# Effects of a Commercial Formulation of Cypermethrin used in Biotech Soybean Crops on Growth and Antioxidant Enzymes of Freshwater Algae

María Elena Sáenz[1,2 *], Walter Darío Di Marzio[1,2], Jose Luis Alberdi[2]

[1]National Council of Scientific and Technical Researches CONICET,

[2]Ecotoxicology Research Program, Department of Basic Sciences, National University of Luján, Argentina

mesaenz@speedy.com.ar

***Abstract*- Commercial formulation of pyrethroid insecticide Cypermethrin used in soybean crops was very toxic to four freshwater algae studied causing harmful effects on algal growth and antioxidative defense system level. Recovery from exposure would be seriously affected since Cypermethrin commercial formulation caused algicidal effects. Antioxidant enzymes were significantly stimulated at concentration below LOEC values derived from algal growth inhibition test. The activation of antioxidant enzymes could be used as sensitive biomarkers for early warning of the action of pyrethroid insecticides. This concentration was below measured field concentration of Cypermethrin (0.092 mg Cyp/L) in a stream from a soybean crop area, indicating enzymatic biomarkers could anticipate adverse actions on recovery. Concentrations of commercial formulation which caused algistatic effects on *P. subcapitata* produced an inhibition of CAT and GR activity, showing oxidative stress damage. The use of insecticides in large areas for growing soybeans is discussed, as toxicity to green algae of formulated Cypermethrin is higher than the others two widely used insecticides, Endosulfan and Chlorpyrifos.**

***Keywords*- Cypermethrin; green algae; algistatic; algicidal; biomakers; soybean crops**

## I. INTRODUCTION

The pyrethroid insecticides are synthetic derivatives of natural products from flowers of *Chrysanthemum cinerariaefolium* and are among the most powerful insecticides. Compared with previous pyrethroids, recent products have very low solubility (10 to 80 μg/L) and greater lipophilicity owing to the increase of halogenation in the molecules. Therefore, modern pyrethroids are more persistent products, with greater power and longer residual half-life. But these features also enhance the possibility of harmful effect occurring in aquatic systems ([1], [2], [3]).

In according to the Argentine Crop Protection Association [4], the most used insecticides in agricultural practice are Cypermethrin, Endosulfan and Clorpiryphos**,** with an increased use in year 2008 respect to 2009 from 25 to 30 millions of Kg or Liters. This was mainly due to the increase of areas cultivated with a Glyphosate tolerant transgenic variety of soybean to an extension of fourteen million hectares, where Cypermethrin is the pyretroid most used with 3,5 millions of liters in the year 2009. That has raised concern about the effects of insecticides in the local aquatic ecosystems, as in most cases, aerial applications is preferred due to the vast areas to be treated. In these cases pesticides are spread directly over de aquatic systems which are inside or near the cultivation areas. In addition, pesticides reach aquatic systems by runoff or drift from aerial applications.

There is little information available about the effects of pyrethroids on microorganisms in spite of their ecologic importance. Algal growth inhibition test are often used to assess ecotoxicological effects of environmental pollutants included pesticides. Several authors have pointed out other endpoints that are potential tools for rapid detection of early effects of these pollutants, such us pigments and activity of antioxidative enzyme system ([5], [6]).

Cypermethrin is the active ingredient of several commercial products available in crop protection market. The half- life in field conditions is estimated to be 30 days ([7], [8]).

The motivation of this study was extend the knowledge about the effects of insecticides on microalgae as in most cases, aerial applications is preferred due to the vast areas to be treated. In these cases pesticides are spread directly over de aquatic systems which are inside or near the cultivation areas. In addition, pesticides reach aquatic systems by runoff or drift from aerial applications. All these routes of entry affect growth and metabolism of algal species of impacted ecosystem.

In virtue of the important role of microalgae in aquatic systems as an essential trophic level which provides the basic energy for food webs and their essential function in nutrient cycles, the effects of a Cypermethrin commercial product most used in Argentine was studied. The aim of the present study was the ecotoxicological assessment of this insecticide upon algal growth of four algal species widely distributed in aquatic systems in pampasic region, as well as early detection of effects using enzymatic biomarkers related to oxidative stress. The main contribution of this study is that the activation of antioxidant enzyme occurs earlier than effects on growth, so these biomarkers could be used as indicator of incipient

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changes in aquatic algal populations provoked by this insecticide widely used in Argentine.

## II. MATERIALS AND METHODS

### *A. Plant Material*

The organisms used were the algae Chlorophycean *Scenedesmus quadricauda* (native strain isolated from an upstream non polluted site of Luján river, Province of Buenos Aires. Identification was done according to Phycology Laboratory, Faculty of Natural Sciences and Museum, National University of La Plata), *Scenedesmus acutus* (SAG 273-3a), *Chlorella vulgaris* (Companhia Saneamento Ambiental Estado de São Paulo, Brazil CETESB) and *Pseudokirchneriella subcapitata* (Korschikov) Hindák 1990 (CCAP 278/4), (formerly, *Selenastrum carpicornutum*). Algae were maintained in batch cultures containing 200 ml of mineral growth medium (pH 7.5) [9]. Algal culture of all species were grown under continuous illumination, (86 µE/m2/s) provided by white fluorescent lamps (General Electric F15W/54) at 24°C +/- 1°C on an orbital shaker at 100 rotations per minute (rpm). Cultures were maintained in exponential growth by subculture every week.

Regular test with $K_2Cr_2O_4$ as reference toxicant were done as validity criteria in relation to the sensibility of the clones used.

### *B. Chemical Substances*

The formulation containing Cypermethrin is produced by Chemotecnica, Buenos Aires, Argentina. The formulation contained 10 % Cypermethrin 7 % emulsifier substances and solvent and 83 % of inert compounds not mentioned. Solutions containing commercial products of Cypermethrin were analyzed according to [10]. In brief, insecticide was extracted twice with methanol ([1], [4]) in an ultrasonic bath (Testlab1) for 60 min and then passed through C18 columns (Supelco). The pesticide analysis was performed after elution of the C18 columns with two ml hexane followed by two ml dichloromethane. The extracts were injected into a GC with mass spectrometer QP 5050A and Mass Spectrometry Workstation Class 5000 (Shimadzu GC/MS). Quantification was done using an external standard method purchased from Supelco. Quantification limit was 10 µg/L. All reported Cypermethrin concentrations are expressed as mg Cypermethrin/L.

### *C. Algal Growth Bioassay*

The toxicity of commercial formulation of Cypermethrin was evaluated by algal growth inhibition bioassays of 96 hours using different species mentioned above. Algal culture medium was prepared by adding macro and micronutrient solutions to Milli-Q water, the pH of medium was adjusted to 7.5 (± 1) using 0.1 N NaOH or HCl solutions, as described in [9]. The resulting culture water had an average hardness of 200 mg/L (as $CaCO_3$) and an average alkalinity of 50 mg/L (as $CaCO_3$). The medium was filtered through a 0.45 µm pore diameter membrane in a vacuum. The medium was sterilised by autoclaving (120º, 15 minutes). The protocol for growth inhibition bioassays was based on standard procedures outlines in [9]. Experiments were conducted in 250 ml Erlenmeyer flasks containing 100 ml of sterilized culture media per replicate, with four replicates per concentration and six concentrations (including the control) per experiment. Nutrient medium was supplied with different pesticide concentrations prepared from commercial formulation. The appropriate volume of product was added to each flask from a single stock solution.

Each experiment was initiated using algae from cultures that were in an exponential growth phase and approximately 5-7 days old. Test flasks were inoculated with algal samples that had been concentrated to a density of 10.000cells/ml. The same initial density was used for all species.

Commercial formulation assays were conducted under controlled conditions in a "Forma" incubation orbital shaker at 24°C +/- 1°C, 86 µE/m2/s of continuous "cool-white" fluorescent lighting operating at 100 rotations per minute (rpm) [11]. Flask position on the shaker was randomized daily by treatment.

At the end of the exposure period algal biomass was measured. Dose response curves for each green algal species were obtained from experiments conducted as described above. The endpoint used was algal growth based on total chlorophyll content measured during the incubation period. Samples were removed from the algal cultures and chlorophyll fluorescence were measured on a Turner TD 700 fluorometer (USA) using an excitation wavelength of 420 nm and measuring the emission at 680 nm.

At 96 hours of exposure, the percentage of growth inhibition with respect to the control culture was calculated using algal density as biomass estimation. Percentage of growth inhibition versus concentration values was plotted for each species.

The procedure of [12] was used for the determination of algistatic (inhibition of growth) and algicidal (cell death) effects [13]. After the contact period of 96 hours only cultures with an apparent inhibition of growth were centrifuged (10 min, 2000 *g*) and the algal pellet was suspended in fresh nutrient medium and centrifuged again. Cells were enumerated and then inoculated in sterile nutrient medium free of toxicant to give an initial inoculum of 5 x $10^4$cell/ml. The recovery period was of ten days and was performed in the same incubation conditions of the contact period, but with no toxicant present. Enumeration of cells was done on days 3, 5, 7, and 10 ([12], [14], [13], [11]).

### *D. Enzyme Assays and Enzyme Extraction*

The enzyme activities were studied in algal cultures exposed to Cypermethrin commercial formulation. Algal suspensions of *Scenedesmus quadricauda* and *Pseudokirchneriella subcapitata* were incubated for 48 hours in 100 ml of medium supplied with different pesticide concentration prepared from commercial formulation, under the conditions described above. After 48 hours of exposure, the cultures were collected by filtration through a 0.45 µm pore diameter. Filters were ground two min in one gr of quartz sand with addition of four ml of 25 mM sodium phosphate buffer (pH 7) at 4 ºC. Enzyme extraction was performed by sonication using three cycles of three minutes each to 70 W.

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At the end of this procedure, the enzyme extract was separated from debris by filtration (Whatman GF/C).

*E. Protein Determination*

Protein concentration was evaluated by the method of [15] using bovine serum albumin as a standard.

*F. Enzyme Determinations*

Catalase (CAT) (1.11.1.6) activity was determined spectrophotometrically by measuring the consumption of $H_2O_2$ at 240 nm (ε: 0.036 $mM^{-1}$ $cm^{-}1$) in a reaction medium containing 50 mM sodium phosphate buffer (pH 7.3), 100 mM of $H_2O_2$ and enzymatic extract ([16], [17]).

Glutathione reductase (GR) (EC 1.6.4.2) activity was measured by monitoring the glutathione-dependent oxidation of NADPH at 340 nm (ε: 6.2 $mM^{-1}$ $cm^{-1}$) in the reaction of reduction of oxidized glutathione (GSSG) ([18], [19]). The reaction mixture contained 50 mM sodium phosphate buffer (pH 7.3), 0.5 mM GSSG, 0.2 mM NADPH, 0.5 mM EDTA and enzymatic extract.

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Threshold concentrations including the no observed effect concentration (NOEC) and the lowest observed effect concentration (LOEC) were estimated using traditional hypothesis testing techniques. Normality of data was first assessed using the Shapiro–Wilks test and homogeneity of variance was assessed with the Bartlett's test. The NOEC and LOEC were determined using Dunnett's test, a parametric test for comparing individual treatment concentrations against controls ([20], [21]). A linear interpolation method was used to estimate inhibition concentrations (IC) for algal growth, using ICPIN software (Version 2.0 (US EPA Duluth MN USA).One-way ANOVA was used to assess differences in algal growth and in enzymatic activity in conjunction with Tuckey's multiple range test and Dunnett's test ($p < 0.05$). All statistical analysis was performed using Statistica v. 8 (StatSoft).

Recovery data were processed according to [12], [13] and [22].

## III. RESULTS

*A. Growth Inhibition Bioassays*

When alga Scenedesmus quadricauda was exposed to commercial formulation of Cypermethrine at concentrations of 0.15 mg Cyp/l not significant inhibition of growth was noticed at 96 hours of treatment. On the contrary, growth inhibition was significant in cultures exposed from 0.3 to 5 mg Cyp/l (Fig. 1). The most affected cultures were those exposed to 2.5 and 5 mg Cyp/L of commercial formulation where growth was inhibited by 79 and 94 %, respectively.

Recovery of these cultures was assessed. Growth of the cultures that have been exposed to 2.5 mg Cyp/L returns to normal growth, because at the end of the recovery period the growth rate was similar to control cultures (ANOVA –Dunnett test, $p < 0.05$). On the contrary, growth of the cultures exposed to 5 mg Cyp/L did not recover as controls, causing an algicidal effect on Scenedesmus quadricauda. The algistatic estimated concentration of commercial formulation was 1.54 mg Cyp/L.

Cultures of Scenedesmus acutus were severely affected after the exposure to 0.6 mg Cyp/L and above. No inhibitory effects of Cypermethrin commercial formulation was observed on cellular growth treated to 0.3 mg Cyp/L (Fig. 1). A total inhibition of growth was observed in algal cultures exposed to highest concentrations, i.e. 5 and 10 mg Cyp/L. At the end of the contact period of 96 hours the growth parameters were significantly different from controls. The two highest concentrations strongly inhibited algal growth, so these cultures were suspended in nutrient medium in the absence of toxic to assess their resilience. The cultures exposed to 5 mg Cyp/L recovered from the action of the insecticide since growth parameters at the end of the recovery period were not significant different from the control (ANOVA - Test de Dunnett, $p < 0.05$). Cultures exposed to 10 mg Cyp/L did not recover, showing that the insecticide exerted irreversible harmful effect, avoiding the recovery of growth even in the absence of insecticide. The estimated algistatic concentration of commercial formulation to Scenedesmus acutus was 4.09 mg Cyp/L. The results presented in Figure 1 showed that Cypermethrin commercial formulation between 0.15 and 2.5 mg Cyp/L inhibited growth of Chlorella vulgaris (ANOVA - Test de Dunnett, $p < 0.05$). Concentrations of 0.07 mg Cyp/L did not affect algal growth at the end of the contact period. The more significant inhibition was recorded at 1.2 and 2.5 mg Cyp/L. Recovery experiments of these cultures indicated that cultures exposed to 1.2 mg Cyp/L developed an exponential growth as the control while the cultures exposed to 2.5 mg Cyp/L did not show any recovery. Commercial formulation exerted algicidal effects at 2.5 mg Cyp/L, while algistatic effect was estimated to be expected at a concentration of 0.71 mg Cyp/L.

Exposure to 0.075 mg Cyp/L did not inhibit culture growth of Pseudokirchneriella subcapitata. Concentrations higher than 0.15 mg Cyp/L produced a significant inhibition of growth respect to the control cultures. At 1.2 mg Cyp/L an almost total inhibition of growth was observed (Fig. 1). The growth parameters of cultures exposed to 0.6 and 1.2 mg Cyp/L at the end of the contact period were significantly different from the controls (ANOVA - Test de Dunnett, $p < 0.05$). The algistatic response was confirmed in cultures exposed to 0.6 mg Cyp/L, since growth parameters at the end of the recovery period did not differ from those of controls (ANOVA - Test de Dunnett, $p < 0.05$). In the case of the higher concentration (1.2 mg Cyp/L) the apparent algicidal response observed in the contact period was confirmed since growth was not recorded. Insecticide was algicidal at 1.2 mg Cyp/L, the algistatic response was estimated to be expected at 0.31 mg Cyp/L.

Inhibitory concentrations (IC) of growth of four green algae species tested are shown is Table 1. There was a significant difference between IC50 from different species.

The most sensitive specie was Pseudokirchneriella subcapitata. Chlorella vulgaris showed a similar sensitivity. Scenedesmus acutus was the least, while Scenedesmus quadricauda presented a median sensitivity. Similar results

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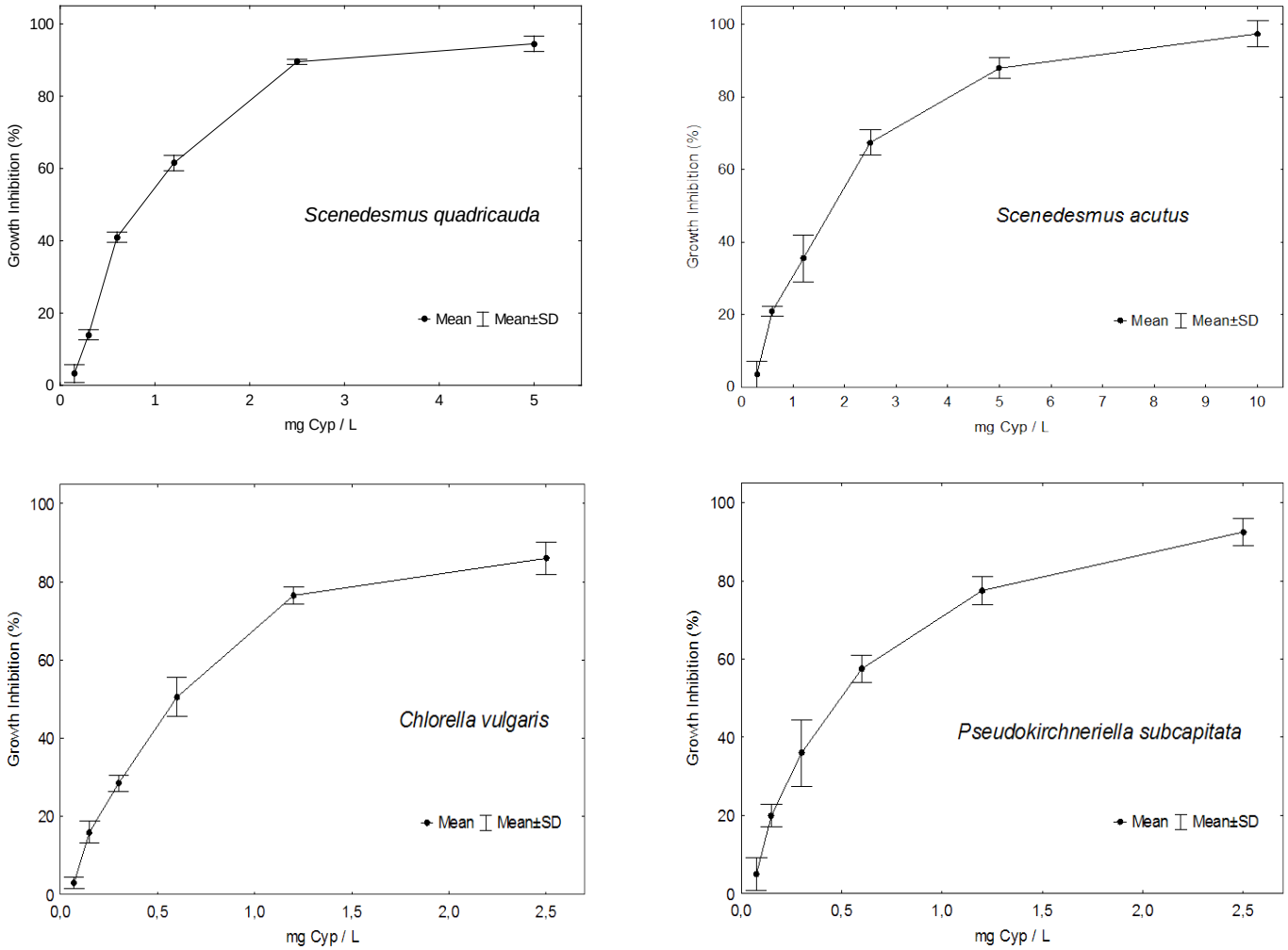


Fig. 1 Growth inhibition of different species after a 96 hours exposure to Cypermethrin commercial formulation expressed in percentage (%) from control

were obtained taken in account the NOEC, LOEC values from different species (Table 1). The NOEC and LOEC values of four species were between 0.075 and 0.3 and 0.15 and 0.6 mg Cyp/l, respectively (Table 1).

TABLE 1.
TOXICITY OF CYPERMETHRIN COMMERCIAL FORMULATION TO FOUR ALGAL SPECIES AFTER A 96 HOURS TREATMENT (MG/L)

| Species | $IC_{25}$ (95% CI) | $IC_{50}$ (95%CI) | NOEC | LOEC |
|---|---|---|---|---|
| S.quad. | 0.23 (0.20-0.27) | 0.48 (0.47-0,50) | 0.15 | 0.3 |
| S. acutus | 0.51 (0.46-0.56) | 0.94 (0.88-1.02) | 0.3 | 0.6 |
| C.vulgaris | 0.10 (0.09-0.12) | 0.23 (0.20-0.26) | 0.075 | 0.15 |
| P.sub. | 0.10 (0.09-0.11) | 0.20 (0.18-0.22) | 0.075 | 0.15 |

The IC25% values showed the same trend. The most sensitive were Pseudokirchneriella subcapitata and Chlorella vulgaris with IC 25 % value of 0.10 mg Cyp/L (95 % CI 0.09-0.011 mg Cyp/L) while the least was Scenedesmus acutus with IC 25 % value of 0.51 mg Cyp/L (95 % CI 0.46-0.56 mg Cyp/L) and a moderate value corresponded to Scenedesmus quadricauda with 0.23mg Cyp/L (95 % CI 0.20-0.27 mg Cyp/L) (Table 1).

Microscopically examinations at effect-level concentrations of cells of Pseudokirchneriella subcapitata and Chlorella vulgaris revealed bloated and colourless or light green cells compared to controls. Cells of Scenedesmus species did not show any cellular abnormalities or colour compared to controls.

### *B. Enzyme Activities*

The activities of antioxidant enzyme CAT and GR of both species exposed to Cypermethrine commercial formulation are shown in Fig. 2 and 3. In the case of Scenedesmus quadricauda cultures it was clear that the CAT and GR activities were stimulated by different concentrations of commercial product. The enzyme activities of all treated cultures showed a significant increase ($p < 0.05$) relative to controls (Fig 2). In the presence of 0.15 mg Cyp/L GR activity was 31 % higher than that of the control.

At higher concentrations increased activity was greater; 0.3 mg/ Cyp/L stimulated the activity by 64 % from control while 0.6 mg Cyp/L stimulated to 73 %. These two last concentrations modified the enzyme activity in a similar manner, as there were not significant differences between

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them (Tuckey $p < 0.05$). The CAT activities of all treated cultures were higher than those of the control, regardless the concentrations of Cypermethrin commercial product. At the three exposure concentrations the stimulation of activity was 37, 62, 87 %, respectively (Fig. 2).

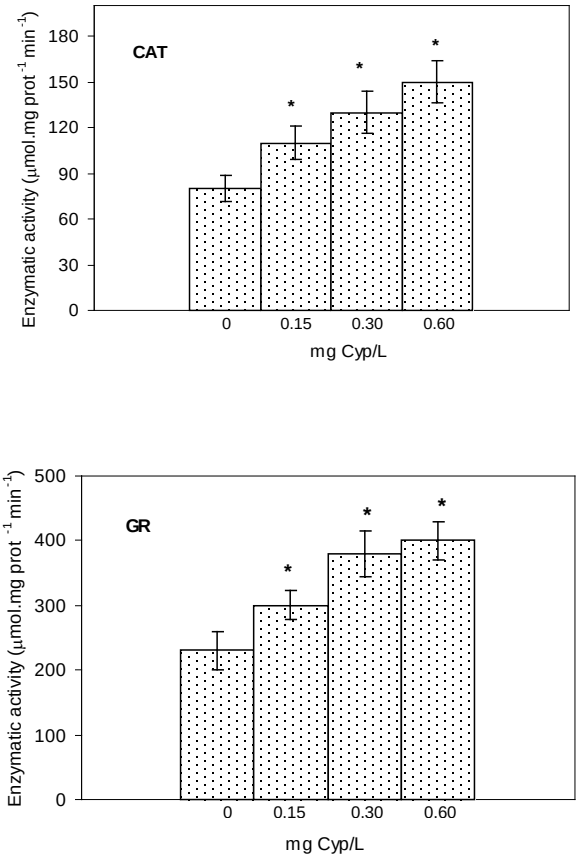


Fig. 2 Effect of Cypermethrin commercial formulation on antioxidative enzyme activities of *Scenedesmus quadricauda* after a 48 hours exposure (* $p < 0.05$)

As shown in Fig. 3, cultures of Pseudokirchneriella subcapitata treated with Cypermethrine commercial product resulted in a significant increase ($p < 0.05$) of both GR and CAT activity in 0.075 and 0.15 mg Cyp/L treatments compared with the controls. On the contrary, at the highest concentration treatment of 0.30 mg Cyp/L both enzyme activities were significantly lower than that of the control, resulting in an inhibition effect of the activities of 28 % (GR) and 20 % (CAT). It is worth noting that action on the activity of both enzymes was significantly different from control at concentrations of 0.15 mg Cyp/L for Scenedesmus quadricauda and 0.075 mg Cyp/L for P subcapitata after 48 hours of exposure. These concentrations are lower to LOEC values derived from algal growth inhibition test of 96 hours of exposure (Table1); therefore effects would be detected through alterations in enzyme activity, prior and at lower exposure concentrations to those affecting algal growth, resulting in more sensitive parameter. In this sense the activation of antioxidant enzymes could be used as sensitive biomarkers for early warning of the action of pyrethroid insecticides.

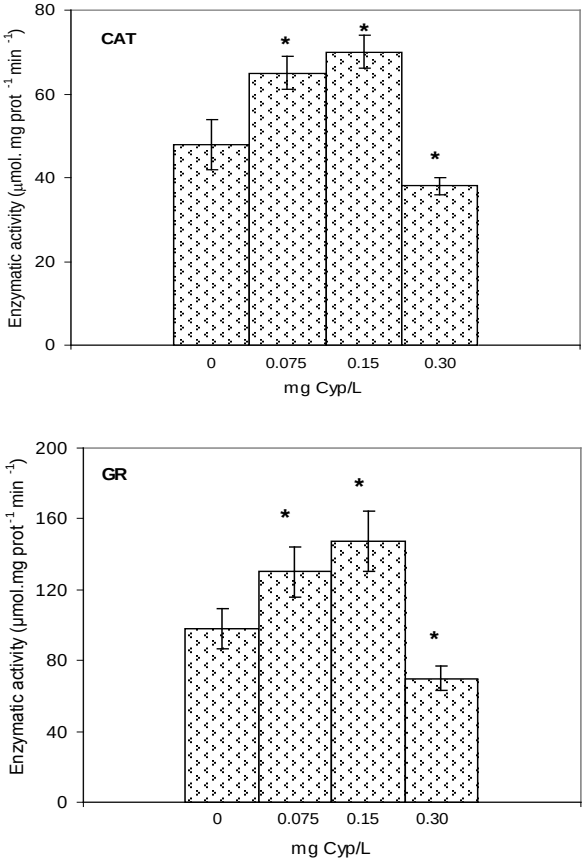


Fig. 3 Effect of Cypermethrin commercial formulation on antioxidative enzyme activities of *Pseudokirchneriella subcapitata* after a 48 hours exposure (* $p < 0.05$)

## IV. DISCUSSION

Studies about the effects of formulations of Cypermethrin and Fenvalerate on algal growth on several species of Cyanophyta and Chlorophyta indicate EC 50 between 5 and 10 mg/L for both pyrethroids [23].

Recovery from exposure would be seriously affected also since Cypermethrin commercial formulation caused algicidal effects. This is an important matter to consider since algal population would not be able to recover after a short exposition to the commercial formulation. Estimated 96 hours IC50 were between 0.20 and 0.94 mg Cyp/L. Toxicity endpoints related to chronic effects were between 0.075 and 0.6 mg Cyp/L. The most sensitive species was Pseudokirchneriella subcapitata. The native strain isolated from Luján river water samples was moderately affected in comparison to the others algae species from Cultures Collections. In this study commercial formulation of Cypermethrin was toxic to algae species, with 96 hours toxicity endpoints lower than those indicated in [23]. The present results would indicate that the commercial formulation of pyrethroid insecticide Cypermethrin would cause harmful effects towards planktonic algae, affecting algal growth when exposed to the insecticide.

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Commercial formulation of insecticide Cypermethrin exerted algicidal effects on Scenedesmus quadricauda, Scenedesmus acutus, Chorella vulgaris and Pseudokirchnerilla subcapitata at concentrations between 1.2 and 10 mg Cyp/L.

Algistatic effects would be expected at concentrations between 0.31 and 4.09 mg Cyp/L. A differential sensitivity was observed between green algae species since the concentrations of commercial formulation that provoked algistatic and algicidal effects on Chlorella vulgaris (algistatic: 0.72 mg Cyp/L; algicidal: 2.5 mg Cyp/L) and Pseudokirchneriella subcapitata (algistatic: 0.36 mg Cyp/L; algicidal: 1.2 mg Cyp/L) were lower than those corresponding to species of genus Scenedesmus: S. quadricauda (algistatic: 2.45 mg Cyp/L; algicidal: 5 mg Cyp/L) and S. acutus (algistatic: 1.34 mg Cyp/L; algicidal: 10 mg Cyp/L). This fact could be explained regarding the different cell wall structure, as species of genus Scenedesmus have a complex cell wall composed of several layers with a particular arrangement and composed of individual chemicals, such as sporopollenine [24].

These ultra structure characteristics confer greater resistance to the coenobium of Scenedesmus, respect to the other two species, which possesses only a cellulose wall as an external covering making them more vulnerable [25].

Assessments on algal growth represent an overall indicator of toxic effects, which integrates different effects of Cypermethrine on different cellular metabolic processes. Especially antioxidative enzyme let see effects at cellular level related to defense mechanisms against active oxygen species, before they are translated in growth. In this study, we found that antioxidative enzymes activities were stimulated by the insecticide at lower exposure concentrations than those found for growth. This fact has also been reported by [6], as they found a 96 hours EC 50 value of 112 mg/L (growth) for Cypermethrin technical grade, but a stimulation of Superoxide dismutase (SOD) activity at a lower concentration of 50 mg/L. [5] reported that fludioxonil induced strong antioxidative activities associated with cytosol enzymes, as catalase, ascorbate peroxidase and glutathione S-transferase and also agreed with the conclusions that enzymatic activities was a sensitive biomarker for early warning of effects.

The inhibition of CAT and GR enzymes at Cypermetrine concentration of 0.3 mg/L observed for P. subcapitata, could be an effect on molecular disruption of the enzyme. Such concentrations of cypermethrin (below IC 50 % value) would cause oxidation of the membranes and the imbalance of enzymatic molecular structure, resulting in a decrease in activity. Faced with these concentrations of insecticide the antioxidant mechanism would be surpassed and would not be effective in protecting the cells from the destructive action of free radicals responsible of cellular oxidative stress. This will take cells to death as observed in studies of recovery.

In natural environments zooplankton communities are seriously affected by pyrethroids insecticides. The recorded effects upon green algae would affect also autotrophic communities with the additional impossibility of recovery from such exposures. This impact on algal community would affect the food chain and the whole aquatic system.

On the other hand, studies on a freshwater community similar to these resulting from field agricultural applications, have demonstrated that concentrations ranging from 0.01 to 6 μg Cyp/L caused harmful effects on crustacean zooplankton. The observed alterations in species composition of autotrophic communities such as periphyton and phytoplankton at such concentrations were indirectly caused by the effects of Cypermethrin on the crustacean grazers ([26], [27], [28]). In the present study commercial formulation of Cypermethrin cause significant effects on algae species at 0.075 mg Cyp/L (enzymatic biomarker) and 0.15 mg Cyp/L (algal growth). According to a study conducting in a stream running through an area of soybean cultivation, Cypermethrin concentration measured in a run off sample were pesticide had been applied was of 0.092 mg Cyp/L [29]. Therefore, in the presence of such pesticide concentrations determined under field conditions, we would expect to find effects on antioxidant enzymes, which would also be used as biomarkers for early impact of adverse and worst actions that would be expected at greater concentrations that would cause impact on population growth and recovery of algal populations. The problem related to recovery of algal community from such exposure would affect the whole freshwater ecosystem.

Green algae species are largely distributed in freshwater phytoplankton community, so they are a suitable model for studies regarding insecticides and herbicides impacts on natural freshwater environments in the pampasic region in relation to the increase in areas for the cultivation of soybeans. The use of other antioxidative enzyme as peroxidase (PX), superoxide dismutase (SOD), glutathione S-transferase (GST), lipid peroxidation together with enzymes studied here, will be of interest as enzymatic biomarkers to be used in ecotoxicological environmental risk assessment. Inhibition of pigment content, chlorophyll fluorescence emission and inhibition of photosynthesis rate also could be considered as biomarkers of harmful effects and detection of the presence of herbicides (Atrazine, Diuron, Paraquat, Terbutryn, flazasulfuron) in aquatic environments. These biomarkers have been pointed out as useful ecotoxicological tools in several studies ([30], [11], [31], [32], [6], [33]).

Degradation products of pyrethroids have been documented as more toxic to bacteria, fungi and algae. The Permethryn EC50 values on algal growth of Cyanophyta of genus Anabaena and some species of Chlorophyta as Chlorella pyrenoidosa were greater than 10 mg/L. However the EC50s for degradation products were between 1.4 and 8 mg/L [34]. Similar results were found regarding the effects on photosynthesis process, with EC50 values greater than 100 mg/L for Permethryn and between 30 and 70 mg/L for degradation products [34]. If together with the effects described here would join those of the degradation products of greater toxicity, the environmental reality from the use of pyrethroid insecticides would be even more risky. Regarding the use of insecticides in large areas for growing soybeans, it is important to note that the toxicity to green algae of formulated Cypermethrin would be higher compared to the others two widely used insecticides, Endosulfan and Chlorpyrifos, as IC 50 96-hour algal growth are among, 1.6 – 6.3 and 1.2 -1.4 mg/L, respectively. ([35], [36]).

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It is worth to mention that it is very important to have information about the ecotoxicity of insecticides towards algae in order to explore combined effects with others agrochemicals products such us herbicides and fungicides also used in soybean crops. Although these combined effects are seldom studied, they reflect a more realistic situation, yet many aquatic ecosystems are in the vicinity or in the middle of agricultural land, they receive all the toxicological pressure being contaminated with a mixture of pesticides. Extending the studies related to detection of early effects to others primary producers such as aquatic floating and rooted macrophytes, will expand the knowledge of the environmental reality enabling the establishment of guidelines or activities for the protection of water resources in areas where cultivation of soybeans take place [33]. Secondary effects are to be considered also that may be as damaging to the ecosystem as direct effects. Due to the large amount of agrochemicals used in the pampasic region related to soybean production, effects on the whole ecosystems must be studied to understand the various risk scenarios resulting from the use of agrochemicals ([37], [10], [33])

## V. CONCLUSION

We conclude that commercial formulation used in soybean crops was very toxic to four algae studied causing algistatic and algicidal effects. Enzymatic biomarkers were also stimulated at concentration below to those that affected algae growth, indicating a more sensitive endpoint. These concentrations were below measured field concentration of Cypermethrin, indicating an important biomarker for ecotoxicological risk assessment. Algistatic concentration of P subcapitata produced an inhibition of CAT and GR activity, showing oxidative stress damage.

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