas da molécula do clomazone indicam sua relativa volatilidade. Em vista do exposto, este estudo foi realizado com o objetivo de avaliar a volatilização de diferentes formulações do herbicida clomazone utilizando espécies bioindicadoras. Para isso, foram utilizadas caixas de vidro hermeticamente fechadas, com a presença de diferentes formulações de clomazone e espécies vegetais. As formulações usadas foram Gamit 360 CS<sup>®</sup>, Gamit 500 EC<sup>®</sup> e Gamit Star<sup>®</sup>. As espécies vegetais avaliadas foram milho, sorgo e arroz. Com os resultados obtidos, foi possível concluir que, entre as formulações, o Gamit 360 CS<sup>®</sup> causou menor fitotoxicidade às espécies bioindicadoras, em comparação com as formulações de Gamit 500 EC<sup>®</sup> e Gamit Star<sup>®</sup>. Gamit 500 EC<sup>®</sup> e Gamit Star<sup>®</sup>, em geral, não apresentaram diferença no potencial de fitotoxicidade para as espécies bioindicadoras.

**Palavras-chave:** fitotoxicidade, Gamit®, volatilização.

## INTRODUCTION

Volatilization represents the overall result of all physical and chemical processes by which a compound is transferred from the soil solution and/or the plant surface to the atmosphere (Bedos et al., 2002). Therefore, a pesticide molecule that passes from its original state to the vapor phase can be easily transported to the atmosphere and out of the area treated by air currents.

Once in the atmosphere, the pesticide may be transported over long distances and be

redeposited on the surface (soil, water and plants) by the wind (dry deposition) and/or by wet deposition (rain, dew, snow and fog) (Gavrilescu, 2005), and this herbicide can, in the vapor phase, contaminate the environment and reach non-target organisms. In the environment, pesticides can affect and infect the biota, the water and air quality (vital for human survival), productivity and the final quality of food of animal and plant origin, and may make them unfit for consumption.

Clomazone {2-[(2-chlorophenyl) methyl] - 4,4-dimethyl-3-isoxazolidinone)} is considered

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a pre-herbicide, because it must be activated by the target plant by means of metabolism, which converts the molecule to the 5-keto clomazone form, which is the metabolite with herbicide activity (Tenbrook et al., 2006). It is selective and used in early pre- or post-emergence in the control of various weeds in irrigated rice crops in southern Brazil (Andres & Machado, 2004); it is also used in crops such as soybeans, cassava, sugar cane and cotton. In Brazil, it has already been marketed under the names of Gamit Star® and Gamit 500 EC®, both formulated as an emulsifiable concentrate at a concentration of 800 and 500 grams of clomazone active ingredient per liter, respectively. It is currently marketed under the name of Gamit 360 CS®, formulated as a microencapsulated suspension and concentrated with 360 grams per liter of active ingredient. It belongs to the isoxazolidinones chemical group and indirectly operates in the process of photosynthesis by inhibiting the desoxixilulose phosphate synthase (DXP synthase; 1-Deoxy-D-xylulose 5-phosphate) enzyme, responsible for the synthesis of isoterpenoids, basic precursors of carotenoids (Ferhatoglu et al., 2006), which have the protection function of chlorophyll to photooxidation. The visual symptoms occur in the leaves of sensitive plants, which lose their green color, becoming white, generating a characteristic symptom of this group of herbicides (Senseman, 2007).

The physicochemical characteristics of the molecules of the herbicides, as well as their commercial formulation, interfere in their dynamics in the environment. Gamit® is a nonionic herbicide with water solubility of  $1.1 \text{ g L}^{-1}$ , molecular weight of 239.70, coefficient of partition n-octanol/water ( $K_{ow}$ ) of 350, coefficient of sorption ( $k_{oc}$ ) equal to  $300 \text{ mL g}^{-1}$ , capacity of adsorption ( $K_d$ ) equal to 1 and vapor pressure of  $1.44 \times 10^{-4} \text{ mm Hg}$  at  $25 \text{ }^\circ\text{C}$  (Worthing & Hance, 1991). Thus, due to some of its physical and chemical characteristics, it is possible to observe that it presents considerable vapor pressure (Senseman, 2007) and relative volatility (Rodrigues & Almeida, 2005).

The herbicide formulations currently used are mainly solutions, liquid suspensions, emulsifiable concentrates, wettable powders

and/or fluids. The active ingredient in these formulations is readily available for processes such as runoff, leaching or losses by volatilization. Losses from 9 to 12% of the active ingredient in formulations with dispersible granules and wettable powder and of 4 to 8% of dispersible emulsion and liquid formulations are reported by Wauchope et al. (1990). Formulations with the active ingredient encapsulated, such as Gamit 360 CS®, may provide a new, more favorable alternative to the environment than conventional herbicide formulations, preventing environmental contamination (Kumbar & Aminabhavi, 2002). Encapsulated formulations are particles comprising an inner core containing the active ingredient and a coating membrane, usually polymeric in nature with a variable thickness (Suave et al., 2006). Most of the active ingredient is retained in the formulation inertly in the matrix or near the soil surface, being less subject to leaching, degradation, runoff and volatilization (Vasilakoglou et al., 1997).

A handy technique to report a possible ecosystem contamination problem, because of its low cost and easiness to perform (Nunes & Vidal, 2009), is the use of bioindicators. These are animal or vegetable organisms with the potential to undergo changes in their vital functions or chemical composition (Lam & Gray, 2003), indicating the presence of a certain contaminant in the environment.

In view of the above, this study was carried out to assess the relative volatilization of different formulations of the herbicide clomazone by means of bioindicator species.

## MATERIALS AND METHODS

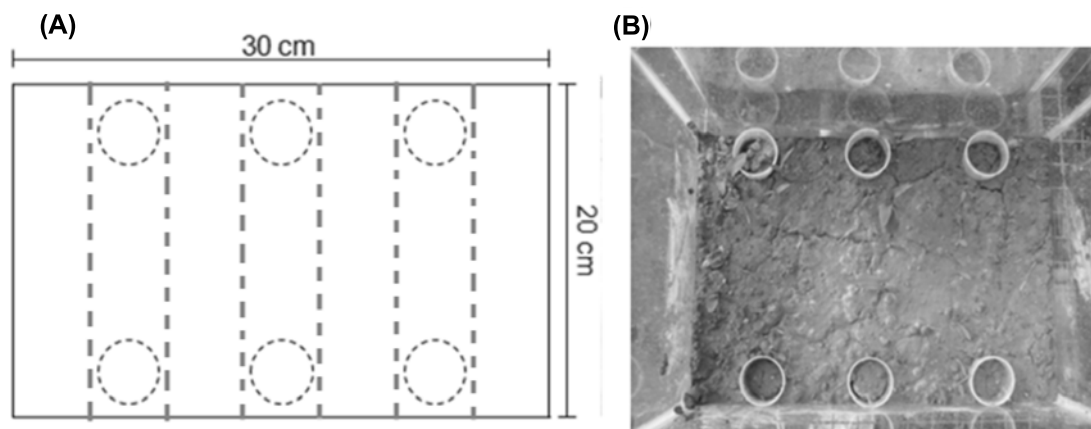
The experiment was conducted in a greenhouse in two periods: October 2011 (season 1) and November 2011 (season 2). The treatments comprised a complete factorial structure (4x3) with a split-plot design and four replications. The main plots were the different formulations of the herbicide clomazone, and the subplots were the plant species. Factor A consisted of the formulations of the herbicide clomazone: Gamit 360 CS® (clomazone  $360 \text{ g i.a. L}^{-1}$ ), Gamit 500 EC® (clomazone  $500 \text{ g i.a. L}^{-1}$ ) e Gamit Star® (clomazone

800 g i.a.L<sup>-1</sup>), besides the control, without the presence of herbicide and factor B by the species of plants: maize, sorghum and rice. The subplots corresponded to the experimental units, which comprised two rows of each species, located within glass boxes with dimensions 30, 20 and 45 cm of length, width and height, respectively (Figures 1 and 2). The boxes were filled with approximately 24 kg of soil previously without clods and sieved, corresponding to 20 cm of height in the box. The soil had no history of herbicide use in the last five years and was collected from the A horizon of a lowland area, being classified as solodic eutrophic Haplic Planosol (Pelotas Mapping Unit).

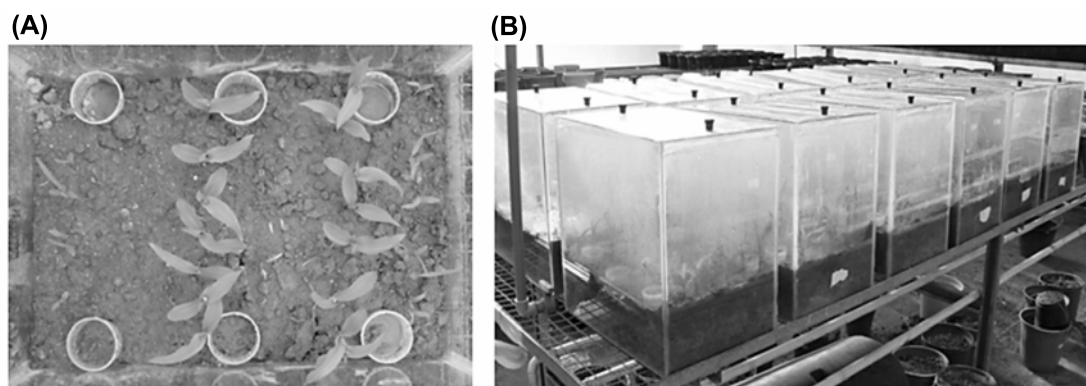
In the choices of the plant species, the work by Schreiber et al. (2013) was taken into account, in which was tested the sensitivity of different species to the herbicide clomazone

in the vapor phase. Each selected species was sown in rows spaced 5 cm, containing ten seeds per row. After plant emergence, one proceeded to thinning, selecting five seedlings per row. The basic fertilization was performed at the time of seeding, using the equivalent of 350 kg ha<sup>-1</sup> of formulation 5-20-20 (N-P-K). The nitrogen fertilization topdressing was applied as urea, using the equivalent of 120 kg ha<sup>-1</sup> of N in stage V<sub>4</sub> of maize.

Seven days after the emergence, six plastic cups of 80 mL with 30 g of soil were evenly distributed within each box, where then the application of different formulations of the herbicide clomazone was made with the aid of graduated pipettes (Figure 1A) in a concentration corresponding to 960 g ha<sup>-1</sup> of active ingredient, that is, in the doses corresponding to 2.7 L ha<sup>-1</sup> of Gamit 360 CS<sup>®</sup>, 1.92 L ha<sup>-1</sup> of Gamit 500 EC<sup>®</sup> and 1.2 L ha<sup>-1</sup> of



**Figure 1** - Provision of the experimental units (represented by the dashed lines) with the plastic cups containing soil for herbicide application (represented by the dashed circles) (A) and top view of the glass box (B).



**Figure 2** - Top view of the experimental units (rows) with the respective allocations of the plastic cups used in the herbicide applications (A) and overview of the experiment (B).

Gamit Star®; each glass box received only one of the formulations. The use of plastic cups aimed to avoid root uptake of the herbicide by the plants and only assess the effect of the volatilization. After application of the herbicide in the cups, the boxes were hermetically sealed in order to avoid losses of herbicide to the environment. After a period of 72 hours of exposure, the boxes were opened and the plastic cups were removed to prevent continued exposure to plants. The average daily temperatures were collected with the aid of a data logger, which was installed in the greenhouse, equipped with calibrated air temperature sensors to perform automatic sampling every 15 minutes during the entire period of the experiments.

The variables analyzed were: phytotoxicity, visually observed in plants at 3, 5, 7, 10, 14, 20 and 24 days after application (DAA) of the herbicide using the percentage scale of zero to 100, where zero represented the absence of symptoms (bleaching) and 100 represented the plant death; and plants fresh dry weight (FW) at 24 DAA. For this, the plant material was harvested and subjected to drying in a forced air circulation oven at 60 °C until constant weight was obtained when weighed.

The data were submitted to normality and homogeneity tests, being transformed into  $\sqrt{Y+0,5}$  when necessary, for not having a normal distribution. Subsequently, they were subjected to analysis of variance ( $P \leq 0.05$ ). When statistical significance was observed, one proceeded to the comparison of the data; for this, the confidence interval of 95% of the formulations under study was calculated.

## RESULTS AND DISCUSSION

The F test of the analysis of variance revealed significant effects ( $P \leq 0.05$ ) among the means of the treatments for plants fresh dry weight and phytotoxicity at 3, 5, 7, 10, 14, 20 and 24 DAA, both on the first and second times of the experiment. Overall, the coefficient of variation found in all variables assessed was low, ranging from 5 to 18%.

Significant effects were observed for factors formulations and plant species, as well as an interaction among them in two periods

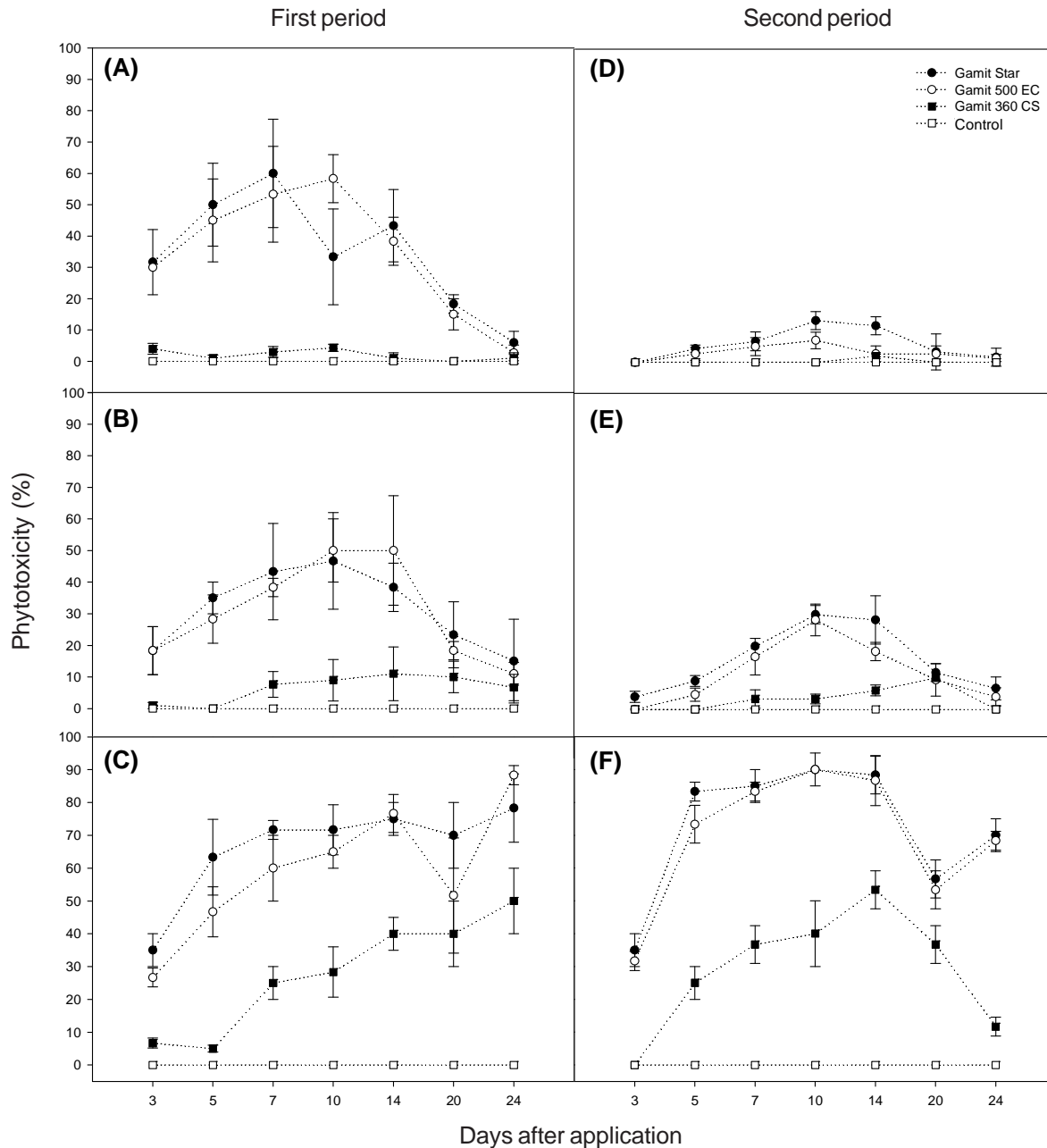
of the experiment; however, to comply with the objective of the work, only the results of phytotoxicity on the basis of clomazone formulations for each species were presented (Figure 3). In the two seasons in which the experiment was conducted, the formulations Gamit 500 EC® and Gamit Star®, generally did not differ significantly in either species, considering the visual effect of phytotoxicity. However, in all species, for Gamit 360 CS® it was possible to observe a significant reduction of phytotoxicity compared to other formulations, probably because it is a microencapsulated product, thus reducing its potential for volatilization.

Overall, phytotoxicity symptoms were observed from 3 DAA. In sorghum, phytotoxicity had a gradual increase during the assessment period. Maize and rice showed potential for recovery of phytotoxicity symptoms from 10 DAA.

The phytotoxic effect for rice and maize was higher during the first time, which can be attributed to lower temperatures observed during the month of October (Figure 4). Low temperatures cause less activation of the herbicide, less volatilization and thus lower the activity of the monooxygenases of the Cytochrome P450 enzymes, which would normally cause low phytotoxicity. However, while there is a decrease of the fluidity of membranes, providing a decrease in the metabolic rate of the plant and impairing its enzyme activity (Murata & Los, 1997), there is a decrease in the herbicide detoxification process, which is mainly based on N-dealkylation reactions followed by glycosidic combination (Elnaggar, 1992).

Significant interaction was observed ( $P \leq 0.05$ ) between the factors studied (species and formulations) for the variable reduction of dry matter of the shoots (Figure 5). Sorghum showed greater reduction of this variable, being higher for formulations of Gamit 500 EC® and Gamit Star®. Rice had little reduction of DM when compared to the control, with a higher reduction in the first period with the Gamit 500 EC®. At the second time there were no differences between the control and the formulations in rice, corroborating phytotoxicity data found.



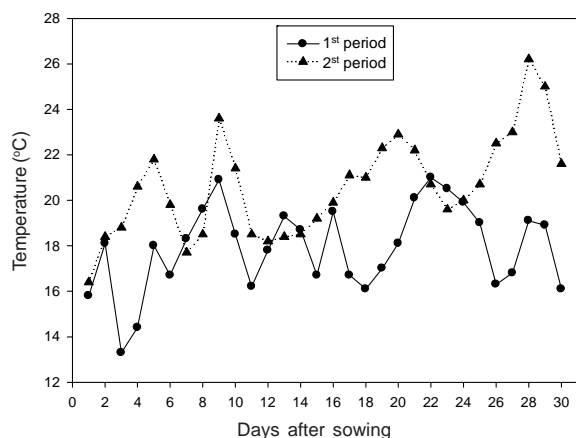


**Figure 3** - Phytotoxicity observed in the 1<sup>st</sup> and 2<sup>nd</sup> period of performance of the experiment in rice (A, D), maize (B, E) and sorghum (C, F) at 3, 5, 7, 10, 14, 20 and 24 days after application, submitted to three formulations of herbicide clomazone (Gamit 360 CS<sup>®</sup>, Gamit 500 EC<sup>®</sup> and Gamit Star<sup>®</sup>) in the vapor phase. The points represent the average values of the repetitions and the bars represent the respective confidence intervals of the average at the level of 95% probability.

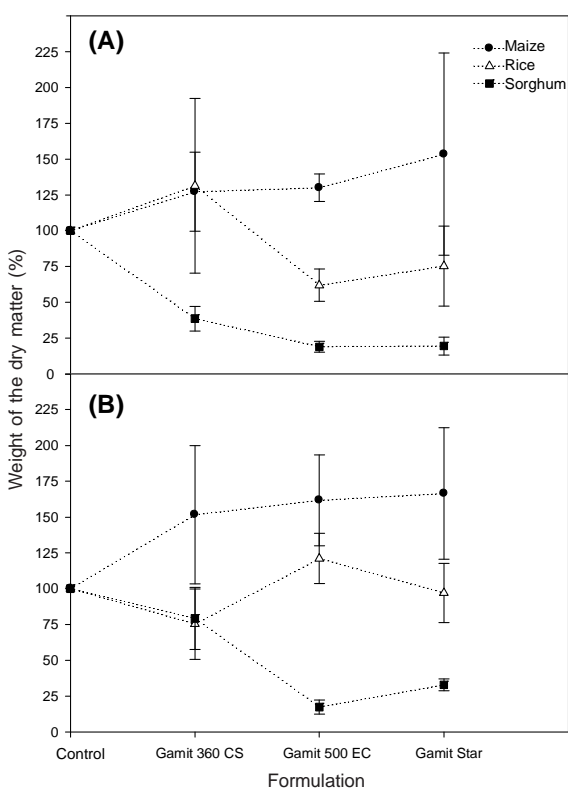
In maize, unlike in sorghum and rice, mass increase of the plants fresh dry weight was observed when compared to control; for formulations Gamit 500 EC<sup>®</sup> and Gamit Star<sup>®</sup> this increase was higher. This occurred probably due to the hormetic effect caused by the herbicide in the plants. Some plant and

animal organisms such as bacteria, fungi, higher plants and animals (Calabrese, 2005), when in contact with toxic substances in low doses, have stimulus in their performance (Calabrese & Baldwin, 2002). This same effect was observed by Wagner (2003) using sub-lethal doses of glyphosate on maize crop.





**Figure 4** - Average daily air temperature (°C) during the period (1<sup>st</sup> and 2<sup>nd</sup> seasons, October and November, respectively) of the experiments, after sowing the species, collected with the aid of a data logger. The points represent the average daily temperature values.



**Figure 5** - Mass percentage of the dry matter of the shoots observed in the 1<sup>st</sup> (A) and 2<sup>nd</sup> (B) seasons of the period of the experiment in relation to the control, of the species of rice, maize and sorghum after 24 days from the application, submitted to three formulations of the herbicide clomazone (Gamit 360 CS<sup>®</sup>, Gamit 500 EC<sup>®</sup> and Gamit Star<sup>®</sup>) in the vapor phase. The points represent the average values of the repetitions, and the bars represent their average confidence intervals of 95% probability.

As conclusions of the study, it is possible to infer that formulation Gamit 360 CS<sup>®</sup> showed lower phytotoxicity to bioindicator species (sorghum, maize and rice) as compared to formulations Gamit 500 EC<sup>®</sup> and Gamit Star<sup>®</sup>. Gamit 500 EC<sup>®</sup> and Gamit Star<sup>®</sup>, in general, showed no difference in the potential of phytotoxicity for the species selected as bioindicators.

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