Original Article: Bioindicators selection to monitoring pyroxasulfone mobility and persistence in soil

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Citation Willian D. Matte, Rubem S. Oliveira Jr., Jamil Constantin, Rafael R. Mendes, Lucas Matheus Padovese, Te-Ming Tseng. 2021. Bioindicators selection to monitoring pyroxasulfone mobility and persistence in soil. Journal of Research in Weed Science, 4(2), 142-150.

http://dx.doi.org/10.26655/JRWEEDSCI.2021.2.1

Article info

Received: **17 November 2020**
Accepted: **26 December 2020**
Available Online: **30 December 2020**
Checked for Plagiarism: **Yes.**
Peer reviewers approved by:
Dr. Mohammad Mehdizadeh
Editor who approved publication:
Dr. Amin Baghizadeh
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Keywords:
Bioassay, Dose-response, Herbicide, Susceptible plants.

ABSTRACT

Pyroxasulfone is a new herbicidal molecule with residual activity to be used in Brazilian agricultural areas, it is necessary to gather information about its behavior in the soil, as well as its persistence in the environment and the risk of environmental contamination. The objective of this work was to evaluate the sensitivity of species to pyroxasulfone in order to select potential plants to be used as bioindicators in herbicide soil activity experiments. Greenhouse experiments were conducted with four species as potential bioindicators including lettuce **(***Lactuca sativa*), cucumber (*Cucumis sativus*), sorghum (*Sorghum bicolor*), and tomato (*Solanum lycopersicum*)**.** The preemergence pyroxasulfone treatments at 0, 3.125, 6.25, 12.5, 25, 50, and 100 g a.i. ha^{-1}. The percentage of injury of the treated species was evaluated by a visual scale of 0-100% at 7 and 14 days after treatment (DAT). We also evaluated the effect of the herbicide on plant height, root length, shoot fresh biomass, root fresh biomass, and total fresh biomass. Using the non-linear regression models was possible to estimate the dose of pyroxasulfone required to obtain 50% of the response for the analyzed variable (I_{50}). I_{50} values were used to determine the susceptibility of the species evaluated. The pyroxasulfone dose-response experiments revealed three species with potential for bioassay studies. Overall, Lettuce was the most sensitive to herbicide. Sorghum may be useful species to detect pyroxasulfone soil activity based on plant height measurements (I_{50} = 9.7 g a.i. ha^{-1}). Cucumber also showed to be a potential candidate as bioindicators. Tomato was considered tolerant of pyroxasulfone doses evaluated.

Introduction

Herbicides applied to soil in pre-emergence play an essential role in agricultural production systems and constitute a useful strategy in weed control during the critical period of competition with crops and in the management of herbicide-resistant weeds (Monquero et al. 2008; Nunes et al. 2018). Raimondi et al. (2010) observed in their study that the residual activity promoted with the application of the recommended doses of prometryne, oxyfluorfen, trifluralin, diuron, pendimethalin and s-metolachlor provided efficient control, with levels of control above 80%, for different species of *Amaranthus* (*A. hybridus, A. spinosus, A. lividus and A. viridis*) up to 30 days after herbicides application. Constantin et al. (2007) concluded that late weed control in soybeans, which occurred 18 days after emergence, resulted in 16.74% reduction in productivity, as a result of the initial competition with the crop. Otherwise, by using herbicides in pre-emergence, no reduction in soybean yield was observed. Pyroxasulfone ((3-[5-(difluoromethoxy)-1-methyl-3-(trifluoromethyl) pyrazol-4-ylmethylsulfonyl]-4,5-dihydro-5,5-dimethyl-1,2-oxazole) inhibits the biosynthesis of very long-chain fatty acids (VLCFA) and provides weed control via pre-emergent applications (Yamaji et al. 2014). Main chemical properties include water solubility of 3.49 mg L^{-1} (20 °C), vapor pressure of $2x10^{-6}$ Pa, log Kow of 2.39, and half-life from 33 to 89 days, depending on the environmental conditions (Westra et al. 2015). Pyroxasulfone is mainly used to control a broad spectrum of grasses and small-seeded broad leaves, and is selective for crops such as corn, soy, wheat, cotton, potato, onion and sunflower (Tanetani et al. 2011; Nakatani et al. 2016). Results obtained so far indicate that rates of 16 and 32 g a.i. ha^{-1} of pyroxasulfone were sufficient to promote more than 90% control of *Echinochloa crusgalli*, *Amaranthus retroflexus* (Yamaji et al. 2014) and *Digitaria sanguinalis* (Nurse et al. 2011).

Understanding ways to identify and quantify herbicide residues, such as that of pyroxasulfone, is a fundamental issue due to its soil activity. Knowledge of herbicide soil residual activity will help growers to identify potential carryover effects. However, herbicide residue quantification in the soil is usually carried out using radioisotopes or chromatography analyses (Inoue et al. 2002), which generally are not affordable methods for many growers and research groups. Alternatively, plant species sensitive to herbicides can be used as bioindicators and as a low cost and practical approach as bioassay methods (Mehdizadeh et al. 2016). In addition, bioassay can be an specific method to detect only the biologicaly active fraction of the herbicide, which better reproduce the field conditions (Kotoula-Syka et al. 1993; Lima et al. 2015). Guerra et al. (2011) used in their study the calculated values of I_{50} (dose required to reduce 50% of the analyzed variable), in order to compare the sensitivity of the species to the herbicides trifloxysulfuron-sodium and pyrithiobac-sodium, and found that cucumber and corn behaved as the most sensitive species, with great potential for use as bioindicator plants in tests of these herbicides in the soil. Khalil et al. (2018 a,b) developed a method to evaluate pyroxasyulfone residues, specifically in soil with high sand contents and low levels of organic matter. Three species were used as bioindicators, ryegrass (*Lolium multiflorum*), cucumber (*Cucumis sativus*), and sugar beet (*Beta vulgaris*). In their research, ryegrass was the most sensitive species to pyroxasulfone, while cucumber and sugar beet were the least sensitive. Shoot and root lengths were adversely affected by herbicide treatment. Szmigielski et al. (2014) also observed a decreased effect on plant and root length of sugar beet, seven days after pyroxasulfone treatment.

Despite it is already studied in a few plants, extra options of species and varieties to be used as bioindicatiors for pyroxasulfone are needed in distinct environment conditions. Therefore, the goal of this research was to evaluate the sensitivity of species to pyroxasulfone in order to select potential plants to be used as bioindicators in herbicide soil activity experiments.

Materials and Methods

Two experiments were carried out in a greenhouse between August and December 2019. The experimental design was completely randomized with a factorial scheme of 7x4, and five replications. The first factor consisted of increasing pyroxasulfone rates in preemergent applications (0, 3.1, 6.2, 12.5, 25, 50, and 100 g a.i. ha^{-1}). The second factor consisted of four putative species selected as bioindicators: *Lactuca sativa* (lettuce var. Elba), *Cucumis sativus* (cucumber), *Sorghum bicolor* (sorghum), and *Solanum lycopersicum* (tomato var. Santa Clara). Experimental units were 5 dm^{-3}-pots filled with soil composed of 70% sand, 23% clay, and 7% silt. The soil pH was 5.3 ($CaCl_2$) and the specific chemical composition was H^+ + AL^{3+} at 1.6 $cmol_c$ dm^{-3}, Ca^{+2} at 1.5 $cmol_c$ dm^{-3}, Mg^{2+} at 0.6 $cmol_c$ dm^{-3}, K^+ at 0.35 cmolc dm^{-3} P (Mehlich) at 17 mg dm^{-3}, and 1.2% of organic matter. Ten seeds per pot of each species were sown 1.5 cm-deep. The pots that received the sowing of the species received 10 mm of water by sprinkler irrigation in order to maintain sufficient soil moisture for the application of the herbicide. Herbicide treatments were applied two hours after irrigation.

A backpack sprayer (CO_2 pressure) equipped with a three nozzles boom (XR-110.02) was used for all herbicide treatments. The pressure was 106.8 KPa (30 psi), delivering 200 L ha^{-1} of spray volume. Climatic conditions at spraying were: air temperature of 25 C, relative air humidity of 70%, and 2 km h^{-1} wind speed. During the experimental period, the pots were watered daily with 9-mm using a sprayer irrigation system. Seven and 14 days after treatment (DAT), plant injury was quantified using a visual scale of 0-100%, where 0 means no symptoms and 100% means plant dead. Fourteen DAT, plant length, and root length were measured in eight plants per experimental unit. Additionally, all plants were collected and weighted to quantify roots, shoots, and plant (root plus shoot) fresh biomass. The data were analyzed in percentage compared to non-treated control to estimate the effect of pyroxasulfone rates in each variable for each bioindicator species. The data were submitted to the normality test (Shapiro-Wilk), after which the individual variance analysis was performed to verify the homogeneity of variance (Cochran's test), and the joint analysis of the data obtained in the different experiments was carried out. The data were subjected to ANOVA and, once significant ($p<0.05$), non-linear regression models were fit to explain the response of pyroxasulfone doses. The statistical analyses were processed using the *drc* package in R software (Ritz et al. 2015), and graphs were constructed using GraphPad Prism version 8.0. The decision to fit each model was made following the significance of the parameters ($p\leq0.05$) as well as the biological response.

The equations used were:

Equation 1: Boltzmann's sigmoidal.

Eq. 1 $$Y = a + \frac{b-a}{1+\exp\left(\frac{EC_{50}-X}{c}\right)}$$

Where Y is the response, a is the response lowest value, and b is the highest response value, X is the pyroxasulfone rate, EC_{50} is the rate to provide 50% of response, and c is the sloop around EC_{50}.

Equation 2: Sigmoidal with three parameters (decreasing).

Eq. 2 $$Y = a + \frac{b-a}{1+\left(\frac{X}{EC_{50}}\right)}$$

Equation 3: Sigmoidal with three parameters (increasing).

Eq. 3 $$Y = a + X * \frac{b-a}{EC_{50}+X}$$

Where Y is the response, a is the response lowest value, and b is the highest response value, X is the pyroxasulfone rate, EC_{50} is the rate to provide 50% of response (I_{50}).

Results and Discussion

The data obtained showed normality and homogeneity. Because there was no difference between experiments, the data were pooled and analyzed. Boltzmann's sigmoidal model was suitable for most of the data on plant injuries (Figures 1 and 2), with another model (Eq3) fit only for *Sorghum bicolor* at 14 days.

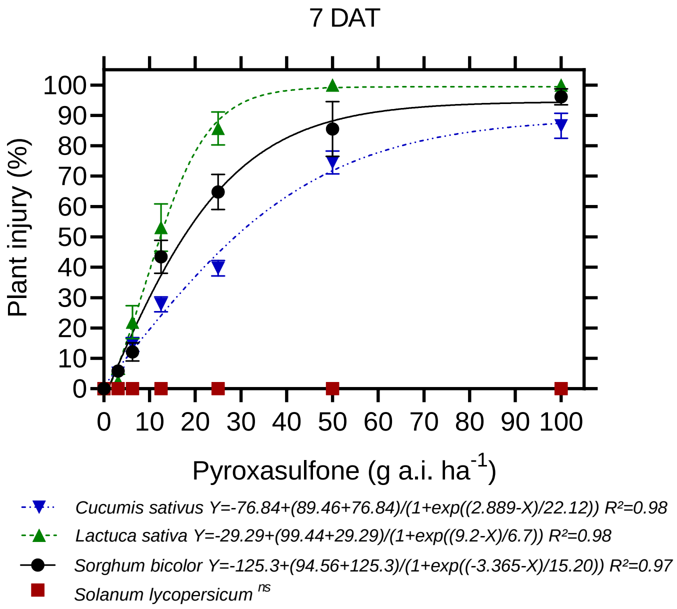

Figure 1. Plant injury (%) of *Cucumis sativus*, *Lactuca sativa*, *Sorghum bicolor*, and *Solanum lycopersicum* at 7 days after pyroxasufone soil-applied treatment.

At 7 DAT, lettuce was the most affected species by rates of 25 g a.i. ha^{-1} or higher, showing an injury level of at least 20% higher than other crops. Sorghum and cucumber were also seriously affected by pyroxasulfone treatments when rates from 12.5 g a.i. ha^{-1} were applied (Figure 1). Higher plant injury levels (>50%) were observed at 14 DAT among the most sensitive species (lettuce, sorghum, and cucumber) when treated with low rates ($\geq$12.5 g a.i. ha^{-1}). Tomato, on the other hand, was the most tolerant species to pyroxasulfone due to the absence of injury even at the highest application rate (100 g a.i. ha^{-1}) (Figure 2).

The effect of herbicide on plant height and shoot fresh biomass is presented in Figures 3 and 4, respectively. The biomass and plant height of sorghum and cucumber decreased as rates applied increased. Nevertheless, these effects were slighter in those species when compared to lettuce due to its high susceptibility. The lowest response (parameter *a*) in plant heigth reduction was 35.3, 31.2, and 12.8% for cucumber, lettuce and sorghum, respectively. Regarding to biomass reduction, these values were 17, 25.8, and 5.4%.

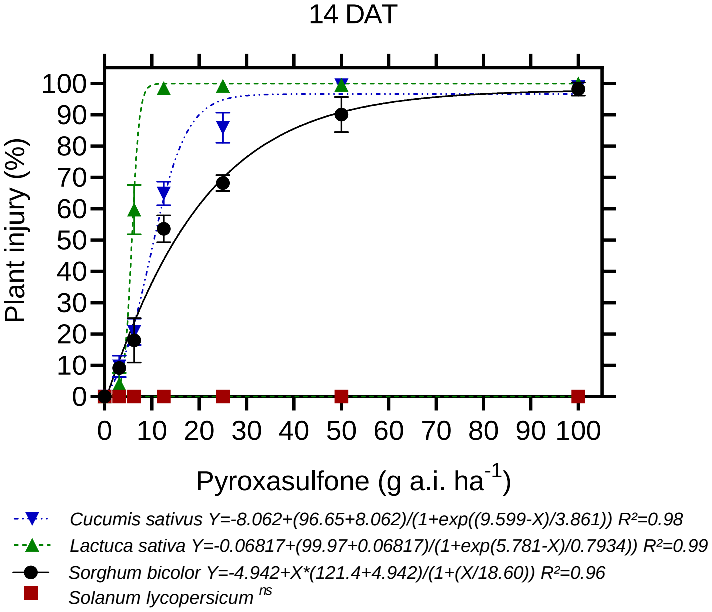

Figure 2. Plant injury (%) of *Cucumis sativus*, *Lactuca sativa*, *Sorghum bicolor*, and *Solanum lycopersicum* at 14 days after pyroxasufone soil-applied treatment.

No differences among all herbicide rates were observed in the plant height and biomass of tomato. About 50% of shoot length reduction in cucumber was observed after application of pyroxasulfone at 102 g a.i. ha^{-1} in the presence of wheat residues of 4 t ha^{-1} (Khalil et al. 2018a). However, as well as mentioned for this study, the same authors verified that the treatment at 102 g a.i. ha^{-1} of pyroxasulfone killed cucumber and ryegrass plants after applied on a lower residue amount (1 t ha^{-1}).

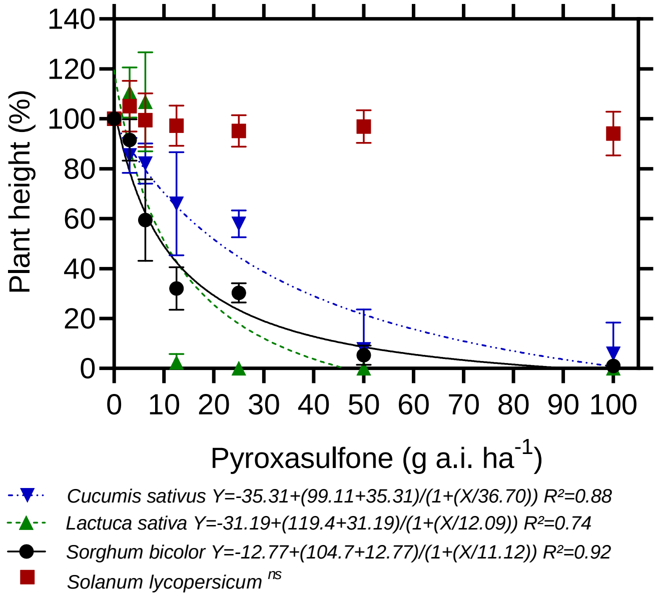

Figure 3. Plant height (%) of *Cucumis sativus*, *Lactuca sativa*, *Sorghum bicolor*, and *Solanum lycopersicum* at 14 days after pyroxasufone soil-applied treatment.

For most species, there was reduced development of roots compared to non-treated control (Figure 5). Only tomato had a normal development on root system meaning no significant response to any herbicide rate. Based on EC_{50} value for root length, lettuce was 7.6 times more sensitive than cucumber and 12.5 times more sensitive than sorghum. The root length of sorghum and cucumber were also affected, however, the response to pyroxasulfone rates was more gradual than for other species. The lowest response was higher than 40% for sorghum and cucumber while this parameter achieves just 13% for lettuce. Similar response was observed for root biomass (Figure 6).

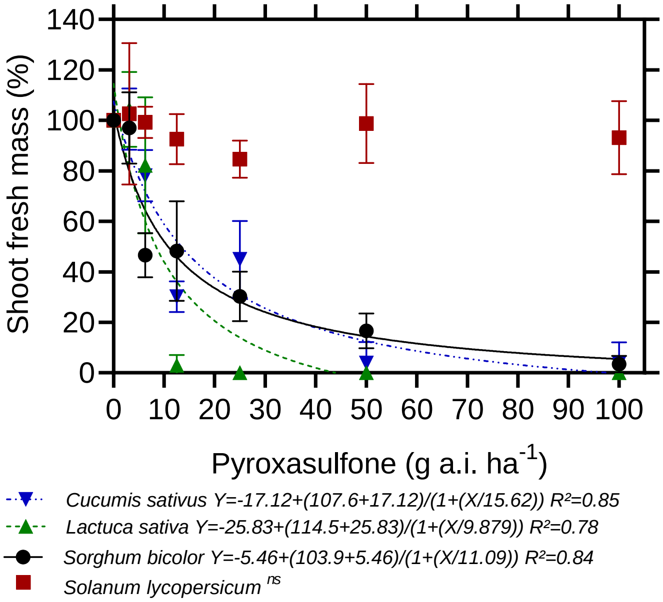

Figure 4. Shoot fresh biomass (%) of *Cucumis sativus*, *Lactuca sativa*, *Sorghum bicolor*, and *Solanum lycopersicum* at 14 days after pyroxasufone soil-applied treatment.

Lettuce was the most susceptible species, while sorghum and cucumber had the root biomass slightly prejudiced. As previously described for other variables, the response of root biomass to the herbicide rates was undetected for tomato. As expected for total plant biomass (root + shoot), the highest effect of pyroxasulfone treatments was observed on lettuce followed by sorghum and cucumber (Figure 7). Total biomass was reduced to 15, 23 and 10% in cucumber, lettuce and sorghum, respectively. Similar to the results from other variables, pyroxasulfone rates did not have any effect on total biomass of tomato, which may indicate the selectivity of the herbicide for that species.

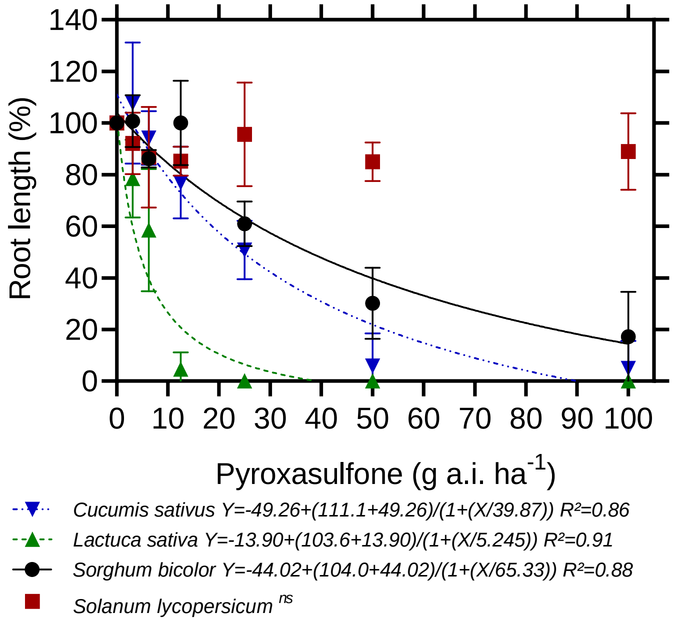

Figure 5. Root length (%) of *Cucumis sativus*, *Lactuca sativa*, *Sorghum bicolor*, and *Solanum lycopersicum* at 14 days after pyroxasufone soil-applied treatment.

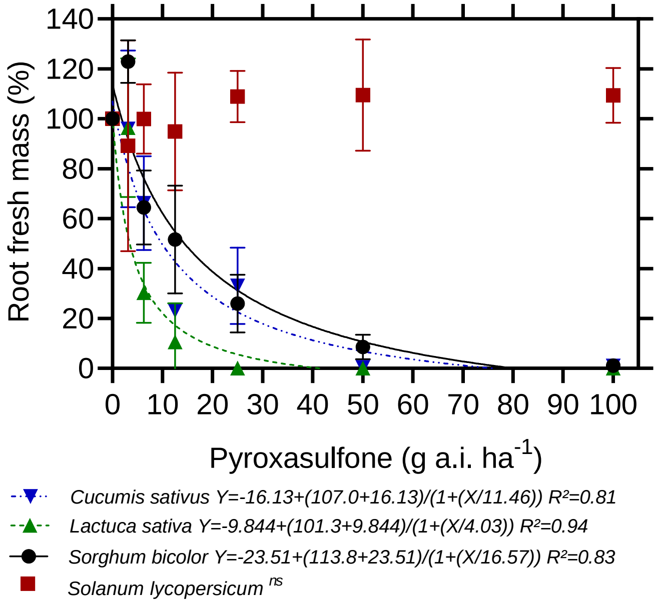

Figure 6. Root fresh biomass (%) of *Cucumis sativus*, *Lactuca sativa*, *Sorghum bicolor*, and *Solanum lycopersicum* at 14 days after pyroxasufone soil-applied treatment.

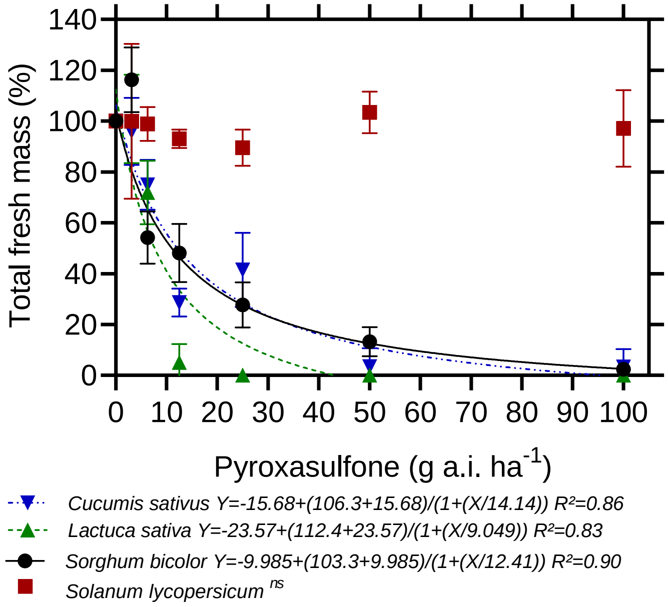

Figure 7. Total fresh biomass (%) of *Cucumis sativus*, *Lactuca sativa*, *Sorghum bicolor*, and *Solanum lycopersicum* at 14 days after pyroxasufone soil-applied treatment.

The I_{50} (pyroxasulfone rate to provide 50% of plant injury or 50% inhibition of height, length, or fresh biomass) has been one of the most plausible parameters to compare sensitivity among plant species (Guerra et al. 2011). For lettuce, the injury levels at 7 and 14 DAT resulted in I_{50} values of 12.4 and 5.8 g a.i. ha^{-1}, respectively (Table 1).

Table 1. Pyroxasulfone rate to provide 50% of plant injury or 50% inhibition of height, length or fresh biomass (I_{50}) in different species in pre-emergent treatment.

Species	I_{50} (g a.i. ha^{-1})*						
	Plant injury		Plant height	Root Length	Fresh biomass		
	7 DAT	14 DAT			Shoot	Root	Total
Cucumis sativus	28.7	10.5	21.2	24.5	13.4	9.8	12.1
Lactuca sativa	12.4	5.8	10.3	4.4	8.4	3.5	7.6
Sorghum bicolor	17.5	14.4	9.7	37.5	10.7	14.3	11.0
Solanum lycopersicum	>100	>100	>100	>100	>100	>100	>100

*Pyroxasulfone rate to provide 50% of plant injury or 50% inhibition of height, length or fresh biomass.

The lowest I_{50} values were found in lettuce for root length (4.4 g a.i. ha^{-1}), shoot fresh biomass (8.4 g a.i. ha^{-1}), root fresh biomass (3.5 g a.i. ha^{-1}), and total fresh biomass (7.6 g a.i. ha^{-1}). Otherwise, the lowest I_{50} value for plant height was estimated for sorghum (9.7 g a.i. ha^{-1}). Khalil et al. (2018b) found I_{50} of 2.4 and 18.4 g a.i. ha^{-1} of pyroxasulfone for shoot height of annual ryegrass (*Lolium multiflorum*) and cucumber (*Cucumis sativus*), respectively. The I_{50} values for shoot height of cucumber found by the authors are close to those obtained in this work (21.2 g a.i. ha^{-1}).

The I_{50} indexes provide evidence that lettuce was most susceptible species to pyroxasulfone, followed by cucumber and sorghum. Assuming the highest rate (100 g a.i. ha^{-1}) did not affect the development of tomato plants, we were not able to estimate $I_{50,}$ and thus suggesting that tomato is not an effective option as a bioindicator. In summary, the order of sensitivity among the species evaluated in this research was lettuce > sorghum ≥ cucumber>tomato.

In order to identify the most useful bioindicator for pyroxasulfone soil activity, it is critical not only to consider the species susceptibility but also its adaptability. In this research, for example, lettuce was the most susceptible species to pyroxasulfone. However, some difficulties in the use of this species in practical tests can be encountered due to sensibility to adverse climatic conditions. Conditions such as high temperatures and tropical rain regimes can hinder their development and damage plants (Bezerra Neto et al. 2005; Gomes et al. 2005; Brzezinski et al. 2017). Because of some of these issues, it is extremely important to evaluate more than one species in bioindicator studies. Here, we demonstrated that sorghum may also be a suitable species to detect pyroxasulfone soil activity, manly based on plant height measurements (I_{50} = 9.7 g a.i. ha^{-1}). In addition to ryegrass (Khalil et al. 2018b) and sugar beet (Szmigielski et al. 2014), the present work provides at least three additional species (lettuce, sorghum, and cucumber) that can be used as a bioindicator for pyroxasulfone.

Conclusion

Lettuce was the most useful species selected for pyroxasulfone soil studies. Sorghum and cucumber also showed to be potential candidates as bioindicators. Tomato was considered tolerant to pyroxasulfone, and therefore not considered a good option as a bioindicator for pyroxasulfone.

Acknowledgements

This research was funded in part by the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) - Brazil, and Núcleo de Estudos Avançados em Ciência das Plantas Daninhas of Universidade Estadual de Maringá (NAPD / UEM) - Brazil.

Conflicts of Interest

The authors have declared no conflicts of interest.

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