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Evaluation of the Indoor Efficacy of Various Foliar-Applied Herbicides on *Sicyos angulatus* Seedlings

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Sicyos angulatus is an increasingly pervasive alien species, has been identified as a significant contributor to the deterioration of biodiversity within the northern quadrant of the china. The objective of this investigation was to assess the efficacy of seven herbicides in suppressing the growth of *S. angulatus* seedlings, specifically targeting the early developmental phase. The application of the herbicides took place during the trileaf stage, a critical growth period. The herbicides under scrutiny included Prometryn, Thifensulfuron methyl, Nicosulfuron, Mesotrione, Bentazone, Imazethapyr, and a composite of Dicamba-Glyphosate-Triclopyr, with water serving as the control. To ensure consistent application and growth conditions, calibrated sprayers and

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standardized soil conditions were utilized. Data collection encompassed observations of toxicity, measurements of fresh weight, assessment of fresh weight effect, and the implementation of ANOVA to discern treatment variances, facilitating graphical comparison. The results indicated that all evaluated agents exhibited inhibitory effects on the seedlings. The Bentazone treatment exhibited an excellent control effect, with a fresh weight inhibition rate exceeding 95% on *S. angulatus* seedlings. Similarly, the Mesotrione and Dicamba-Glyphosate-Triclopy treatments demonstrated a good control effect, resulting in a fresh weight inhibition rate of over 85%. When treated with Prometryn, the *S. angulatus* seedlings displayed a moderate control effect, as indicated by a fresh weightinhibition rate exceeding 55%. However, Thifensulfuron methyl, Nicosulfuron, andImazethapyr showed poor control effects with a fresh weight inhibition rate below 55%. In light of these findings, it is posited that Mesotrione, Bentazone, and the Dicamba-Glyphosate-Triclopyr blend manifest as viable candidates for the prophylaxis and remediation of the early-stage growth of *S. angulatus*. Paramount to the study's conclusions is the lack of statistically significant disparity in the suppression outcomes across the concentration spectrum of the aforementioned efficacious agents. Hence, when juxtaposing ecotoxicological ramifications and economic considerations, the adoption of a concentration commensurate with the standard unitary dosage is proposed.

Keywords: Sicyos angulatus; seedling stage; foliar-applied herbicide; indoor experiments; fresh weight.

1. INTRODUCTION

The burcucumber (*Sicyos angulatus* L.), an annual herbaceous vine belonging to the *Sicyos* genus in the Cucurbitaceae family, is native to North America [1]. This species exhibits a wide distribution across Europe and Asia due to its strong adaptability, high reproductive capacity, and diverse modes of dispersal [2,3,4]. Moreover, it has been observed in various regions such as Taiwan, Hebei, Liaoning, and Beijing [5,6,7]. The invasive nature of *S. angulatus* is characterized by its ability to aggressively overgrow and intertwine with native plant species, thereby obstructing their access to sunlight. This competitive advantage ultimately results in the restriction of growth and, in severe cases, the demise of the affected native plants. The consequences of this behavior extend beyond individual plants, as it can lead to a detrimental impact on the overall biodiversity of the ecosystem and may even precipitate ecological catastrophes. Extensive research has been conducted by both domestic and international scholars on the control of *S. angulatus*, encompassing physical, chemical, biological, and other control methods [8,9,10,11,12]. Among these methods, chemical control has emerged as the primary approach for large-scale management of *S. angulatus* due to its cost-effectiveness, rapid efficacy, and convenience. Specifically, chemical control methods have been primarily applied to crops and have demonstrated good weed control effects. For instance, Esbenshade et al. [9] evaluated the efficacy of postemergence (POST) soybean herbicides against *S. angulatus* by applying them during the early-POST and mid-POST stages (6-8 leaves), as well as the late-POST stage (more than 10 leaves). The results indicated that nine herbicides, including glyphosate, exhibited an efficacy exceeding 87% when applied during the late-POST stage. Messersmith et al. [8] assessed the herbicide efficacy in an established *S. angulatus* population within a corn field, with applications made at 8, 11, and 14 weeks after planting. CGA 152005, primisulfuron, and their combination showed over 85% efficacy in controlling *S. angulatus* at the 14-week post-planting stage. Notably, the vulnerable growth period for postemergence weeds, including *S. angulatus*, occurs at the 3-5 leaf stage (approximately 4 weeks after planting), and applying herbicides during this specific timeframe yields optimal results [13]. However, no research has focused specifically on herbicide screening for this phase of *S. angulatus* growth, leading to uncertainty regarding the potential enhancement of control efficacy during this period. Therefore, in order to rapidly identify herbicides that can effectively manage *S. angulatus* post-emergence, this experiment employed seven foliar-applied herbicides with diverse mechanisms of action during this critical growth period to determine the most efficacious ones for controlling *S. angulatus* seedlings.

In accordance with the tenets of high efficacy, safety, and precise selectivity, a set of 7 foliar-applied herbicides with distinct mechanisms of action was deliberately chosen to undertake an indoor experimentation on *S. angulatus* seedlings. The objective was to identify the foliar-applied herbicides yielding the most effective therapeutic impact on *S. angulatus* seedlings, as evidenced by evaluations of phytotoxicity grade fresh weight effect, and efficacy evaluation. This investigation offers a theoretical framework for the accurate chemical management of *S. angulatus*.

2. MATERIALS AND METHODS

2.1 Experimental Materials

In October of 2021, *S. angulatus* fruits were harvested from a maize field located in Pulandian, Dalian City, within the province of Liaoning. Subsequently, the fruits underwent an indoor air drying process before being carefully packaged and stored in a refrigerated environment at a temperature of 4℃, until their intended utilization.

The selected herbicides for the experiment include Prometryn, Thifensulfuron methyl, Nicosulfuron, Mesotrion, Bentazone, Imazethapyr, and the herbicide mixture Dicamba-Glyphosate-Triclopyr. The characteristics and profiles of these herbicides are presented in Table 1.

2.2 Experimental Design

The experiment was carried out on January 3, 2022, utilizing the greenhouse pot method for cultivating *S. angulatus*. Nutritive soil with a minimum organic matter content of 40%, neutral pH levels, and good permeability (with a matrix of vermiculite and soil in a ratio of 3:1) was employed. Plastic basins, measuring 5cm in diameter and 10cm in height, were filled with moist nutrient soil up to 4/5th of their capacity. The seeds were planted at depths ranging from 0.5cm to 2cm below the soil surface and placed in a greenhouse with diurnal temperatures maintained at 25°C and nocturnal temperatures at 15°C. Adequate soil moisture was consistently maintained throughout the experiment. The dosage of the herbicide was determined based on the manufacturer's instructions, and two treatment groups (Table 2) were established, with each group having 4 replicate samples. Treatment group 1 represented a single application, while treatment group 2 represented two applications. A water control group was also included concurrently. When the seedlings reached the 3-5 leaf stage, the stems and leaves were treated using a sprayer with a spray volume of 450L/hm^2, following the guidelines outlined in " Pesticides guidelines for laboratory bioactivity tests Part 4: Foliar spray application test for herbicide activity. Subsequently, the plants were transferred to the greenhouse for routine incubation, and remaining damage was observed and recorded for a period of 5 consecutive days. The fresh weight effect was calculated as part of the evaluation process. To investigate the indoor efficacy of different foliar-applied herbicide sprays on *S. angulatus* seedlings, this study assessed the phytotoxicity grade and the impact on fresh weight as part of the efficacy evaluation.

2.3 Phytotoxicity Grade

Following the completion of the treatment protocol, the daily monitoring of the growth status of *S. angulatus* was undertaken, with attentiveness to any noticeable indicators of detrimental effects. The primary manifestations identified, as documented by ICAMA [14], entailed:

——Changes in color (etiolation, bleaching, etc.)

——Morphological alterations (new leaf deformity, distortion, etc.)

——Modifications in growth patterns (dehydration, wilt, dwarfism, etc.)

The assessment of the herbicidal efficacy of the agents was conducted by considering the discernible symptoms and the level of damage inflicted on the tested *S. angulatus* seedlings. To ensure consistency and uniformity, the grading system outlined by ICAMA was adopted for this purpose [14]:

- Level 1: all dead
- Level 2: it was equivalent to 0% to 2.5% of the control group
- Level 3: it was equivalent to 2.6% to 5 % of the control group
- Level 4: it was equivalent to 5.1% to 10% of the control group
- Level 5: it was equivalent to 10.1% to 15% of the control group
- Level 6: it was equivalent to 15.1% to 25% of the control group
- Level 7: it was equivalent to 25.1% to 35% of the control group
- Level 8: it was equivalent to 35.1% to 67.5% of the control group
- Level 9: it was equivalent to 67.6% to 100% of the control group

Table 1. Herbicide characteristics and profiles

Herbicides	Main ingredients (dosage forms)	Manufacturer	Feature	Mechanism of action
Prometryn	50% Prometryn (WP)	Shangdong Vicome Greenlan And Chemical Co. LTD	Wide spectrum, extended efficacy, low toxicity, and chemically stable.	Its mode of action involves the inhibition of photosynthesis in the leaves of weeds, thereby hindering their proper growth and ultimately leading to wilting and death.
Thifensulfuron methyl	25% Thifensulfuron methyl (WP)	Anhui Fengle Agrochemical Co. LTD	Wide spectrum, low toxicity, minimal residue, and high activity	It acts by suppressing the biosynthesis of branched-chain amino acids such as valine, leucine, and isoleucine, which hinders cell division in weeds and causes them to cease growth and die.
Nicosulfuron	10% Nicosulfuron (OD)	Shandong Aokun Crop Science Co. LTD	Wide spectrum，comprehensive weed control, good safety, and mixability	Its mechanism of action involves the inhibition of branched-chain amino acid production in plants, thereby preventing normal mitosis and gradually leading to weed demise.
Mesotrion	15% Mesotrion (SC)	Sichuan Runer Technology Co. LTD	Wide spectrum，high activity, low dosage, and good environmental compatibility	It functions by reducing the carotenoid content in weeds through the inhibition of HPPD, rendering the weed unable to absorb nutrients and leading to its death.
Bentazone	480g/L Bentazone (WS)	Anhui Fengle Agrochemical Co. LTD	Wide spectrum, effective prevention, and drug resistance properties	Its mode of action targets the D1 protein of PSⅡ in weeds to inhibit the photosynthetic electron transport of PS ii , thereby achieving weed control.
Imazethapyr	5% Imazethapyr (EW)	Shandong Cynda (Chemical) Co. LTD	Wide spectrum, high selectivity, and high activity	It acts by inhibiting acetolactate synthase, thereby inhibiting the biosynthesis of branched-chain amino acids and resulting in plant growth inhibition and death.
Dicamba-Glyphosate-Triclopyr	10% Dicamba + 50% Glyphosate + 2% Triclopyr (WP)	Shandong Yilan Technology Co. LTD	To address the issue of drug resistance in malignant weeds by expanding the spectrum of weed-killing	Its mode of action involves impeding normal weed hormone activity, inhibiting protein synthesis, and embolizing or rupturing vascular bundles to cause weed death.
Check	Water			

Table 2. Experimental herbicide doses for *Sicyos angulatus* control

Herbicides	Dose 1/(g a.i./hm^2)	Dose 2/(g a.i./hm^2)
Prometryn	1499.25	2998.50
Thifensulfuron methyl	119.94	239.88
Nicosulfuron	749.62	1499.25
Mesotrion	974.51	1949.02
Bentazone	2998.50	5997.00
Imazethapyr	1499.25	2998.50
Dicamba-Glyphosate-Triclopyr	1349.32	2698.65

2.4 Fresh Weight Effect

Following the application, the above-ground component will be sampled continuously for a period of 5 days. The fresh weight of the samples will be determined, and subsequently, the fresh weight effect will be calculated and expressed as a percentage (%). The resulting calculation will be rounded to two decimal places and retained.

$$\boldsymbol{E} = \frac{\boldsymbol{C}-\boldsymbol{T}}{\boldsymbol{C}} \times \mathbf{100}$$ [15]

In the formula：

- *E*——fresh weight effect
- *C*——Fresh weight of *S. angulatus* above ground in control group
- *T*——Fresh weight of *S. angulatus* above ground in treatment group

2.5 Efficacy Evaluation

Based on the low dosage of each medication, *S. angulatus* was classified according to its fresh inhibition rate [16].

- I≥95%: excellent
- 85≤I＜95%: good
- 55%≤I＜85%: moderate
- I＜55%: poor

2.6 Statistical Analysis

The information from Excel 2016 was summarised and plotted, and SPSS version 22.0 was employed to perform a one-way analysis of variance (ANOVA). To detect any significant variances among the group means, Duncan's multiple range test was applied, with the level of significance established at $P < 0.05$.

3. RESULTS

3.1 Herbicidal Activity of Different Foliar-Applied Herbicides on *S. angulatus* Seedlings

Following the administration of diverse foliar-applied herbicidal treatments, a range of phytotoxic symptoms were detected in *S. angulatus* saplings. Specifically, by the fifth day of exposure, the application of Mesotrion, Bentazon, and a combination of Dicamba-Glyphosate-Triclopyr led to the death of all seedlings (Table 3, Fig. 1). Three days after treatment, saplings treated with Bentazone showed symptoms of leaf etiolation, phytotoxic effects, deformities, and wilting. In parallel, those treated with the Dicamba-Glyphosate-Triclopyr blend exhibited symptoms through leaf etiolation, distortion, and wilting. The herbicides in question inflicted severe harm upon *S. angulatus*, with damage reaching a level 4 rating. By the end of the fourth day of treatment, both Bentazone and the Dicamba-Glyphosate-Triclopyr blend had caused sapling mortality, with *S. angulatus* experiencing level 1 damage. Mesotrion-treated leaves exhibited irregular bleaching, deformity, and wilt symptoms, while leaves exposed to a double dose of Mesotrion presented complete wilt, leading to a level 4 damage rating for *S. angulatus*. On the fifth day post-treatment, signs of herbicide damage were evident on the dead seedlings, with the phytotoxicity achieving a level 1 rating in *S. angulatus*. In summary, the effectiveness of Bentazone and the Dicamba-Glyphosate-Triclopyr mixture was the most pronounced, followed by Mesotrion, in terms of their rapid impact on *S. angulatus*. Each herbicide acted through a unique mechanism: Bentazone primarily interferes with photosynthesis, the Dicamba-Glyphosate-Triclopyr blend disrupts plant hormones to cause mortality in *S. angulatus* seedlings, and Mesotrion inhibits the HPPD enzyme, thereby affecting carotenoid synthesis and leading to the death of *S. angulatus*.

Table 3. Phytotoxicity grade of different foliar-applied herbicides on *S. angulatus* seedlings

Herbicides	Dose	Phytotoxicity grade				
		1 d	2 d	3 d	4 d	5 d
Prometryn	1	9	8	6	4	2
	2	9	8	6	4	2
Thifensulfuron methyl	1	9	9	9	8	7
	2	9	9	9	8	7
Nicosulfuron	1	9	9	9	9	9
	2	9	9	9	9	9
Mesotrion	1	9	9	8	4	1
	2	9	9	8	4	1
Bentazone	1	9	8	4	1	1
	2	9	8	4	1	1
Imazethapyr	1	9	9	9	9	9
	2	9	9	9	9	9
Dicamba-Glyphosate-Triclopyr	1	9	8	4	1	1
	2	9	8	4	1	1

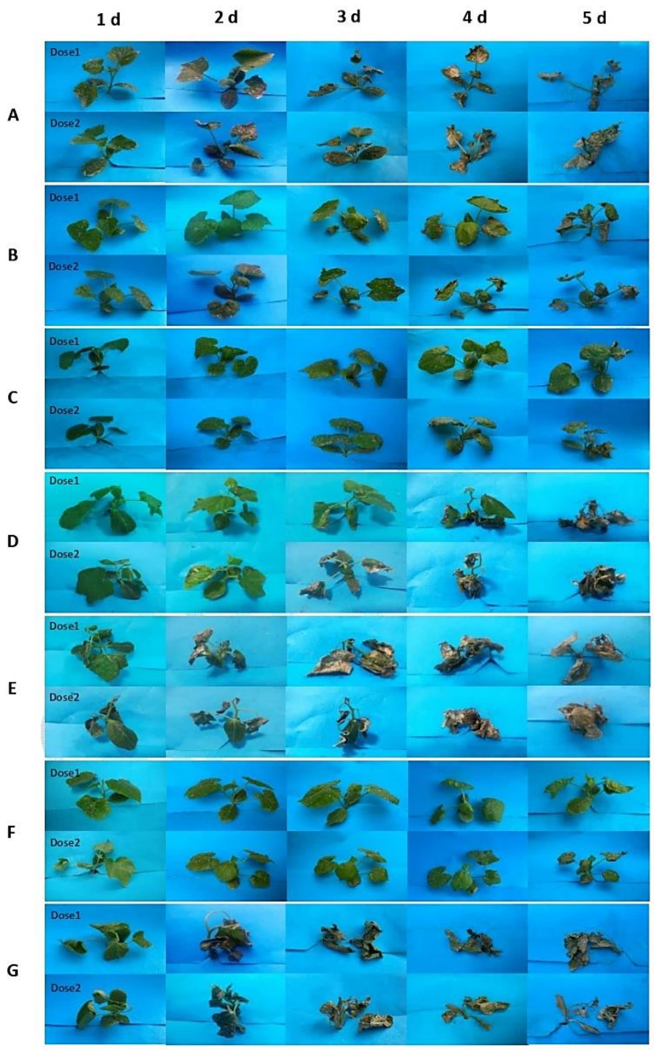

Fig. 1. Victimization symptom of different foliar-applied herbicides on *S. angulatus* seedlings
Note: A: Prometryn; B: Thifensulfuron methyl; C: Nicosulfuron; D: Mesotrion; E: Bentazone; F: Imazethapyr; G: Dicamba-Glyphosate-Triclopyr; d: Number of days

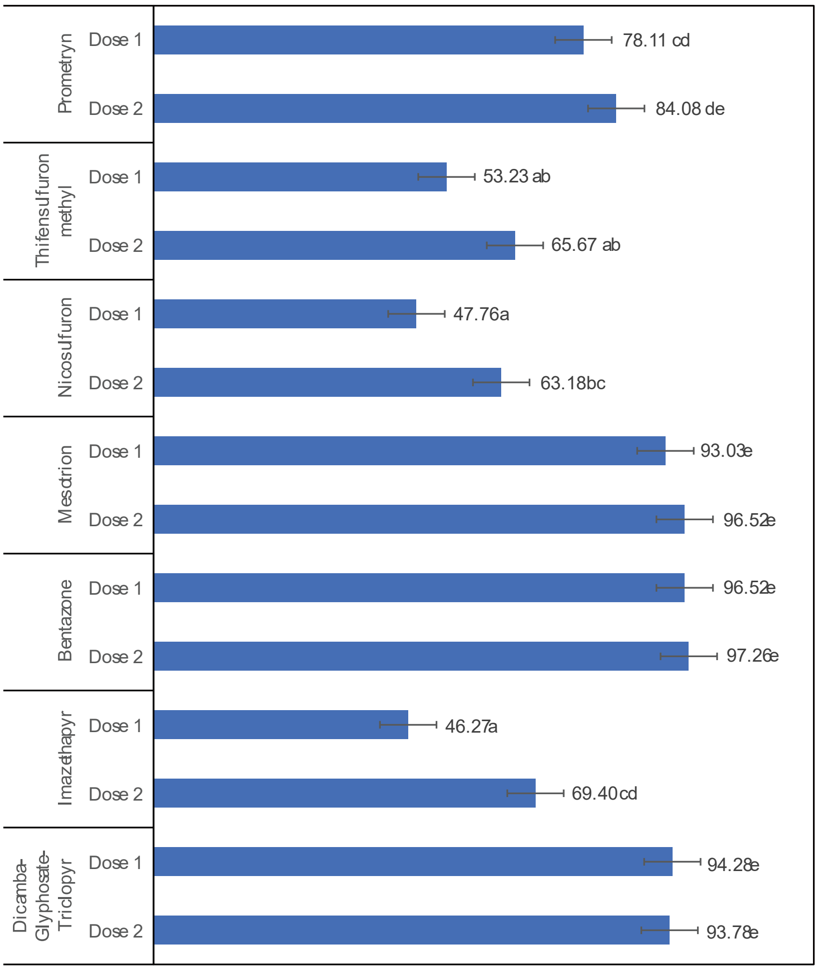

Fig. 2. Fresh weight effect of different foliar-applied herbicides on *S. angulatus* seedlings

Note: Different letters indicate significant differences between herbicide types as well as between doses (one-way ANOVA, P < 0.05)

Table 4. Efficacy evaluation of different foliar-applied herbicides on *S. angulatus* seedlings

Efficacy evaluation	Fresh weight inhibition rate	Herbicides
Excellent	≥95%	Bentazone
Good	85≤I＜95%	Mesotrione and Dicamba-Glyphosate-Triclopy
moderate	55%≤I＜85%	Prometryn
Poor	I＜55%	Thifensulfuron methyl, Nicosulfuron and Imazethapyr

3.2 Fresh Weight Effect of Different Foliar-Applied Herbicides on *S. angulatus* Seedlings

When assessing the impact of various herbicides on *S. angulatus* seedlings five days after their application, the reduction in fresh weight was observed to vary from 46.27% to 97.27% (Fig. 2). Bentazone was particularly effective, causing a significant reduction in fresh weight, with recorded effects of 96.52% and 97.26%. The treatment involving a combination of Bentazone and Dicamba-Glyphosate-Triclopyr also showed a strong effect, with fresh weight reductions ranging from 93.03% to 94.28%. Statistical analysis indicated that there was no significant variation in the efficacy of these three treatments. On the other hand, the 1-fold concentration of

Imazethapyr and Nicosulfuron had a much weaker impact, with fresh weight reductions both under 50%. It is also important to note that the effects of the 1-fold concentration of Nicosulfuron and Imazethapyr were significantly different from those observed with a 2-fold concentration. These findings suggest that Mesotrion, Bentazone, and Dicamba-Glyphosate-Triclopyr are effective in managing *S. angulatus* seedlings.

3.3 Efficacy Evaluation of Different Foliar-Applied Herbicides on *S. angulatus* Seedlings

All seven agents tested demonstrated inhibitory effects on the seedlings of *S. angulatus*. Bentazone showed exceptional effectiveness in controlling the seedlings, while Mesotrione and Dicamba-Glyphosate-Triclopy similarly exhibited substantial efficacy. Prometryn exhibited a moderate degree of control over the seedlings. However, Thifensulfuron methyl, Nicosulfuron, and Imazethapyr demonstrated poor efficacy in controlling the seedlings (Table 4). These findings suggest that Mesotrione, Bentazone, and Dicamba-Glyphosate-Triclopyr could serve as viable herbicides in the prevention and control of *S. angulatus* seedlings.

4. DISCUSSION

The swift expansion of *S. angulatus* has led to a considerable reduction in crop yields. A Japanese research initiative assessed the impact of *S. angulatus* on agricultural fields through comprehensive surveys. The findings indicated a significant decrease in the size of the fields, where an 80% reduction was observed at population densities of 15-20 individuals per 10 m^2. Moreover, as the population density escalated to 28-50 individuals per 10 m^2, a striking 90-98% reduction was noted [17]. Averill et al. [18] further explored the effects of *S. angulatus* on *Z. mays* via a field-based experiment, where varying densities of *S. angulatus* seedlings were introduced to corn rows. The outcomes revealed that diverse planting densities of *S. angulatus* caused a decline in corn production. Notably, planting *S. angulatus* at a density of one square meter per hectare resulted in a corn yield decrease of approximately 1700 kg ha^{-1}. These results highlight the destructive influence of *S. angulatus* and the urgent need for effective strategies to mitigate its negative effects on agricultural output. To date, large-scale management strategies for *S. angulatus* are under investigation, with chemical control emerging as the most prevalent and potent method for weed management. This includes pre-seedling soil treatment herbicides and post-seedling foliar-applied treatments [19]. Among these, post-seedling foliar-applied herbicides work by absorbing and translocating the chemical through the weeds' stems and leaves, offering benefits such as minimal soil interaction, high flexibility and selectivity, and broad applicability [20]. Given their efficacy in preventing and managing *S. angulatus* seedlings, it is imperative to administer the correct dosages to crops like *Z. mays* and Glycine max to prevent harm. Research by Liang [21] into the optimal dosage for controlling weeds with Mesotrion, a prevalent herbicide in corn cultivation, indicated that a 15% Mesotrion suspension concentrate at 146.3 g a.i./hm^2 achieved the lowest residual weed density in corn fields, with an elimination rate surpassing 90% within three days. The Mesotrion treatment rates in this study are in line with the minimum effective control dosages established by previous research. Chen et al. [22] determined that the advisable application rate for Bentazone ranged from 2248.87 g to 2998.50 g a.i./hm^2, without inflicting drift damage on adjacent crops. The Bentazone concentration used in this study is consistent with the recommended dosages from earlier studies. As for Dicamba-Glyphosate-Triclopyr, a combined herbicide, no relevant research has been discovered, preventing the establishment of residue limit standards.

5. CONCLUSION

In conclusion, the impact of varying concentrations of Mesotrion, Bentazone, and Dicamba-Glyphosate-Triclopyr on the fresh weight of *S. angulatus* seedlings showed no notable disparities. Taking into account the suggested application rates from prior investigations and with regard to safety and cost-efficiency, it is advisable to opt for a concentration that corresponds to a single application.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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