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Effects of a pyraclostrobin-based fungicide in plant and green microalgae models

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Abstract

Pyraclostrobin-based fungicides play an effective role in the control of fungal diseases and are extensively used in the agricultural sector. However, there is growing concern regarding the potential effects of these fungicides on nontarget organisms and the influence they exert on ecosystem functioning. Given this concern, it is essential to conduct comprehensive studies with model organisms to understand the impacts of these fungicides on different groups of living organisms. In this study, the ecotoxicity of a commercial fungicide containing pyraclostrobin was evaluated. The focus of the analysis was on the germination and initial development of seedlings of four plant models (*Lactuca sativa*, *Raphanus sativus, Pennisetum glaucum* and *Triticum aestivum*), in addition to evaluating the population growth rate and total carbohydrate content in the microalga *Raphidocelis subcapitata*. The fungicide negatively influenced the growth and development of the tested plants, indicating its toxic effect. The fungicide had a significant impact on the initial development of seedlings of all the model species evaluated, and *T. aestivum* plants exhibited the greatest susceptibility to pyraclostrobin. Plants of this species exhibited inhibitory effects on both the aerial parts and roots when treated with at a concentration of 4.75 mg/L. In addition, the green microalga *R. subcapitata* was also significantly affected by the fungicide, especially at relatively high concentrations, which resulted in a reduction in the total carbohydrate content. The pyraclostrobin-based fungicide showed phytotoxic potential for the model plant species tested in this study and was shown to be a highly toxic contaminant for the aquatic environment.

1 INTRODUCTION

Pesticides are chemical products widely used to control pests in large plantations and small gardens. Although they are useful in crop protection, the excessive and inappropriate use of pesticides can have negative impacts on the environment and human health. One of the problems associated with the large-scale use of these products is the contamination of soil, water and air due to leaching from agricultural areas where they are applied and being carried into rivers, lakes and groundwater (Ribeiro et al. 2007) .

Pesticides are classified according to their chemical composition, mode of action and purpose (Hassaan and El Nemr 2020). The most common classification approach is to group them by target organism into insecticides, fungicides, herbicides, nematicides and acaricides, which are used to control insects, fungi, weeds, nematodes and mites, respectively. Fungicides can be divided into two chemical groups, triazole and strobilurin. Triazole fungicides act by inhibiting the synthesis of ergosterol (a component of the fungal cell membrane), and strobilurin fungicides act as inhibitors of electron transport in the mitochondria of fungal cells, thus inhibiting the synthesis of adenosine triphosphate (ATP), which is essential for metabolic processes in cells (Wang et al. 2020; Zhang et al. 2020).

Pyraclostrobin is an active ingredient of strobilurin fungicides present in commercial products widely used in agriculture. In 2014, pyraclostrobin-based fungicides were ranked as the second best sellers on the market (Mao et al. 2020). They accounted for 20% of sales in the global market (Zhang et al. 2020)

Currently, pyraclostrobin-based fungicides can be applied to more than fifty different crops, such as cotton, garlic, coffee, sugarcane, corn, radish, soybeans, and wheat. Although these fungicides are recognized as effective, there is great concern about their effects on nontarget flora and fauna and ecosystem functioning. Studies have been conducted to evaluate the presence of pyraclostrobin in water bodies, such as rivers and lakes, and its presence has been reported at varying concentrations. Mao et al. (2020) reported that pyraclostrobin was detected in different surface waters, such as rice fields and rainwater basins, at concentrations ranging from 0.1 to 17.3 μg/L. Several studies with plant models have shown that pyraclostrobin stimulates development (length of the root and shoot) in soybean plants and increases the levels of chlorophyll and root activity (Li et al. 2020). Therefore, there is a need for studies with model organisms to understand their effects on different groups of living organisms.

In this context, ecotoxicity tests, which can be used to reveal the effects of chemical agents on an organism, with model organisms where a particular *endpoint* is evaluated are essential tools for evaluating the risks of environmental pollution by contaminants and protecting biota. They can also be used to determine the effects of chemical agents on population diversity and communities of an ecosystem (Clements and Rohr 2009). Several standardized tests and protocols based on international standards can be applied to evaluate ecotoxicological risks, and plant models (Palmieri et al. 2014) and microalgae, such as *Raphidocelis subcapitata*, which is widely used for this purpose (Franklin et al. 2007), are specimens of easy access and cultivation that produce fast and reliable responses to tests. Thus, in a hierarchical context, the use of models that represent the base of the aquatic food chain, such as green microalgae and terrestrial microalgae, is important for predicting the toxicity of a given compound to the ecosystem.

Green microalgae can be used as models of aquatic photosynthetic producers of great ecological importance; in addition to sustaining the trophic chain in these environments, they are great producers of organic carbon and are responsible for the release of oxygen (Martínez-Ruiz and Martínez-Jerónimo 2015). In addition to presenting toxicological responses, microalgae are examples of plants in the biotechnological context and natural sources of a variety of biomolecular compounds of commercial interest, as well as proteins, carbohydrates (high potential for bioethanol production) (Harun et al. 2010a, b) and carotenoids. Plants are producers within the terrestrial environment, and their roots represent the first organ to contact pollutants adsorbed in the soil solution. The advantages of using plants as models for the purpose of environmental risk assessment studies are related to their low cost and easy handling and the ability to test their responses to different conditions, in addition to having responses that corroborate those of animal models, including human cells (Andrade-Vieira et al., 2014; Reis et al. 2017). Finally, microalgae and plants used as experimental models share the advantage of not requiring approval from bioethics committees, which is in accordance with the guidelines for reducing the use of animals in ecotoxicological tests of the 21st century agenda (Hartung 2009).

Thus, the aim of the present study was to evaluate the ecotoxicity of a pyraclostrobin-based commercial fungicide in four plant models, namely, *Lactuca sativa*, *Raphanus sativus*, *Pennisetum glaucum* and *Triticum aestivum*, and in an aquatic model, the green microalga *Raphidocelis subcapitata*. The results

may provide important information for evaluating the environmental risk of fungicides and for developing more effective and sustainable management strategies for the control of pests and diseases in plants.

2 MATERIALS AND METHODS

2.1 Study models

Plant model species used for the germination and growth inhibition tests were representative of terrestrial producers: two dicotyledonous representatives, *L. sativa* L. var. (Blueline – 659) and *R. sativus* (Blueline), and two monocots, *P. glaucum* and *T. aestivum*. Lettuce and radish seeds were purchased from local stores, millet seeds were purchased *online*, and wheat seeds were purchased from the Germplasm Bank of the Federal University of Lavras (UFLA – MG).

The green microalga *R. subcapitata* was obtained from the algae culture of the Laboratory of Limnology, Federal University of Alfenas/MG. The seaweed was cultivated in a 1-litre Erlenmeyer flask containing 500 mL of LC Oligo culture medium (Afnor, 1980). Before use, the LC Oligo culture medium was sterilized in an autoclave at 121°C for 20 minutes. The culture temperature was 23+/-2°C, and the plants were illuminated with 6800 lux light with a photoperiod of 12 hours of light and 12 hours of darkness. The flask was shaken daily to prevent removal of the cells. Healthy algae in the logarithmic exponential growth phase were subjected to contamination.

2.2 Test solutions

The test solutions were prepared in distilled water from the commercial product COMET® containing 250 g/L pyraclostrobin, the active ingredient. The test concentrations for the plant models were determined based on preliminary tests with the fungicide, literature searches and determined doses for field application according to the manufacturer's package insert, with a denominator factor of 3.2 between them. The predefined doses were 0.0 (control), 0.25, 0.5, 1.5, 4.75, 15, 48, 153.5, 491.5, 1572.75 and 5033.25 mg/L.

The test concentrations for the inhibition assay with the aquatic model were determined based on the EC_{50} values previously calculated and presented in the PPDB (Pesticide Properties DataBase) for *R. subcapitata* from the active principle of pyraclostrobin and concentrations that encompassed those found in water bodies. The following doses of the active ingredient of pyraclostrobin were tested, and 1.8 was the factor used: 0.0 (control), 2.51, 8.03, 25.72, 82.32, 263.43, 843.00 and 2697.60 µg/L.

2.3 Seed exposure and experimental design

The tests were performed according to the Organization for Economic Cooperation and Development (OECD) 208 (2006), the European Community and the BRICS. For all tested plant models, the experiment was plotted with a completely randomized design with 6 replicates. Each replicate was represented by a polyethylene Petri dish (9 cm diameter) containing 10 seeds. The seeds were placed on germination

filter paper soaked in 3 mL of the test solution. The plates were stored in BOD (biochemical oxygen demand) at 24°C without lighting for 72 hours.

For the macroscopic analyses, the germination of the seeds was evaluated every 12 hours for a total of 72 hours. The seeds with root protrusions were considered germinated and the percentage of germination after 72 h of exposure and the germination speed index (GVI) were calculated (Mangure 1962; Carmello and Cardoso 2018). The fresh weight of the germinated seeds in each Petri dish after 72 h of exposure was obtained on a precision scale (Shimadzu® AUY220) and then kept at -4°C. To measure the roots and shoots, the plates were left at room temperature, and the plants were arranged and stretched across a dark surface. The lengths of the roots and shoots were measured with a digital calliper to two decimal places.

2.4 Green microalgae growth inhibition test

The growth inhibition test was performed according to the NF EN ISO 8692 (2012) standard, with 6 replicates per concentration. Each replicate was represented by a 100 mL glass test tube containing 30 mL of the test solution and an initial cell density of $1x10^4$ cells mL^{-1}. The tests were conducted in an acclimatized room at 23 ± 2°C with an intensity of 6800 lux and periodic manual agitation at each sampling. Sampling for determination of cell density was performed every 24 hours, and after 72 hours of exposure to the fungicide, the samples were removed for biochemical analyses.

Determination of the microalgae (*R. subcapitata)* cell density was performed with the aid of Fuchs Rosenthal counting chambers. For this purpose, 0.5 mL of each sample was removed daily and fixed with 0.5 mL of 4% formalin for subsequent counting. The specific growth rates were calculated based on the cell abundance considering the exponential growth phase from 24 to 72 h, where the specific growth rate was calculated according to the following equation:

$$r = [Ln (N2) – Ln (N1)]/Dt$$

where r is the intrinsic growth rate, N_1 is the population size at the beginning of a time interval, N_2 is the population size at the end of the time interval and Dt is the time change.

2.5 Biochemical analysis of microalgae: Total carbohydrates

The total carbohydrate content was determined according to a methodology previously described by Albalasmeh et al., (2013). The method involves a colorimetric reaction with sulfuric acid that consists of the quantification of total intracellular carbohydrates present in the microalgae. Samples from each treatment were collected (28 mL) within 72 hours of exposure and added to test tubes. The tubes were then centrifuged (BL-206/1 FANEM®) for 10 minutes at 1600 rpm. After centrifugation, the supernatant was discarded, 1 mL of distilled water was added to the resulting pellet, and the samples were frozen until the final analysis.

For analysis, the samples were thawed and shaken to homogenize the cells. Then, 3 mL of concentrated sulfuric acid was added to each sample, and the samples were shaken for 3 minutes and placed in an ice bath for cooling. After cooling, each sample was transferred to a 2 mL quartz cuvette, and the absorbance was read with a spectrophotometer at 315 nm (Biospectro® spectrophotometer SP-220). A calibration curve was constructed following the same definitions as for microalgae and using a glucose standard.

2.6 Statistical analyses

The results were subjected to analysis of variance (ANOVA) followed by Dunnett's test using Minitab software 17, and the differences between treatments were considered significant at the 95% significance level ($p < 0.05$). The growth inhibition concentration (EC_{50}), no observed effect concentration (NOEC) and lowest observed effect concentration (LOEC) were calculated using GraphPad Prism software (version 8).

3 RESULTS

3.1 Dose–effect relationship of fungicides: development of plant model seedlings

The fungicide containing pyraclostrobin as the active ingredient strongly inhibited germination, as shown in Fig. 1, reaching a maximum inhibition of 20% in the seeds of *L. sativa* and *T. aestivum*. The fungicide affected the GVIs of the seeds (Fig. 1) of all species when it was administered at relatively high concentrations. In the case of *L. sativa*, both germination and GVI were affected at the highest concentration of the fungicide tested (5033.25 mg/L); for *R. sativus*, there was no inhibition of germination, but the GVI was affected at concentrations greater than 491.5 mg/L. The highest concentrations also had an inhibitory effect on the GVI of the representative monocots *P. glaucum* and *T. aestivum*.

The fungicide had a significant impact on the initial development of seedlings of all the evaluated species, as illustrated in Fig. 2. *T. aestivum* plants exhibited greater susceptibility to the contaminant, as indicated by the inhibitory effects on both the aerial parts and roots of the plants at a concentration of 4.75 mg/L fungicide. This dose was notably 10 times lower than the standard recommended concentration for field use, which is 48 mg/L. For *L. sativa* and *R. sativus*, the aerial portion of the plants was the most affected in terms of development, with both exhibiting decreases starting at a concentration of 48 mg/L fungicide. On the other hand, *P. glaucum* was the least sensitive species in terms of the initial developmental parameters of the seedlings compared to those of the other tested species. Consequently, the reductions observed in the initial development of the plants influenced their final fresh mass, and there were significant reductions in the final weight (Fig. 2) compared to those in the control treatment.

The ecotoxicological values calculated for the concentration that inhibited 50% of growth (EC_{50}) were determined for several parameters, including germination, the GVI, weight, and shoot and root counts, as shown in Table 1. The effective concentration represents the amount of fungicide necessary to achieve 50% of the maximum response in the evaluated parameters. In general, for all the tested species, there was greater sensitivity to the contaminant in terms of the seedling development parameters (weight, aerial part and root), as evidenced by the lower values of EC_{50} in these categories.

3.2 Dose–effect relationship of fungicides on green microalgae

The population growth of the green microalga *R. subcapitata* was notably inhibited by treatment with the commercial product COMET® at the sixth concentration tested, as shown in Fig. 3 (b). The toxic effect of the fungicide, whose active ingredient is pyraclostrobin, resulted in a reduction in the growth rate (Fig. 3 a) of the microalgal population by up to 71.4% in the treatments, except T3 and T4, in which the rates were equivalent to those of the control treatment. For the ecotoxicological parameters evaluated for the population growth of *R. subcapitata*, a concentration of 746.3 µg/L represents the EC_{50} after 72 hours of exposure to the fungicide. This indicates the effective concentration at which there is a 50% inhibition of microalgal population growth after the aforementioned period.

3.3 Total carbohydrate content in microalgae

Understanding the biochemical responses of microalgae is crucial for evaluating the impacts of fungicides on their physiology and ability to adapt to stressful conditions. As a result, there was an increase in total carbohydrates (µg mL^{-1}) in the microalgae in the treatments with the lowest concentration of the fungicide (T1 to T3), as shown in Fig. 4. Among the remaining treatments, which had the highest concentrations of the pyraclostrobin-based fungicide, there were significant reductions of up to four times in the carbohydrate content compared to that of the control, indicating the effect of stress induced by the fungicide.

4 DISCUSSION

Strobilurins are a group of chemical compounds whose mode of action is based on the inhibition of mitochondrial respiration in fungi—more specifically, on the inhibition of complex III of the electron transport chain within the mitochondria (Anke 1995; Bartlett et al. 2002; Zhang et al. 2020). Cellular respiration occurs within mitochondria via electron transport between specific proteins to generate a proton gradient and, consequently, adenosine triphosphate (ATP) production (Hekimi et al. 2011; Mailloux 2015). The action of strobilurins, including the active ingredient pyraclostrobin, interferes with the normal transfer of electrons along the transport chain, causing a blockage in the flow of electrons. This process results in reduced ATP production in fungal cells (Anke 1995; Bartlett et al. 2002). Consequently, the interruption of ATP production compromises the essential metabolic functions of fungi, causing damage to their physiology, proliferation and growth. With fungal death or inhibition of fungal development, there will be prevention and advancement of controlling fungal diseases in treated

crops. In addition, plants and animals not targeted by strobilurins may also be affected, and there is a need to elucidate the extent of toxicity in these cases.

In this study, we used the seed germination and initial development of *L. sativa* and *R. sativus* seedlings, which are representative dicots, as well as *P. glaucum* and *T. aestivum*, which are representative monocots, as indicators of toxicity to evaluate the efficacy of a pyraclostrobin-based fungicide. In addition, we considered the microalga *R. subcapitata* an additional model organism for investigations of toxicity. These organisms were selected for their representativeness and common application in toxicity studies. For the terrestrial representatives, the effects of the fungicide were more notable during the initial development of the seedlings, while the germination and GSI were less sensitive parameters, as indicated by the analysed ecotoxicological data.

Germination represents an essential physiological process associated with root protrusion that plays a key role in the reproduction of seed plants and involves the activation of the embryo contained in the seed (Nonogaki et al. 2010; Resentini et al. 2015). Despite its complexity, once the radicle becomes apparent, the seed is considered germinated. Regarding the initial development of the seedling, the cells of the radicle and shoots require nutrients and energy to perform various cell divisions and promote growth. In this context, many chemical compounds do not directly affect germination, decreasing the sensitivity of this parameter (El-Temsah 2010). However, these compounds may delay or inhibit growth due to their mode of action.

Pyraclostrobin inhibits the production of ATP, a molecule crucial for the function of the mitotic spindle and the completion of cell division, in fungal cells and may compromise the development of seedlings. In plants, cell division and organ development are intrinsically linked (Harashima and Schnittger 2010; Sablowski 2016). This study showed that seedling development may be impaired by pyraclostrobin.

Previous studies on the effects of pyraclostrobin in other plant models revealed different results in the early development of seedlings. In *Zea mays* L., pyraclostrobin caused a significant reduction in chlorophyll content and vegetative growth, as demonstrated by Kuswanto and collaborators (2013). In contrast, Li et al. (2020), when exposing *Glycine max* L. to different concentrations of pyraclostrobin, reported opposite effects, revealing a stimulus stimulating the growth of both the roots and the aerial parts of the plants. However, even with this growth stimulus, toxicity was observed, with an EC_{50} of 1.59 and a concentration of 1.24 μg mL^{-1}. In the present study, doses starting at 4.75 mg/L pyraclostrobin inhibited the development of *T. aestivum*, which is the most sensitive species in terms of root and shoot development. Importantly, these doses are significantly less than the concentration recommended for application in the cultures indicated in the package insert of the commercial product; 48 mg/L was also tested in this study. Notably, international databases, such as the PPDB, still lack references regarding the levels of toxicity for the plant models used in this study. To date, this study is pioneering in regards to performing tests with model plants, including references that are easy to handle and access, such as radish and wheat. Plants, as terrestrial producers, play a key role in ecosystems. The presence of fungicide residues in these plants not only represents a risk of contamination for primary consumers but

also implies potential adverse effects at higher levels of the trophic chain, depending on the dynamics and kinetics of the active ingredient. Therefore, the extensive application and accumulation of pyraclostrobin in ecosystems are important concerns, as they may pose a risk to nontarget organisms. This complex interaction between terrestrial producers and the presence of fungicide residues and the potential impacts on primary consumers highlights the importance of comprehensive assessments to understand the environmental risks associated with these agricultural practices because, in addition to affecting the terrestrial environment, fungicides also occur in aquatic ecosystems.

In the context of aquatic ecosystems, algae play a key role as primary producers and constitute excellent models for assessing the risk of contamination and environmental quality. The species *R. subcapitata* is frequently used in ecotoxicity bioassays involving pesticides, and these studies showed high sensitivity to the commercial fungicide COMET. The pyraclostrobin-based fungicide demonstrated dose-dependent toxicity to *R. subcapitata*, as reflected by the increase in growth inhibition as the concentration increased. Similar findings were reported by Liu and collaborators (2018), who reported that strobilurin-based compounds inhibited the growth of *Chlorella vulgaris*. Lu and collaborators (2019) also reported that pyraclostrobin caused growth inhibition in *C. vulgaris* and that, in addition, it reduced the microbial population of *Microcystis aeruginosa*.

In our growth inhibition assays, a significant reduction in the growth of the microalgae was observed after 24 hours of exposure to the fungicide, especially at the highest concentrations, indicating that pyraclostrobin rapidly promoted toxicity in *R. subcapitata*. In addition, the toxicity levels attributed to this active ingredient, as reported by Liu et al. (2018) for *C. vulgaris* and *Chlorella pyrenoidosa*, were 190 µg/L and 11538 µg/L, respectively, which differed from the results obtained in this study. A total of 746.3 µg/L was recorded for *R. subcapitata*. However, when these data were compared with the EC_{50} value of 843 µg/L obtained from the PPDB database for *R. subcapitata*, a remarkable similarity was observed, corroborating our findings. However, it is crucial to highlight the possible physiological and sensitivity differences between the various microalgae in their response to fungicides (Lu et al., 2019). In addition, it is necessary to distinguish between commercial products, which may contain auxiliary ingredients such as adjuvants, solvents or stabilizers, and the active ingredients used in the aforementioned comparisons, which have a high degree of purity. These discrepancies can significantly influence the results obtained in comparative studies and reinforce the importance of a judicious analysis when interpreting the effects of fungicides in different contexts.

The growth rate of the microalgal population has emerged as a highly sensitive parameter for evaluating the toxicity of chemical agents. In the present study, cell growth was more sensitive than *R. subcapitata* growth, which showed adverse effects at the lowest concentration tested. The marked reduction in the long-term growth rate observed for *R. subcapitata* may culminate in a decrease in the global production of biomass. In other words, the lower production of microalgae cells resulting from this decrease in growth rate may lead to a reduction in productivity in terms of biomass or substances of interest (Church et al. 2017; Pandit et al. 2017). This observation underscores the relevance of the population growth rate as a sensitive and predictive indicator of the long-term impacts of toxic agents on microalgae.

The present study stands out as a pioneer in the investigation of the direct effects of fungicides on carbohydrate production in microalgae. It was observed that lower concentrations of the commercial fungicide pyraclostrobin resulted in an accumulation of carbohydrates in the cells of *R. subcapitata*. This accumulation may have provided the energy necessary for the microalgae to maintain its growth throughout the 72 hours of exposure to the contaminant, even with a reduction in the specific growth rate. An increase in carbohydrate production in microalgae under physiological stress may be an adaptive response to overcome adverse conditions and maintain cellular homeostasis. An increase in the production and accumulation of carbohydrates may benefit microalgae by serving as a source of energy and carbon reserve for cells (Chen et al. 2013; Samiee-Zafarghandi et al. 2018) Moreover, microalgae can maintain their metabolism and growth during the stress period. Understanding the mechanisms that lead to the accumulation of carbohydrates in microalgae is important for optimizing the production of biomass and compounds of commercial interest from these microalgae (Markou et al. 2012).

At the highest concentrations of the fungicide, where there were considerable reductions in the levels of total carbohydrates in the cells, it is likely that there were changes in the photosynthetic apparatus. These changes contributed to the decreases in the growth rate and biomolecule levels. The mechanisms by which microalgae acclimatize to the environment involve changes in the synthesis of biomolecules and in the light-harvesting complex (Torzillo and Vonshak 2013). During the production of carbohydrates, microalgae perform enzymatic activities related to the synthesis of sugars, such as ribulose-1,5-bisphosphate carboxylase oxygenase (Markou et al., 2012). This may result in an increase in the use of carbon dioxide, affecting the photosynthetic rate and nutrient uptake, as strobilurin fungicides inhibit mitochondrial complex III, blocking the transfer of electrons between cytochromes b and c1 and, consequently, preventing ATP production (Liu et al. 2018; Zhang et al. 2020).

Considering the entry of the fungicide into aquatic ecosystems via terrestrial runoff and/or leaching, our results suggest that pyraclostrobin may threaten the health of algae and other organisms present in these environments. Importantly, many of the concentrations tested in this study included those indicated for field use, which had already been recorded in natural water bodies. These results offer valuable insights for future studies that use plant models and aquatic bioindicators to evaluate fungicide toxicity. These observations highlight the importance of understanding the potential impacts of fungicides on the dynamics of terrestrial and aquatic ecosystems and underscore the need for sustainable agricultural practices and precautions in the use of these agents to mitigate possible adverse effects on biota in general.

5 CONCLUSIONS

We conclude that the pyraclostrobin-based fungicide has phytotoxic potential for the model plant species tested in this study and is considered a highly toxic contaminant for the aquatic environment. This fungicide inhibited the growth of the microalga *R. subcapitata* while altering its biochemical properties, notably the total carbohydrate content. From an ecotoxicological point of view, this study

contributes to a deeper understanding of the potential risks associated with contamination of nontarget plants in agriculture, as well as for the aquatic environment in general.

Declarations

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COMPETING INTERESTS

The authors have no relevant financial or non-financial interests to disclose.

AUTHOR CONTRIBUTIONS

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Tamires de Freitas Oliveira, Maria Fernanda Barbosa Vaz da Costa and Tamara Alessandra Costa Santos. The first draft of the manuscript was written by Tamires de Freitas Oliveira and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.”

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DATA AVAILABILITY

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.”

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Tables

Table 1: Values of the growth inhibition concentration (EC_{50}), in mg/L, of the fungicide COMET® in the model plants treated for 72 hours.

Parameters	**Species**			
	Lactuca sativa	*Raphanus sativus*	*Pennisetum glaucum*	*Triticum aestivum*
Germination	5729,0	865,9	-	1096
GVI	1729,0	520,9	405,1	1444
Weight	136,0	100,7	28,66	38,07
Aerial part	14,24	75,70	0,5275	14,03
Root	34,699	58,52	16,68	4,714

Figures

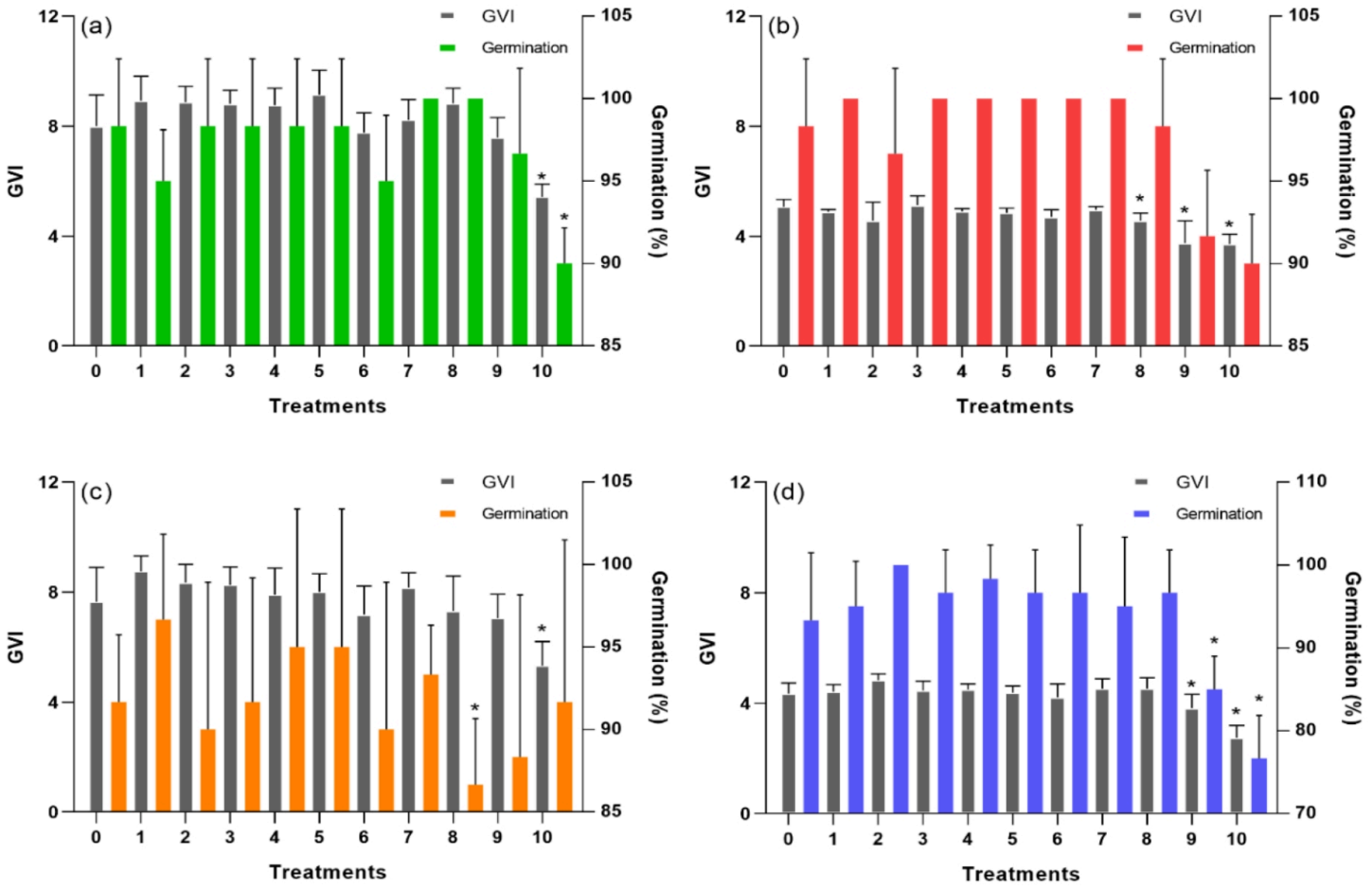


Figure 1

Germination speed index (GVI) and percentage of germination of the plant models (a) *Lactuca sativa*, (b) *Raphanus sativus,* (c) *Pennisetum glaucum* and (d) *Triticum aestivum* after 72 hours of exposure to pyraclostrobin. 0: negative control; 1: 0.25 mg L^{-1}; 2: 0.5 mg L^{-1}; 3: 1.5 mg L^{-1}; 4: 4.75 mg L^{-1}; 5: 15 mg L^{-1}; 6: 48 mg L^{-1}; 7: 153.5 mg L^{-1}; 8: 491.5 mg L^{-1}; 9: 1572.75 mg L^{-1}; 10: 5033.25 mg L^{-1}. The * symbol indicates a significant difference (Tukey $p<0.05$) compared to the control treatment. The bars represent the standard deviation of the mean.

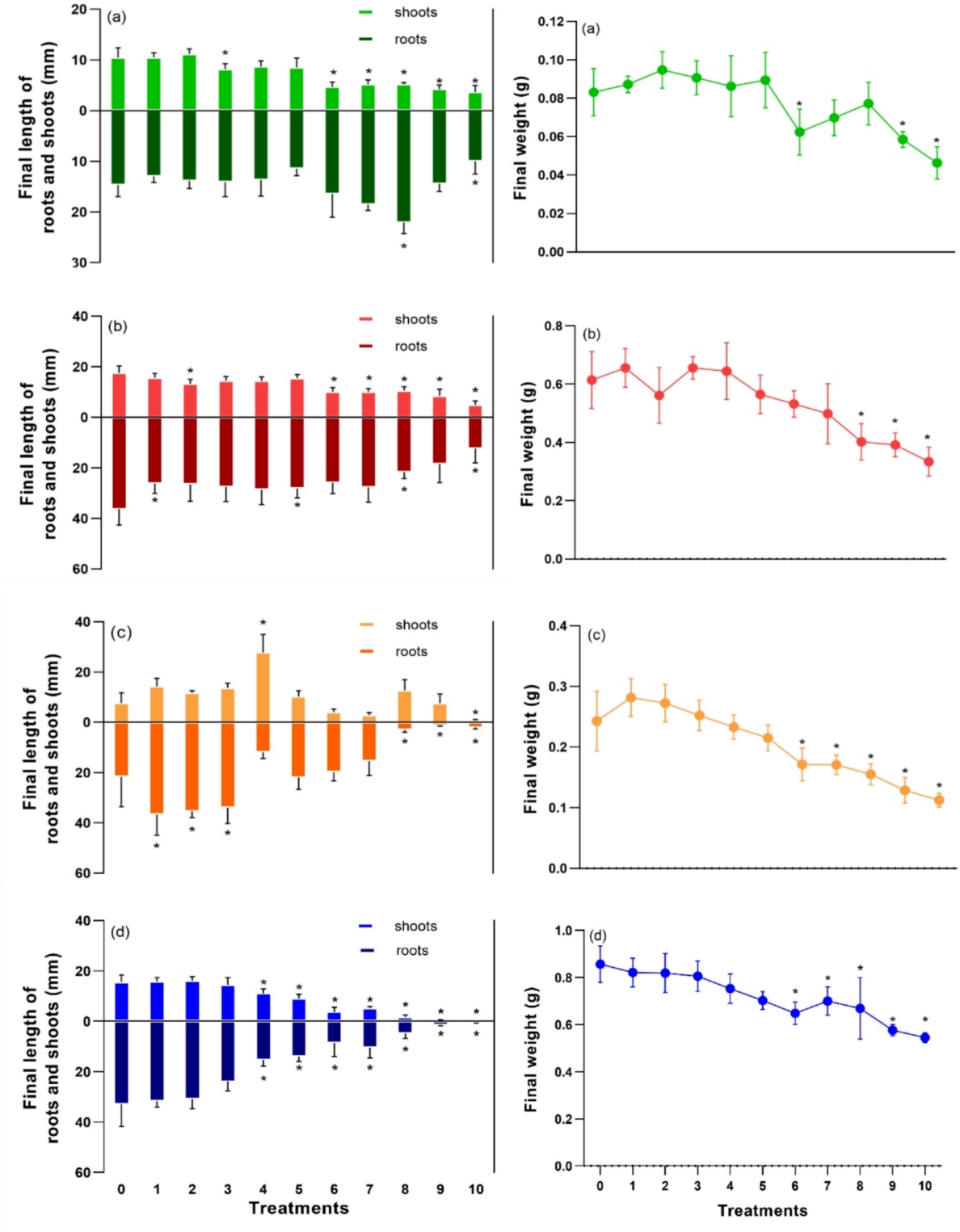


Figure 2

Final length of roots and shoots (mm) and final weight (g) of plants of the plant models (a) *Lactuca sativa*, (b) *Raphanus sativus,* (c) *Pennisetum glaucum* and (d) *Triticum aestivum* after 72 hours of exposure to pyraclostrobin-based fungicide. 0: negative control; 1: 0.25 mg L^{-1}; 2: 0.5 mg L^{-1}; 3: 1.5 mg L^{-1}; 4: 4.75 mg L^{-1}; 5: 15 mg L^{-1}; 6: 48 mg L^{-1}; 7: 153.5 mg L^{-1}; 8: 491.5 mg L^{-1}; 9: 1572.75 mg L^{-1}; 10:

5033.25 mg L $^{-1}$. The * symbol indicates a significant difference (Tukey p<0.05) compared to the control treatment. The bars represent the standard deviation of the mean.

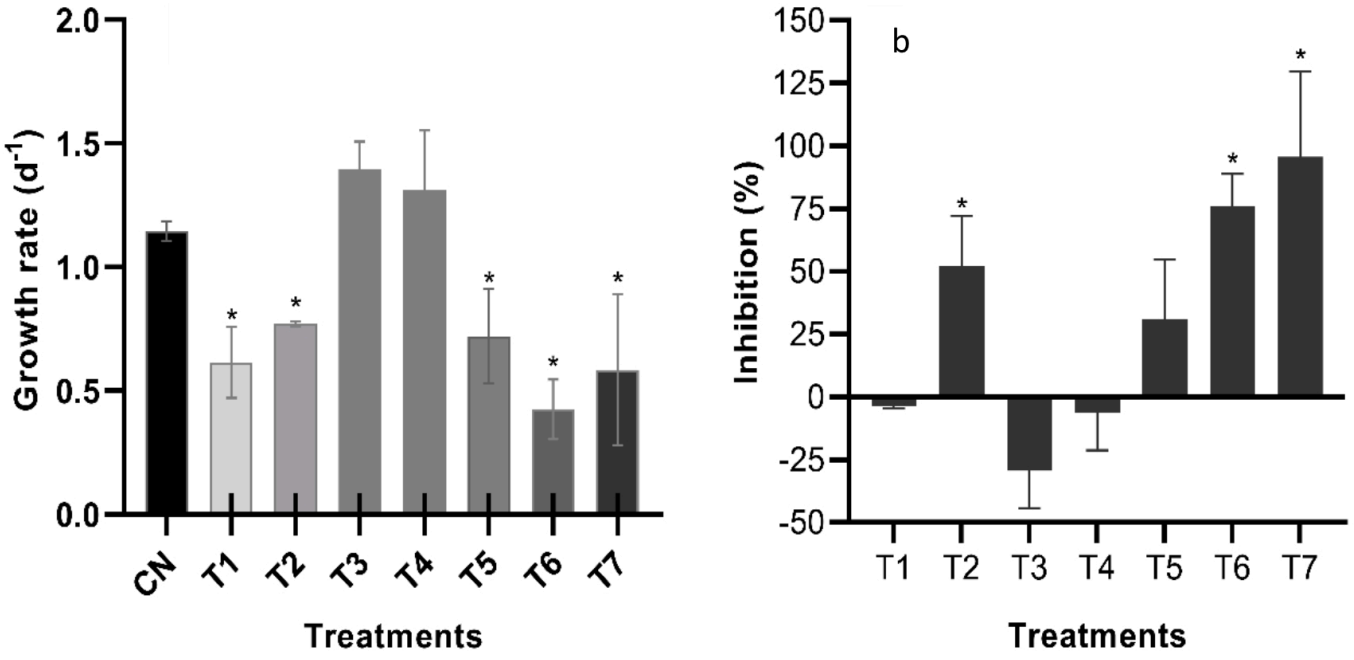


Figure 3

Growth and inhibition parameters of *Raphidocelis subcapitata* after exposure to pyraclostrobin-based fungicides. (a) Growth rate per day (d $^{-1}$) and (b) percentage of growth inhibition at 72 hours. NC: negative control; T1: 2.51 µg L $^{-1}$; T2: 8.03 µg L $^{-1}$; T3: 25.72 µg L $^{-1}$; T4: 82.32 µg L $^{-1}$; T5: 263.43 µg L $^{-1}$; T6: 843.00 µg L $^{-1}$; and T7: 2697.60 µg L $^{-1}$. The * symbol indicates a significant difference (Tukey p<0.05) compared to the control treatment. The bars represent the standard deviation of the mean.

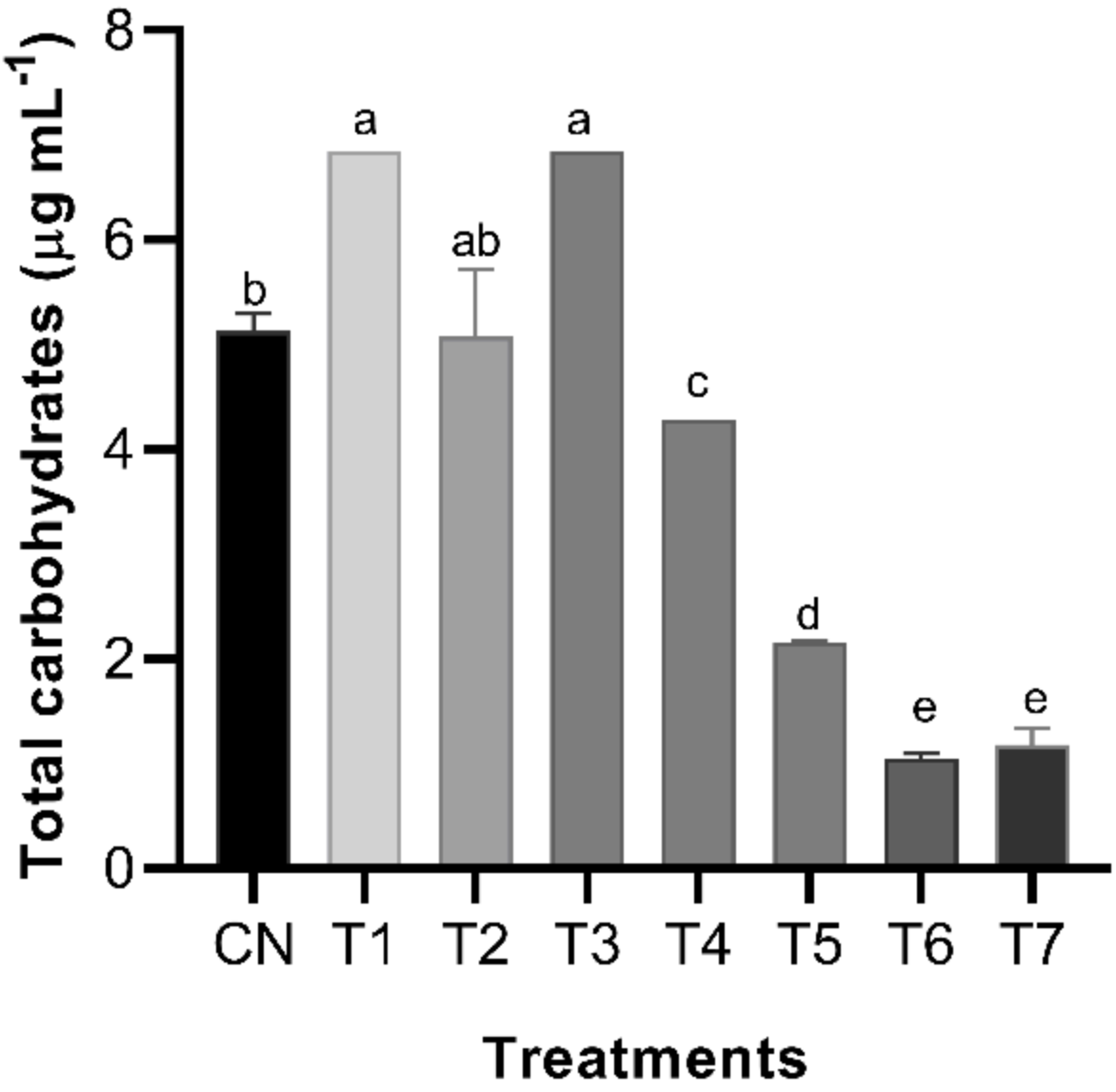


Figure 4

Total carbohydrate content of the microalga *Raphidocelis subcapitata* quantified after 72 hours of exposure to the pyraclostrobin-based fungicide. NC: negative control; T1: 2.51 µg L^{-1}; T2: 8.03 µg L^{-1}; T3: 25.72 µg L^{-1}; T4: 82.32 µg L^{-1}; T5: 263.43 µg L^{-1}; T6: 843.00 µg L^{-1}; and T7: 2697.60 µg L^{-1}. Different letters indicate significant differences (Tukey $p<0.05$) between the treatments and the control. The bars represent the standard deviation of the mean.