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Dose–Response Relationships of flumioxazin, S-Metolachlor, and the Apresa® Mixture in Vegetables: Implications for Germination and Root Development

Relação Dose-Resposta de Flumioxazina, S-metolacloro e da Mistura Apresa® em Espécies Olerícolas: Implicações para Germinação e Desenvolvimento Radicular

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Abstract

This study aimed to evaluate the phytotoxicity of flumioxazin, S-metolachlor, and their combined formulation (Apresa®) at different concentrations on lettuce (*Lactuca sativa*), onion (*Allium cepa*), and cucumber (*Cucumis sativus*). Germination and root growth tests were performed using seeds exposed to the field-recommended dose (FD), as well as to dilutions of 1%, 3%, 12.5%, 25%, and 50% FD, and at a 300% FD. The different herbicide concentrations identified dose–response relationships, allowing determination of safe thresholds and potential toxic effects. The Apresa® was more toxic than the individual herbicides, particularly to lettuce, with significant reductions in germination at FD ($p < 0.01$) and at 300% FD ($p < 0.001$). Cucumber demonstrated greater tolerance, except at 300% FD of S-metolachlor. Phytotoxic responses varied by crop and formulation: lettuce was more sensitive to S-metolachlor, while cucumber and onion were more affected by the Apresa®. Given the economic and nutritional importance of these vegetables and the widespread use of no-till systems and herbicide combinations, rigorous monitoring of residual effects is essential to guide safer agricultural practices.

Keywords: Phytotoxicity. Residual Herbicides. Vegetable Crops.

Resumo

O objetivo deste estudo foi avaliar a fitotoxicidade da flumioxazina, do S-metolacloro e de sua formulação combinada em diferentes concentrações, utilizando as culturas de alface (*Lactuca sativa*), cebola (*Allium cepa*) e pepino (*Cucumis sativus*). Para isso teste de germinação e crescimento da raiz foram realizados com sementes destas espécies expostas a concentração utilizada em campo (d.c), diluições desta dose à 1%, 3%, 12,5%, 25% e 50% d.c; e também a concentração elevada de 3x d.c. O uso de diferentes doses dos herbicidas permite identificar a relação dose-resposta, determinando os limites seguros para as culturas e os potenciais efeitos tóxicos. A formulação Apresa® demonstrou maior toxicidade em relação aos herbicidas isolados, especialmente para alface, com redução significativa da germinação na dose de campo ($p < 0,01$) e 3x d.c ($p < 0,001$). O pepino mostrou maior tolerância, exceto na concentração de 3x d.c. de S-metolacloro. Assim, nossos resultados indicam que a resposta fitotóxica aos herbicidas varia de acordo com a cultura e a formulação utilizada, sendo a alface mais sensível ao S-metolacloro e o pepino e a cebola mais afetados pela combinação dos compostos. Considerando a importância econômica e nutricional dessas hortaliças, bem como a ampla adoção do plantio direto e o uso crescente de misturas de herbicidas no manejo de plantas daninhas, destaca-se a necessidade de um monitoramento criterioso dos efeitos residuais, sendo fundamentais para orientar práticas agrícolas mais seguras.

Palavras-chave: Fitotoxicidade. Herbicidas Residuais. Olericultura.

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1 Introduction

The carryover effect is one of the main environmental impacts associated with herbicides and is characterized by the persistence of herbicide residues in the soil after a previous crop, impairing the development of subsequent crops. Incomplete degradation of herbicides may hinder plant physiological processes, particularly germination, which is critical for seedling establishment (Mancuso; Negrisoli; Perim, 2011). Although these effects are not always visible, they reduce crop vigor, growth, and productivity, and interfere with reserve mobilization and defense against pathogens (Felix, 2012; Gomes *et al*., 2016).

Irrigated areas commonly support two to three crops per year, which increases the risk. The lack of information on safety intervals in herbicide labels for sensitive successive crops raises further concern. Therefore, assessing herbicide persistence in soil, usually by half-life, guides safe management of these substances.

S-metolachlor is a pre-emergence herbicide used to manage weeds in several crops, including corn and soybeans. Its soil half-life varies from 2.5 to 289 days, depending on edaphic factors, such as texture, pH, moisture content, temperature, aeration, and microbial activity (Gehrke *et al*., 2021; Rice; Anderson; Coats, 2002). This variability, associated with its high environmental persistence, raises concerns about contamination of soil and neighboring ecosystems. Controlled studies found that S-metolachlor persisted longer in dry soils, as low soil moisture reduced its availability and the microbial activity that degrades it t (Christoffoleti *et al*., 2008 ; Gehrke *et al*., 2021).

The half-life of flumioxazin varies from 8.2 to 50.6 days. It depends on soil texture, organic matter content, microbial activity, pH, and environmental conditions (e.g., moisture and precipitation) (Chen *et al*., 2021; Ferrell; Vencill, 2003). Despite its low mobility in clay soils due to the strong adsorption, sandy soils pose a leaching risk that may result in groundwater contamination (Alister *et al*., 2008). Additionally, the use of flumioxazina near planting may cause the carryover effect in sensitive subsequent crops, hindering their growth (Camacho *et al*., 2022).

Continuous herbicide use also fosters weed resistance, reducing the effectiveness of chemicals and threatening agricultural sustainability (HEAP, 2021). To minimize this issue, herbicide combinations with different mechanisms of action have been used to reduce selective pressure and hinder selection of resistant biotypes. This method controls weeds more effectively, but it may pose environmental risks due to compound interactions and soil persistence (Gazziero, 2015; Vargas *et al.*, 1999).

The herbicide Apresa® (flumioxazin + S-metolachlor) was developed to manage weeds effectively across several crops. Despite its widespread use in crops, the literature lacks information on its effects on vegetables and the efficacy of the combination. Several herbicides and their combinations, such as glyphosate, sulfentrazone, and diquat, may cause metabolic damage in vegetables, resulting in chlorosis and necrosis (Coêlho *et al.*, 2024; Perboni *et al.*, 2018; Qi *et al.*, 2017; Silva; Palmieri; Andrade-Vieira, 2022;). Thus, investigating interactions among compounds in commercial formulations is fundamental for effective crop management and minimizing adverse effects on target species (Barbieri *et al.*, 2022).

This study aimed to evaluate the phytotoxicity of flumioxazin, S-metolachlor, and their combination (Apresa®) at different concentrations on lettuce, onion, and cucumber.

2 Material and Methods

The study used the following herbicides: Sumisoya® (flumioxazin, 500 g/kg, Sumitomo), Dual Gold® (S-metolachlor, 960 g/L, Syngenta), and Apresa® (flumioxazin, 42 g/L + S-metolachlor, 840 g/L, ADAMA). Their concentrations were calculated considering the amount of active ingredient in the formulations. For Apresa®, the amount of S-metolachlor stated on the packaging was considered.

A randomized (three × three × eight factorial) design was used, with three plant species, three herbicides, and eight treatments (seven concentrations and a negative control).

The concentrations (Table 1) were defined based on the field-recommended dose (FD): 1%, 3%, 12.5%, 25%, 50%, 100%, and 300%, plus a distilled water control (0%). A positive control (zinc sulfate heptahydrate [6 mg/mL]) was included as a reference, but it was excluded from the analysis.

Table 1 - Pesticide concentrations

Treatment	Flumioxazin (mg mL^{-1})	S-metolachlor (mg mL^{-1})	Mixture (mg mL^{-1})
1% (C1)	0.0025	0.064	0.063
3% (C2)	0.0075	0.192	0.190
12.5%(C3)	0.0313	0.800	0.790
25% (C4)	0.0625	1.600	1.580
50% (C5)	0.1250	3.200	3.150
Field (C6)	0.2500	6.400	6.300
3× (C7)	0.7500	19.200	18.900

Source: research data.

Each herbicide × plant × concentration combination had three independent replicates, totaling 216 experimental units. Each unit comprised a plate or box containing seeds exposed to a fixed volume of the test solution at a controlled temperature (25 ± 1 °C). The design allowed the assessment of main and interactive effects and the evaluation of dose-dependent responses and variation in sensitivity among species.

The response variables included the seed germination rate and radicle length. Since the three pesticides were evaluated across distinct absolute concentration ranges, doses were grouped into seven concentration levels (C1 [lowest] to C7 [highest]) for each compound.

Concentrations were standardized to ensure equivalent exposures regardless of the absolute concentrations used (mg/mL), guaranteeing comparability among herbicides. Rather than directly comparing doses with different values, herbicides were analyzed based on their relative positions within the experimental concentration scale.

The evaluation of statistical assumptions revealed violations of the normality of residuals and homogeneity of variances, precluding the application of conventional parametric ANOVA. Therefore, the aligned rank transformation ANOVA (ART-ANOVA) was employed by using the art() function of the ARTool package. This method was suitable for factorial designs with nonparametric data.

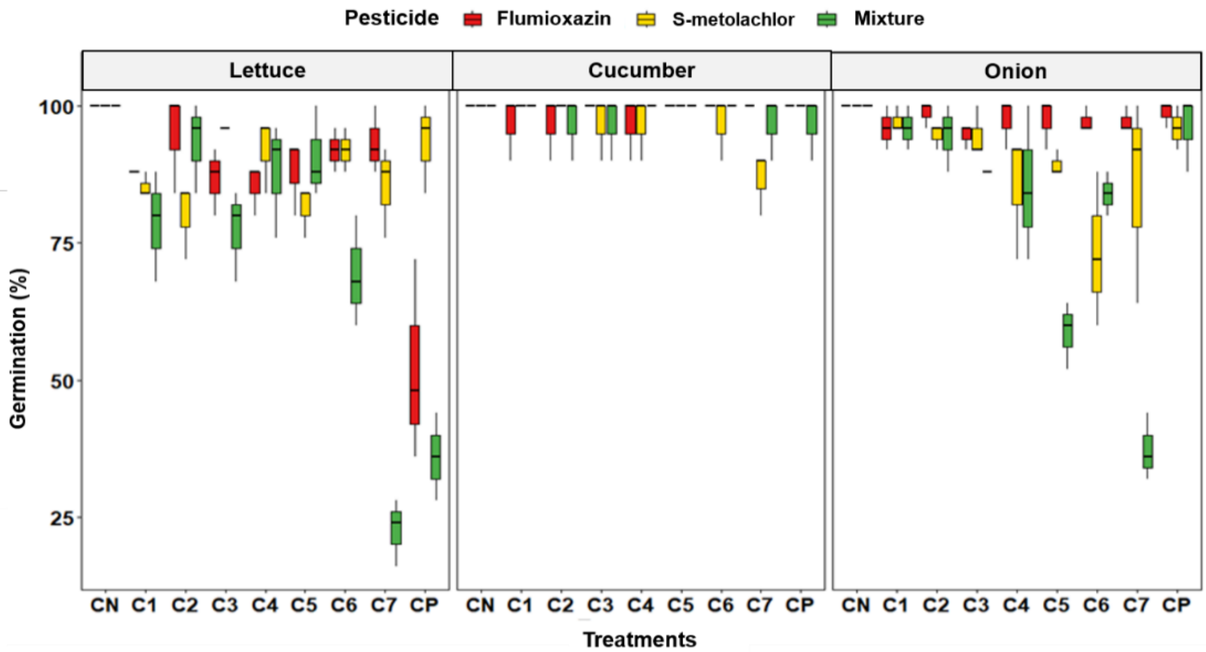
The model included main effects and all interactions. After detecting a significant three-way interaction, the herbicides were compared within each plant and concentration level, using the emmeans() function with a Tukey adjustment to control Type I error. Post hoc analyses focused on contrasts between Apresa® and the other herbicides (Sumisoya® and Dual Gold®).

The effective concentration required to reduce seedling growth by 50% (EC50) was determined using nonlinear log-logistic regression. A negative control was assigned to 0 mg/mL; the positive control was excluded from the analysis. Models were fitted using the drm() function in the drc package in R software, with the LL.4() parameterization (four-parameter log-logistic model). This model was recommended for data that followed a sigmoid pattern, a common feature in toxicological studies (AN *et al*., 2019).

3 Results and Discussion

The analysis using ART-ANOVA revealed significant effects ($p < 0.001$) for all main factors (herbicides, plant species, and treatment) , as well as for all second-order interactions and the three-way interaction (herbicide × plant species × treatment). The significant three-way interaction indicated that the effect of each herbicide on seed germination varied according to plant species and herbicide concentrations. The toxicity of the combination (flumioxazin + S-metolachlor) varied with plant species and exposure level (Figure 1). These interactions underscored the need to evaluate commercial formulations across experimental contexts, because their effects cannot be generalized across species or exposure conditions.

Figure 1 - Distribution of the germination rate (%) of lettuce (*Lactuca sativa*), cucumber (*Cucumis sativus*), and onion (*Allium cepa*) seeds exposed to commercial formulations containing flumioxazin, S-metolachlor, or the combined formulation (mixture), at different concentrations (C1 to C7), negative control (NC), and positive control (PC). The boxplots show the median (central line) and the quartiles (box limits)



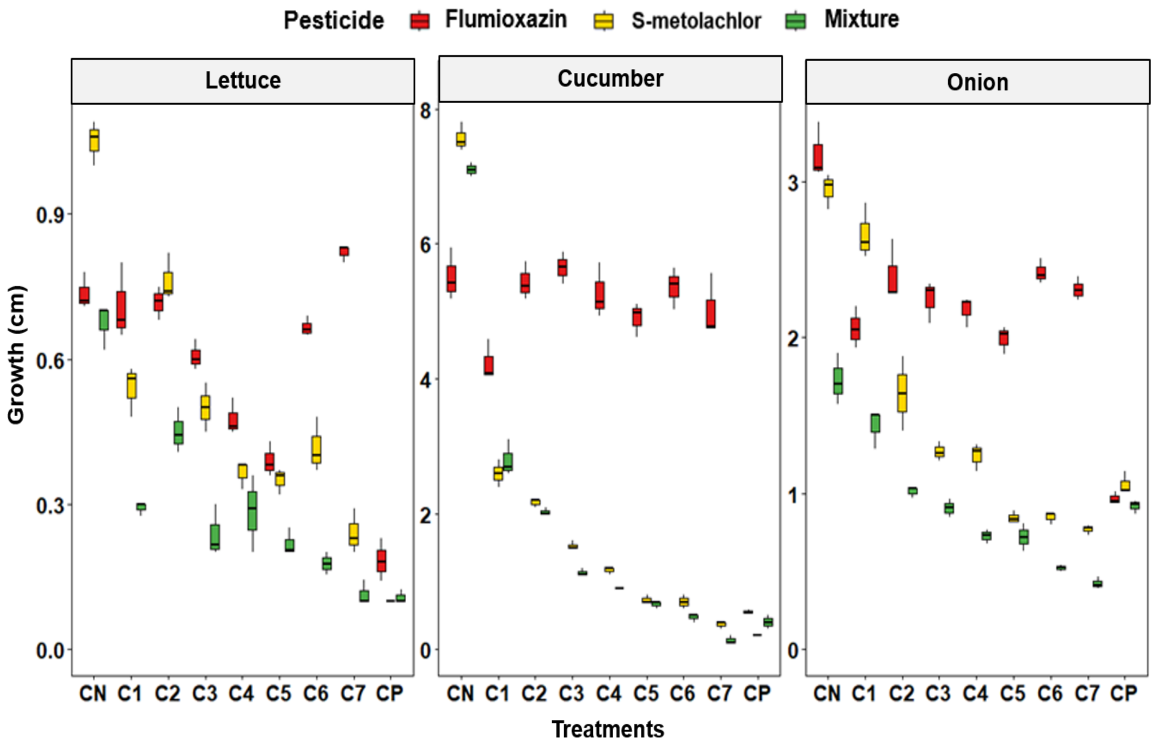
Source: research data.

The combination of flumioxazin + S-metolachlor served as the reference in multiple comparisons. Doses of the three herbicides were grouped into seven concentration levels (C1 to C7), representing increasing exposure within the range of each product. Standardizing doses enabled an equitable assessment of herbicide toxicity across exposure levels.

In general, the combination of flumioxazin + S-metolachlor was more toxic than the isolated active ingredients. In lettuce, the concentration of the combination at C6 and C7 significantly reduced the germination ($p < 0.01$ and $p < 0.001$, respectively). In onion, the C5 and C7 treatments produced similar effects ($p < 0.001$). Cucumber showed a higher tolerance, maintaining germination at all tested concentrations except at S-metolachlor C7.

A significant three-way interaction (herbicide × plant species × concentration; $p < 0.001$) affected radicle length. This result indicated that herbicide effects on root growth depended on species and concentration (Figure 2).

Figure 2 - Distribution of radicle growth of lettuce (*Lactuca sativa*), cucumber (*Cucumis sativus*), and onion (*Allium cepa*) seeds exposed to commercial formulations containing flumioxazin, S-metolachlor, or the combined formulation (mixture), at different concentrations (C1 to C7), negative control (NC), and positive control (PC). The boxplots show the median (central line) and the quartiles (box limits)



Source: research data.

Residual herbicides are widely used in weed management in intensive agricultural systems. This persistence may cause a carryover effect, in which residues remain active after a crop and hamper germination and development of subsequent crops, particularly those more sensitive (i.e., vegetables) (Fontes; Atroch; De Morais, 2025). Germination is vulnerable to residual herbicides even at low concentrations. As herbicide combinations are increasingly adopted to mitigate weed resistance, the toxicity of S-metolachlor, flumioxazin, and their combined formulations must be assessed. Although few studies address residual effects on vegetables, this gap underscores the need to clarify risks to germination and early seedling growth and to support safer, more sustainable herbicide use (Melo *et al*., 2016).

Flumioxazin reduced root growth in all three species, especially in lettuce. Its EC50 was estimated at 0.0176 mg mL^{-1}. Onion and cucumber showed greater tolerance, possibly due to more efficient metabolism or lower susceptibility to cytotoxic effects (Vollmer *et al*., 2024 ; Zhang; Yang, 2021). Tavares *et al*. (2020) reported that bean seed germination was maintained after flumioxazin at recommended doses, highlighting the variability in response among crops.

Lettuce was more sensitive to S-metolachlor, while cucumber and onion showed smaller growth reductions (Boschiero, 2022; Schaeffer *et al*., 2024). S-metolachlor impairs lettuce germination and vigor by inhibiting essential lipid synthesis, which explains its phytotoxicity. Soil residues can affect subsequent crops, underscoring the need to consider persistence and crop rotation.

The combination of flumioxazin + S-metolachlor acted synergistically, increasing toxicity compared with the individual products and significantly reducing onion and cucumber root growth. Synergism likely reflects complementary modes of action: flumioxazin impairs chlorophyll synthesis, and S-metolachlor disrupts cuticle formation and energy accumulation. However, information on Apresa® remains limited. The results suggested that the combination increased the absorption and phytotoxicity of the active ingredients, potentially generating synergistic interactions, depending on herbicide compatibility (Matos, Borges, 2024).

All treatments affected species growth. The combination of flumioxazin + S-metolachlor was more toxic to onion and cucumber, even at low concentrations. Lettuce showed reduced growth but maintained better germination with the combination, highlighting interspecific differences in herbicide response.

4 Conclusion

S-metolachlor and flumioxazin impaired germination and early growth in horticultural species, when applied alone or combined in the commercial formulation Apresa®. Sensitivity varied among crops: lettuce was more sensitive to S-metolachlor, whereas onion and cucumber were more sensitive to the combination. This result indicated species- and formulation-dependent responses to herbicides.

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