

## Mitigating selective herbicide stress in maize (*Zea mays* L.) with biostimulant intervention

Saeed Reza YAGHOobi<sup>1</sup> (✉), Ali MOKHTASSI-BIDGOLI<sup>2</sup>, Saba HAJHEIDARI<sup>3</sup>

<sup>1</sup> Department of Agricultural Engineering, Faculty of Agriculture, Technical and Vocational University (TVU), Postal code: 1435661137, Tehran, Iran

<sup>2,3</sup> Department of Agronomy, Faculty of Agriculture, Tarbiat Modares University, P.O. Box: 14115-336, Tehran, Iran

✉ Corresponding author: sr-yaghoobi@tvu.ac.ir

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### ABSTRACT

Biostimulants, integral to organic crop management, offer a sustainable approach to enhancing plant growth and resilience. Integrating biostimulants with selective herbicides may reduce application costs while mitigating herbicide-related stress. This study investigates the combined effects of maize-selective herbicide (mesotrione + nicosulfuron at 0, 105, 157.5, 210, and 315 g active ingredient (a.i.) per hectare) with various biostimulants amino acid fertilizers (1 and 3 g/L), seaweed extracts (4 and 12 mL/L), potassium chloride (2 and 6 g/L), and a mixture of potassium chloride (2 g/L) + amino acid (1 mL/L) compared to a control with no fertilizer on maize and redroot pigweed (*Amaranthus retroflexus*). Biostimulant addition, particularly amino acids or potassium chloride at 105 g/ha a.i., significantly improved maize total dry matter, leaf area, and root volume. Moreover, incorporating seaweed extract into the herbicide mixture significantly enhanced chlorophyll a and b and carotenoid levels in maize. Notably, seaweed treatments raised the maize mortality ED<sub>50</sub> from 223.5 g/ha a.i. (control) to 448.8 and 579.6 g/ha a.i., indicating increased herbicide tolerance. Conversely, biostimulant addition had a minimal impact on redroot pigweed mortality. These findings suggest that combining biostimulants with herbicides can improve maize tolerance without reducing weed control efficacy.

**Keywords:** eco-friendly farming practices, fertilizer, herbicide tolerance, weed management

### INTRODUCTION

Weeds pose significant challenges to maize cultivation, necessitating the use of selective herbicides for effective management in crop fields. At the same time, foliar fertilization plays a key role in improving crop health and yield. The practice of combining fertilizers with herbicides in a single tank mix is increasingly popular among farmers. This approach not only minimizes application time and soil compaction but may also reduce crop stress and enhance productivity (Mesarović et al., 2019).

The practice of mixing herbicides and fertilizers in a single tank mix primarily aims to enhance herbicide effectiveness (Devkota and Johnson, 2016), prevent crop damage (Hooker et al., 2018), and ultimately boost crop yields (Nelson et al., 2012). Additionally, integrating fer-

tilizers with selective herbicides can offset the costs associated with separate applications (Nelson et al., 2012). Biostimulants, natural products that enhance plant vigor and stress tolerance, have gained traction in sustainable agriculture (Bulgari et al., 2019; Shahrajabian et al., 2021). The review by Shahrajabian et al. (2021) focuses on the diverse class of compounds known as biostimulants, which include substances or microorganisms that positively impact plant growth, yield, chemical composition, and stress tolerance. This paper highlights the major categories of biostimulants, ranging from plant and animal protein hydrolysates to humic substances, seaweed extracts, and microbial origin compounds and their potential mechanisms of action. These mechanisms predominantly relate to improved physiological processes

and plant morphology, such as enhanced root formation, increased nutrient uptake, and improved seed germination rates. The review underscores the growing interest and market potential for biostimulant products, which are expected to significantly contribute to sustainable crop management and organic farming practices.

Seaweed extracts are extensively utilized as biostimulant fertilizers, attributed to their rich content of plant growth regulators, including cytokinins, auxins, gibberellins, and betaines, alongside essential micronutrients and macronutrients, as well as beneficial natural components such as polysaccharides (Begum et al., 2018). A study by Vijayakumar et al. (2019) highlighted the positive response of *Capsicum annum* crop traits to seaweed liquid fertilizer, noting significant increases in leaf area, dry weight, and chlorophyll concentration, particularly with the application of a 20% seaweed liquid fertilizer solution. The efficacy of seaweed extracts in boosting crop yields has been extensively documented across a variety of crops, such as maize (Basavaraja et al., 2018), underscoring their versatile role in sustainable agriculture.

Amino acid fertilizers and protein hydrolysates, derived through the chemical or enzymatic hydrolysis of plant and animal feedstocks, represent a significant category of biostimulants aimed at enhancing plant growth and soil health (Radkowski and Radkowska, 2018). Aamlid et al. (2017) observed that the application of amino acid fertilizers led to a 75% turfgrass coverage, a significant improvement over the 35% coverage achieved with conventional nitrogen fertilizers within 26 days post-fertilization. Radkowski and Radkowska (2018) found that applying 3.0 L/ha of amino acid fertilizer increased the yield of timothy seeds by 11% compared to untreated plots.

The integration of biostimulants with herbicides has been investigated in a variety of crops, including potato (Ginter et al., 2022), oilseed rape (Neshev et al., 2022), wheat (Matysiak et al., 2018; Soltani et al., 2015a), maize (Panfili et al., 2019), soybean (Soltani et al., 2015b), and dry bean (Soltani et al., 2015b). These studies aimed to achieve efficient weed management, optimize crop yields,

and minimize herbicidal damage. Panfili et al. (2019) observed that biostimulants increased maize's resilience to metolachlor through the augmentation of antioxidant enzyme activities. Conversely, several studies indicated that combining biostimulants with herbicides such as glyphosate, chlorimuron, imazethapyr, fomesafen, or quizalofop in soybean (Soltani et al., 2016) and a mixture of glyphosate, topramezone, and atrazine in corn (Soltani et al., 2015a) did not affect crop injury, weed control, or yield outcomes. For instance, Matysiak et al. (2018) noted that combining biostimulants with herbicide mixtures in wheat did not impact herbicide efficiency but improved grain yield and gluten content. Potassium plays a pivotal role in plant growth and metabolism, contributing significantly to a plant's defense mechanisms against both biotic and abiotic stresses (Srinivasarao and Kundu, 2017). The strategic inclusion of foliar mineral fertilizers in herbicide mixtures offers a promising approach to achieve effective weed management while enhancing crop yields and reducing costs (Lawrence et al., 2020). Nelson et al. (2012) found that potassium sulfate improved the performance of glyphosate in resistant soybean fields.

The present study tested the hypothesis that combining biostimulants (seaweed extract, amino acid fertilizer, and potassium chloride) with a maize-selective herbicide can enhance weed control while reducing herbicide-induced crop damage. Specifically, we aim to 1) evaluate the effectiveness of biostimulants in enhancing redroot pigweed suppression when mixed with herbicides; 2) assess their role in improving maize growth and stress tolerance; 3) identify optimal biostimulant and herbicide combinations for weed control and crop safety; 4) explore potential physiological mechanisms underlying the observed effects.

## MATERIALS AND METHODS

### *Experimental site and design*

The maize (*Zea mays* L. var. NS 640) seeds for this study were obtained from the Seed and Plant Improvement Institute in Karaj, Alborz province, Iran. Redroot pigweed (*Amaranthus retroflexus* L.) seeds were harvest-

ed from the fields of the Faculty of Agriculture, Tarbiat Modares University, Tehran, Iran, between July and August 2017, and were subsequently stored under dry conditions at room temperature to preserve their viability.

The greenhouse experiments were conducted in 2018 at the Faculty of Agriculture, Tarbiat Modares University, located along the Tehran-Karaj highway (35°44'N, 51°10'E and 1,265 m above sea level), Tehran, Iran. The experimental design was a factorial arrangement in a randomized complete block design (RCBD) with three replications. The experimental factors in the study were factor A) Herbicide application rate of mesotrione + nicosulfuron (Elumis®, 10.5% OD, Syngenta, Switzerland); Factor B) the application of different biostimulants and fertilizers: amino acid fertilizer (Amino Plus, Biofert Co., Canada) at concentrations of 1 and 3 g/L, seaweed extract at concentrations of 4 and 12 mL/L, and potassium chloride at concentrations of 2 and 6 g/L. Additionally, a mixture of potassium chloride (2 g/L) with amino acid (1 mL/L) and a control (no fertilizer). All fertilizers and herbicides were applied as tank mixtures. Maize and redroot pigweed seeds were sown in plastic pots (40 cm diameter × 60 cm height), filled with a soil mixture composed of 50% clay, 30% sand, and 20% compost to ensure adequate drainage and nutrient availability. Both species were thinned at the 3-leaf stage to maintain 5 maize and 10 pigweed plants per pot, optimizing growth conditions and minimizing competition. The application of herbicide and fertilizer mixtures was carried out at the 4-leaf stage of both maize and redroot pigweed using a 16-litre knapsack sprayer (HECHT 4166) at a 3-bar pressure to ensure uniform coverage.

### Sample collection

Fifteen days post-treatment, leaf samples from maize were collected to quantify chlorophyll *a*, chlorophyll *b*, and carotenoids, using the method described by Hiscox and Israelstam (1979). Three maize plants per treatment were carefully removed from the pots for leaf area measurements, which were conducted using a leaf area meter (DELTA-T DEVICES, UK). Subsequently, these plants

were oven-dried at 70 °C for 72 hours and then weighed to determine dry biomass. Root volume was assessed by water displacement in a 5-L graduated cylinder, filled with water. The water volume displacement caused by the submersion of the roots was used to calculate root volume, following the method outlined by Pang et al. (2011): Root volume = Final volume - Initial volume. This precise methodology ensures accurate assessment of the impacts of treatments on plant growth and development.

### Statistical analysis

Data were analyzed using two-way ANOVA under the General Linear Model (GLM) procedure in SAS 9.0. The main effects and interaction of herbicide dose and biostimulant type were treated as fixed effects. Residual normality was assessed using PROC UNIVARIATE. Significant two-way F-tests warranted mean separation using the slicing method at  $P \leq 0.05$ , applying the protected least significant difference (LSD) to dissect herbicide dose × biostimulant type interactions. This approach, appropriate for analyzing significant interactions, partitions the dataset to allow detailed comparisons of biostimulant types within each herbicide dose. A four-parameter log-logistic model (Eq. 1) was used to analyze dose-response relationships, based on Alizadeh et al. (2020), using the NLIN procedure in SAS. This analysis plotted the dry biomass of maize or redroot pigweed ( $y$ ) against the herbicide doses ( $x$ ), where 'b' represents the slope at the  $ED_{50}$  point, 'min' denotes the minimal response limit at an exceedingly high herbicide dose ratio (approaching zero maize biomass loss at maximum herbicide dosage), ' $ED_{50}$ ' is the herbicide dose leading to a 50% reduction in maize or redroot pigweed dry weight, and 'max' indicates the maximal response limit (the utmost maize or redroot pigweed biomass at zero herbicide application).

Equation 1: Four-parameter logistic model

$$y = \min + (\max - \min) / (1 + \exp[b \times (\ln(x) - \ln(ED_{50}))])$$

- min = minimum response (at the highest herbicide dose)
- max = maximum response (no herbicide)

- $b$  = slope around  $ED_{50}$
- $ED_{50}$  = herbicide dose causing 50% biomass reduction

To estimate  $ED_{10}$  and  $ED_{90}$ , equations were modified as:

Equation 2:

$$ED_{10} = \exp[\ln(ED_{50}) - (1/b) \times \ln(9)]$$

Equation 3:

$$ED_{90} = \exp[\ln(ED_{50}) + (1/b) \times \ln(9)]$$

Model performance was assessed using the coefficient of determination ( $R^2$ ) and root mean square error (RMSE):

Equation 4: Coefficient of Determination ( $R^2$ )

$$R^2 = 1 - [\sum(M_i - P_i)^2 / \sum(M_i - A_i)^2]$$

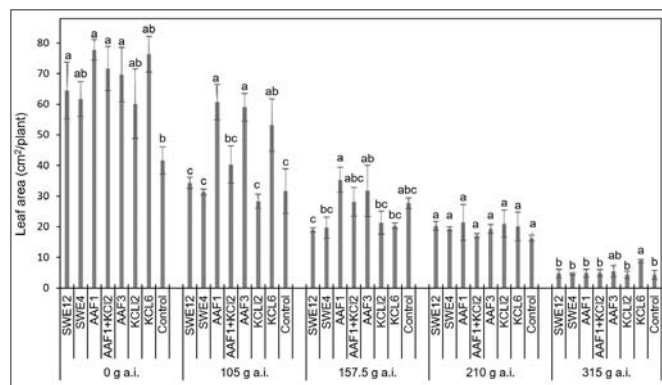
Equation 5: Root Mean Square Error (RMSE)

$$RMSE = \sqrt{[\sum(P_i - M_i)^2 / n]}$$

- $M_i$  = measured biomass
- $P_i$  = predicted biomass
- $A_i$  = mean measured biomass
- $n$  = number of observations.

## RESULTS

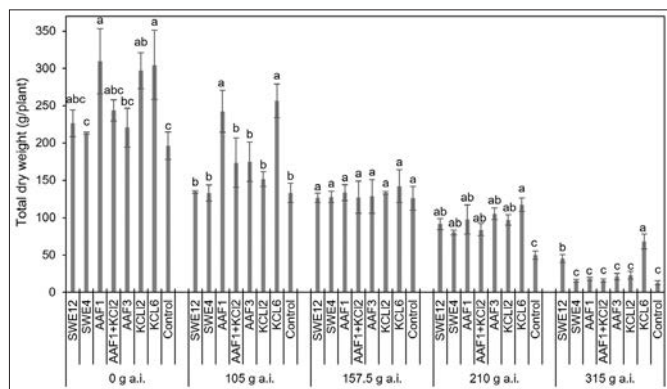
The analysis of variance revealed that the two-way interaction between the experimental factors significantly influenced all the measured traits. The interaction between mesotrione + nicosulfuron significantly influenced all measured maize growth traits and redroot pigweed mortality (Figures 1-5 and Table 1-2). These included leaf area, total dry weight, root volume, chlorophyll and carotenoid concentrations. Redroot pigweed mortality was also interestingly affected by these treatment combinations. When no herbicide was applied (control), all biostimulant treatments significantly enhanced maize leaf area and dry weight. Among these, 1 and 3 mL/L amino acid and 6 g/L potassium chloride, combined with 105 g/ha a.i. mesotrione + nicosulfuron produced the greatest improvements in maize leaf area and biomass.



**Figure 1.** Effect of tank mixing mesotrione + nicosulfuron (0–315 g/ha a.i.) with various biostimulants on maize leaf area. Treatments include seaweed extract (SWE4, SWE12), amino acid fertilizer (AAF1, AAF3), potassium chloride (KCl4, KCl12), and combined AAF1 + KCl2. Error bars represent standard error. Within each mesotrione + nicosulfuron dose, means sharing the same letter are not significantly different at  $P \leq 0.05$ .

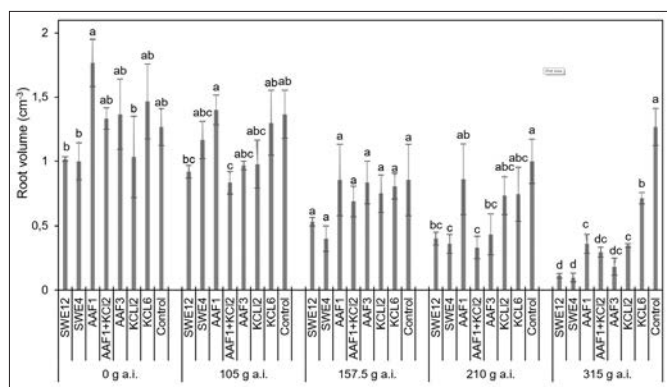
An observed increase in the mesotrione + nicosulfuron dose from 105 to 157.5 g/ha a.i. resulted in a significant reduction in both maize leaf area and total dry weight (Figures 1 and 2). However, 1 mL/L amino acid or 6 g/L potassium chloride mitigated these reductions in maize leaf area and total dry weight associated with this herbicide application. At doses beyond 157.5 g/ha a.i., the protective effects of all biostimulants were lost. Importantly, maximum dry matter accumulation (310–304 g per plant) was observed in treatments with no herbicide but with amino acid and potassium chloride additions. With 105 g/ha a.i. of mesotrione + nicosulfuron conditions alone, maize dry weight dropped to 133 g per plant. However, tank mixing with 1 mL/L amino acid or 6 g/L potassium chloride significantly restored dry weight to 242 and 256 g, respectively (Figure 2). Herbicide doses above 157.5 g/ha a.i. did not improve maize dry weight, though a marginal benefit was noted when 6 g/L potassium chloride or 12 mL/L seaweed extract was added at 315 g/ha a.i.

Under herbicide-free conditions, 1 mL/L amino acid or 6 g/L KCl significantly increased maize root volume (Figure 3). However, introducing seaweed extract at concentrations of 4- or 12-mL/L reduced root volume to  $\sim 1.01$  cm<sup>3</sup>. When mesotrione + nicosulfuron was applied at a rate of 105 g/ha a.i., maize root volume reached 1.36 cm<sup>3</sup>.



**Figure 2.** Effect of mesotrione + nicosulfuron dose and biostimulant co-application on maize dry weight. Treatments include seaweed extract (SWE4, SWE12), amino acid fertilizer (AAF1, AAF3), potassium chloride (KCl4, KCl12), and combined AAF1 + KCl2. Error bars represent standard error. Within each mesotrione + nicosulfuron dose, means sharing the same letter are not significantly different at  $P \leq 0.05$ .

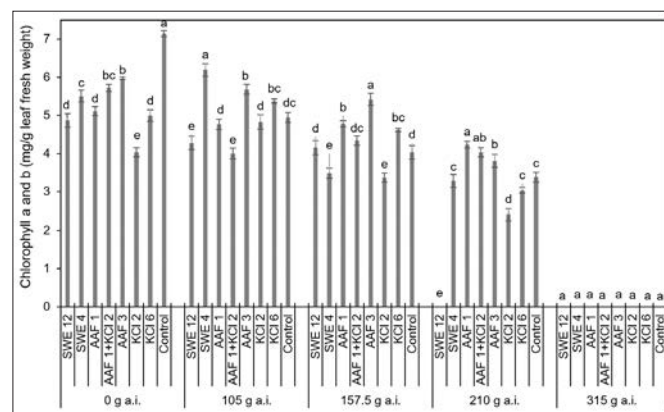
Combining this herbicide treatment with 1 mL/L amino acid or 6 g/L potassium chloride was statistically similar to the untreated control. However, incorporating seaweed extract at 4- and 12-mL/L with mesotrione + nicosulfuron at 105 g/ha a.i. decreased maize root volume. Higher herbicide doses nullify these root-growth benefits.



**Figure 3.** Maize root volume in response to herbicide dose and biostimulant treatments. Treatments include seaweed extract (SWE4, SWE12), amino acid fertilizer (AAF1, AAF3), potassium chloride (KCl4, KCl12), and combined AAF1 + KCl2. Error bars represent standard error. Within each mesotrione + nicosulfuron dose, means sharing the same letter are not significantly different at  $P \leq 0.05$ .

Under conditions devoid of both herbicide and fertilizer, the concentrations of chlorophyll and carotenoids in maize leaves were 7.14 mg/g and 0.61 mg/g fresh weight, respectively. In the control group without herbicide ap-

plication, chlorophyll concentration was maintained, whereas the introduction of fertilizers did not significantly alter carotenoid levels in maize leaves (Figures 4 and 5). 105 g/ha a.i. herbicide with 1 mL/L amino acid led to the lowest carotenoid concentration (0.43 mg/g), while 2 g/L KCl reduced chlorophyll to 4.03 mg/g (Figures 4–5). A significant difference in chlorophyll and carotenoid concentrations in maize was observed when fertilizers were tank mixed with mesotrione + nicosulfuron at doses of 157.5 g/ha a.i. or higher. Specifically, tank-mixing 3 mL/L amino acid fertilizer with mesotrione + nicosulfuron at 157.5 and 210 g/ha a.i. significantly boosted the concentrations of both chlorophyll and carotenoids in maize leaves, achieving chlorophyll levels of 5.4–5.6 mg/g of leaf fresh weight, and carotenoid concentrations of 0.66–0.77 mg/g of leaf fresh weight, respectively.



**Figure 4.** Chlorophyll (a and b) and carotenoid concentrations in maize leaves across treatments. Treatments include seaweed extract (SWE4, SWE12), amino acid fertilizer (AAF1, AAF3), potassium chloride (KCl4, KCl12), and combined AAF1 + KCl2. Error bars represent standard error. Within each mesotrione + nicosulfuron dose, means sharing the same letter are not significantly different at  $P \leq 0.05$ .

Conversely, the addition of 12 mL/L seaweed extract significantly suppressed pigment concentrations. Increasing the dose of mesotrione + nicosulfuron from 105 to 210 g/ha a.i. resulted in a decrease in chlorophyll concentration from 4.9 to 3.38 mg/g of leaf fresh weight and a reduction in carotenoid levels from 0.55 to 0.49 mg/g of leaf fresh weight. At the highest tested dose of mesotrione + nicosulfuron at 315 g/ha a.i. resulted in the complete depletion of chlorophyll and carotenoids. (Figures 4 and 5).

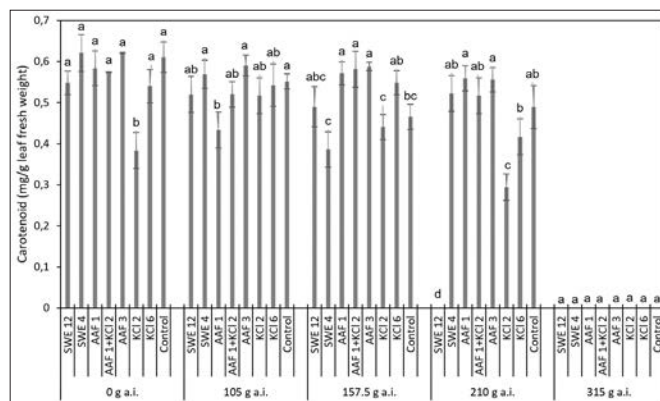
In the control group without any fertilizer, the effective dose to achieve 90% reduction, ED<sub>90</sub> for maize biomass, was determined to be 223.5 g/ha a.i. However, 4 and 12 mL/L of seaweed extract increased ED<sub>90</sub> to 448.8 and 579.6 g/ha a.i., indicating enhanced tolerance (Table 1). The addition of an amino acid (1 mL/L) in a tank mix with mesotrione + nicosulfuron significantly reduced herbicidal damage to maize, with the effective doses for 10% reduction (ED<sub>10</sub>) and 50% reduction (ED<sub>50</sub>) being 114.6 / 162.2. Similarly, mixing KCl (6 g/L) with mesotrione + nicosulfuron resulted in ED<sub>10</sub> and ED<sub>50</sub> values of 109.8 / 167.1 g/ha a.i. for maize. Conversely, combining

1 mL/L amino acid or 6 g/L potassium chloride with mesotrione + nicosulfuron slightly decreased maize's herbicide tolerance. Their ED<sub>90</sub> values were moderately elevated to 237.8 and 253.1 g/ha a.i., respectively (Table 1). Overall, biostimulants increased maize's tolerance to mesotrione + nicosulfuron without fully negating herbicidal injury. In redroot pigweed, ED<sub>90</sub> in the control group was 135.6 g/ha a.i., consistent across most treatments except 12 mL/L seaweed extract, which slightly increased ED<sub>90</sub> to 144.2 g/ha a.i. (Table 2). Seaweed extract raised pigweed ED<sub>10</sub> and ED<sub>50</sub> values to 63.0 / 91.7 and 69.7 / 100.2 g/ha a.i., indicating some tolerance shift (Table 2).

**Table 1.** Logistic model coefficients and ED<sub>10</sub>, ED<sub>50</sub>, and ED<sub>90</sub> values for maize biomass under different treatments

Biostimulants & fertilizer	Model coefficient (standard error)						Model accuracy Parameter	
	a	b	c	ED <sub>10</sub>	ED <sub>50</sub>	ED <sub>90</sub>	R <sup>2</sup>	RMSE
		%		g/ha a.i.				
Seaweed extract 4 mL/L	0.00	-1.60 (0.25)	100	28.65 (8.59)	113.6 (12.40)	448.8 (77.30)	0.99	7.75
Seaweed extract 12 mL/L	0.73 (0.72)	-1.36 (0.34)	100	22.92 (12.4)	115.5 (21.90)	<b>579.6</b> <b>(187.00)</b>	0.97	11.64
Amino acid fertilizer 1 mL/L	0.00	-6.00 (0.00)	100	114.6 (1.91)	165.2 (2.86)	237.8 (3.82)	0.99	3.82
Amino acid fertilizer 3 mL/L	0.78 (0.77)	-2.13 (0.30)	100	49.66 (9.55)	138.5 (12.40)	388.6 (51.50)	0.98	8.29
Potassium chloride 2 g/L	0.00	-2.23 (0.04)	100	35.33 (9.55)	94.5 (9.55)	252.1 (42.00)	0.99	8.96
Potassium chloride 6 g/L	0.00	-5.26 (2.36)	73.98 (5.75)	109.8 (24.8)	<b>167.1</b> <b>(14.30)</b>	253.1 (45.80)	0.97	9.94
Amino acid fertilizer 1 mL/L+ Potassium chloride 2 g/L	1.00 (0.00)	-2.33 (0.42)	100	48.7 (10.5)	125.1 (10.50)	321.8 (52.50)	0.98	10.55
Control	0.05 (0.03)	-1.32 (0.51)	100	7.64 (8.59)	42.02 (10.20)	223.5 (62.10)	0.99	9.22

The symbols a, b, and c represent the lower limit, slope, and upper limit of the response curve, respectively. ED<sub>10</sub>, ED<sub>50</sub>, and ED<sub>90</sub> denote the effective doses required to reduce maize total dry weight by 10%, 50%, and 90%, respectively. Standard errors are enclosed in parentheses for clarity. Bold values indicate the highest ED estimates for maize within each column. Bold values indicate the highest ED estimates within each column; higher ED values denote greater maize tolerance to herbicide injury.



**Figure 5.** Chlorophyll (a and b) and carotenoid concentrations in maize leaves across treatments. Treatments include seaweed extract (SWE4, SWE12), amino acid fertilizer (AAF1, AAF3), potassium chloride (KCl4, KCl12), and combined AAF1 + KCl2. Error bars represent standard error. Within each mesotrione + nicosulfuron dose, means sharing the same letter are not significantly different at  $P < 0.05$ .

**Table 2.** Logistic model coefficients and effective dose estimates for redroot pigweed biomass under various treatments

Biostimulants & fertilizer	Model coefficient (standard error)						Model accuracy Parameter	
	a	b	c	ED <sub>10</sub>	ED <sub>50</sub>	ED <sub>90</sub>	R <sup>2</sup>	RMSE
	%			g/ha a.i.				
Seaweed extract 4 mL/L	0.00	-6.00	100	63.03 (5.73)	91.68 (6.68)	135.60 (0.00)	0.95	19.40
Seaweed extract 12 mL/L	0.00	-6.00	100	69.71 (5.73)	<b>100.2</b> <b>(7.64)</b>	<b>144.20</b> <b>(11.4)</b>	0.95	19.49
Amino acid fertilizer 1 mL/L	0.00	-6.00	100	49.66 (1.01)	66.86 (2.05)	135.60 (4.08)	0.99	4.68
Amino acid fertilizer 3 mL/L	0.75 (0.28)	-6.00	100	49.66 (1.07)	66.86 (1.09)	135.60 (5.01)	0.99	3.97
Potassium chloride 2 g/L	0.04 (0.09)	-3.86 (1.05)	100	49.66 (1.05)	66.86 (1.90)	135.60 (3.70)	0.99	8.47
Potassium chloride 6 g/L	1.00	-6.00	100	49.66 (1.03)	66.86 (2.02)	135.60 (3.99)	0.99	4.68
Amino acid fertilizer 1 mL/L+	0.0005	-4.29	100	49.66 (1.00)	81.17 (17.20)	135.60 (7.05)	0.98	13.74
Potassium chloride 2 g/L	(0.003)	(0.65)						
Control	1.00	-6.00	100	49.66 (1.02)	66.85 (2.08)	135.60 (2.50)	0.99	4.68

The symbols a, b, and c represent the lower limit, slope, and upper limit of the response curve, respectively. ED<sub>10</sub>, ED<sub>50</sub>, and ED<sub>90</sub> denote the effective doses required to reduce redroot pigweed total dry weight by 10%, 50%, and 90%, respectively. Standard errors are enclosed in parentheses for clarity. Bold values indicate the highest ED estimates within each column; higher ED values denote greater redroot pigweed tolerance and potentially reduced herbicide efficacy.

The application of mesotrione + nicosulfuron combined with either 1 or 3 mL/L amino acids, or 2 or 6 g/L potassium chloride, produced effects on redroot pigweed similar to those of the control treatment, with ED<sub>10</sub> and ED<sub>50</sub> values of 49.66 and 66.85 g a.i./ha, respectively. Interestingly, when 1 mL/L amino acid and 2 g/L potassium chloride were used in a tank mix with mesotrione + nicosulfuron, the ED<sub>50</sub> for redroot pigweed was determined to be 81.17 g/ha a.i. slight interference with herbicide efficacy (Table 2).

## DISCUSSION

Biostimulants have long been recognized as organic resources capable of enhancing crop yields. In recent years, the practice of combining biostimulants with herbicides in tank mixes has gained attention for its potential to not only increase crop yield (Ginter et al., 2022) but also to lower the costs associated with foliar spraying. Despite the widespread adoption of selective herbicides for effective weed management, phytotoxic effects, particularly oxidative stress, can negatively affect crop development, affecting post-emergence applications (Dragičević et al., 2012). Safeners included in herbicide formulations play a critical role in mitigating herbicide-induced damage to crops and enhancing selectivity between crops and weeds (Jia et al., 2022), although not all herbicides are formulated with these protective agents. Katsenios et al. (2023) reviewed the application of biostimulants and herbicides in agriculture, focusing on their co-implementation as a new cultivation practice. It discussed how biostimulants, when combined with herbicides, can serve as a safener strategy by reducing their harmful effects, potentially introducing a sustainable agricultural technique. However, it also acknowledges that the effects can vary, being either positive, negative, or non-significant, largely depending on the specific interactions between the plant species, biostimulant, and herbicide used. In addition, Kanatas et al. (2022) suggested that using biostimulants with herbicides could potentially allow for reduced herbicide application rates, aligning with the Green Deal and agroecological approaches. However, they also noted the need

for further research to explore this possibility in more depth across various crops, weed species, and environmental conditions.

In the current study, biostimulants significantly improved maize tolerance to mesotrione + nicosulfuron, as reflected by increases in ED<sub>10</sub>, ED<sub>50</sub>, and ED<sub>90</sub> values. In contrast, the ED<sub>90</sub> values for redroot pigweed across all treatments mirrored the control, except for the 12 mL/L seaweed extract treatment. Similarly, the ED<sub>50</sub> values for redroot pigweed in all treatments were comparable to the control, except for treatments involving 4 and 12 mL/L seaweed extract and a combination of amino acid fertilizer (1 mL/L) and potassium chloride (2 g/L), which led to a slight increase in redroot pigweed's ED<sub>50</sub>. Consequently, tank mixing 4 and 12 mL/L seaweed extract with mesotrione + nicosulfuron significantly enhanced maize tolerance, with ED<sub>90</sub> values reaching 448.8 and 579.6 g/ha a.i., respectively, while the highest maize ED<sub>50</sub> was achieved with the addition of 1 mL/L amino acid fertilizer or 6 g/L potassium chloride in combination with mesotrione + nicosulfuron, registering at 165.2 and 167.1 g/ha a.i., respectively. Although certain biostimulants, particularly seaweed extract, marginally increased the ED<sub>50</sub> for redroot pigweed, weed control was not significantly compromised. In most treatments, pigweed mortality metrics remained similar to the herbicide-only control, suggesting that biostimulant application primarily benefits the crop without reducing herbicide efficacy. Notably, the physiological indicators measured, such as increased maize dry weight, leaf area, and root volume, were under biostimulant-herbicide combinations. Additionally, the concentration of chlorophyll and carotenoids in maize increased when treated with a tank mix of seaweed extract and mesotrione + nicosulfuron at the 105 g/ha dose, indicating the potential benefits of biostimulant and herbicide combinations for enhancing crop physiological traits and stress recovery mechanisms.

Applying biostimulants post-herbicide treatment emerges as a promising strategy to mitigate crop damage caused by herbicide drift or misapplication. Neshev et al. (2022) observed that sunflowers, adversely affected by incorrect herbicide applications, exhibited varied

degrees of recovery following biostimulant treatment. Similarly, Neshev et al. (2022) documented that although herbicide drift led to chlorosis and growth delays in rape-seed until the early flowering stage, subsequent biostimulant applications expedited recovery, enhancing crop growth and development.

Proactive tank-mixing biostimulants with herbicides presents another effective approach to diminishing the adverse impacts of selective herbicides on crops. These results support the hypothesis that biostimulants act as physiological buffers, helping maize withstand herbicide-induced stress. Constantin et al. (2016) demonstrated that applying biostimulants with glyphosate post-emergence in glyphosate-resistant soybean reduced leaf damage and averted yield loss. Moreover, Matysiak et al. (2018) reported that biostimulant addition to herbicides boosted the gluten content in winter wheat grains. Conversely, some research indicates that biostimulants do not always affect crops' tolerance to selective herbicides and the resultant crop damage. Soltani et al. (2016) found that incorporating biostimulants with common post-emergence herbicides in Ontario did not significantly impact soybean yield damage. Likewise, adding biostimulants to bromoxynil/MCPA showed no significant effect on crop injury and yield in oats or winter wheat (Soltani et al., 2015a), with minimal effects observed on white and cranberry beans, highlighting the importance of product specificity (Soltani et al., 2015b).

In our experiment, redroot pigweed control was largely unaffected by biostimulant treatments. Generally, past research indicates that biostimulants do not alter the effectiveness of herbicides nor the level of weed control achieved (Matysiak et al., 2018; Soltani et al., 2015a; Soltani et al., 2016; Srinivasarao and Kundu, 2017), suggesting the need for further exploration into the specific conditions under which biostimulants may offer benefits. Nonetheless, the crop-specific benefits observed suggest that biostimulants may serve a valuable role in herbicide risk management, particularly in systems seeking to minimize chemical stress.

## CONCLUSIONS

The adoption of biostimulants in organic crop production has significantly increased over the past decades, underlining their value not only as growth enhancers but also as mitigators of stress, including that induced by selective herbicide applications. Our findings highlight the efficacy of biostimulants, such as amino acids, potassium chloride, and seaweed extracts, when tank-mixed with mesotrione + nicosulfuron, significantly improve maize tolerance to herbicide stress. Remarkably, this increased tolerance did not compromise the herbicide's effectiveness against redroot pigweed, maintaining its control efficacy. Among the tested treatments, 1 mL/L amino acid and 6 g/L potassium chloride offered the greatest improvement in maize biomass and pigment content at the recommended herbicide dose. Additionally, seaweed extract enhanced chlorophyll and carotenoid levels, indicating improved photosynthetic performance. This dual benefit underscores the potential that biostimulants may serve a safener-like role, helping protect crops from herbicide-induced injury while maintaining weed control. Furthermore, the research underscores the importance of selecting appropriate biostimulant formulations to achieve a desired promising strategy for integrated crop and weed management. By mitigating the phytotoxic effects of herbicides on crops while retaining their weed control efficiency, biostimulants offer a promising avenue for developing more resilient agricultural systems. Future research should continue to explore underlying physiological and molecular mechanisms of biostimulants and expand the range of biostimulant and herbicide combinations to optimize crop health and productivity in diverse cultivars, environmental conditions, and weed spectra. Optimizing biostimulant-herbicide combinations could pave the way for more resilient, cost-effective, and sustainable cropping systems.

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