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The impact of herbicides on weed control and physiological traits in corn (*Zea mays* L.)

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Abstract
Objective: This research aimed to evaluate the effect of herbicides on weed control and physiological traits in corn (*Zea mays* L.).
Methods: An experiment was performed using a randomized complete block design with four replications at the Agricultural and Natural Resources Research Center of Moghan, Iran, during the 2021 growing season. The herbicides included Amaize-ing (Terbuthylazine, 50% SC) at the rates of 1, 1.2, 1.5, 1.8, and 2 L/ha, Isoxaflutel + Tincarbazone (Adango 46.5% SC), Mesotrione + S-metolachlor + Lumax (Lumax 53.75% SE), U46 Combi Fluid (2,4-D + MCPA 67.5% SL), Bromicide MA (Bromoxynil + MCPA 40% EC), and Bromicide MA (Bromoxynil + MCPA 40% EC) + Cruze (Nicosulfuron 4% SC).
Results: The analysis of variance revealed a significant difference among treatments in terms of weed density, weed dry weight, corn grain yield, chlorophyll index (SPAD), leaf weight, proline, and catalase activity. The use of 1.8 and 2 L/ha concentrations of Amaize-ing, Adengo, Lumax, U46 Combi Fluid, Bromicide MA, and Bromicide MA + Cruze herbicides had a favorable effect on redroot pigweed and common lambsquarters. It resulted in a 20 to 30% increase in corn yield. The highest chlorophyll content and leaf weight of corn were associated with treatments of Adengo, Lumax, U46 Combi Fluid, Bromicide MA, and Bromicide MA + Cruze. However, the Amaize-ing herbicide had a smaller effect on the leaf weight of corn. Also, increasing the concentration of Amaize-ing increased the proline content and activity of catalase in corn.
Conclusion: In conclusion, it seems that the use of 1.8 L/ha of A-mazing and Adengo herbicides can have an effective role in controlling weeds, leading to an increase in leaf weight, photosynthetic capacity, and ultimately corn grain yield.

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Introduction

Corn ranks first globally in terms of yield per hectare and production, and it is the second highest cultivated crop after wheat in terms of acreage (Statista 2021). Among various methods of weed control, the use of herbicides plays a crucial role in weed management due to their effectiveness and cost efficiency. It is widely accepted that the use of herbicides has been successful (Fetyukhin *et al*. 2022), and every year, new compounds are proposed for the chemical control of weeds. New compounds may include new active ingredients or previous active ingredients that have been blended together in new formulations with optimal ratios (Minbashi *et al*. 2020). Among the herbicides used in Iran for weed control in corn, we can mention U46 Combi Fluid, Eradican, Bromoxynil + MCPA, Foramsulfuron, Nicosulfuron, Rimsulfuron, Nicosulfuron + Rimsulfuron, Mesotrione + S-metolachlor + Terbuthylazine, and Topramezone (Zand *et al*. 2019). Some of these herbicides have been used in Iranian corn fields for years. Therefore, in addition to the environmental risks, there is also a high risk of weed resistance to some herbicides such as Nicosulfuron (Zand *et al*. 2019). Therefore, the limited mechanism of action of the recommended herbicides and the risks associated with consecutive use of the herbicides with similar mechanisms of action are the main reasons for utilizing herbicides with a wide control spectrum, especially with diverse target sites, for weed management in Iranian corn fields.

Despite the inherent selectivity mechanisms that facilitate crop production, herbicides can cause some phytotoxicity to crop plants and reduce shoot dry weight, plant height, yield, and alterations in plant metabolism by generating reactive oxygen species (ROS) (Cobb and Reade 2010). However, the yield reduction depends on the stress intensity, duration, and incidence at the crop stage (Ghassemi *et al.* 2020). Most of the perturbations caused by herbicide treatment in plants are related to ROS generation and consequent oxidative stress (Cobb and Reade 2010). The mode of action for some herbicides is the generation of ROS in plants as a secondary effect after the specific target site is sufficiently inhibited.

Overall, due to the low number and diversity of registered herbicides for weed control in corn in Iran, this research aimed to evaluate the effectiveness of new and commonly used herbicides on annual broadleaf weeds in corn fields.

Materials and Methods

The experiment was conducted in 2021-2022 using a randomized complete block design with 11 treatments and four replications at the Agricultural and Natural Resources Research Center of Moghan, Iran. The details of the experimental treatments are provided in Table 1.

Table 1. Characteristics of the experimental treatments.

	Hand weeding	
	Herbicides:	
Commercial name	**Common name**	**Concentration**
Amaize-ing	Terbuthylazine (500 g.L)	1 L/ha
Amaize-ing	Terbuthylazine	1.2 L/ha
Amaize-ing	Terbuthylazine	1.5 L/ha
Amaize-ing	Terbuthylazine	1.8 L/ha
Amaize-ing	Terbuthylazine	2 L/ha
Adango	Isoxaflutole (225 g/L)+ Thiencarbazone (90 g/L)+ Ciprosulfamide safener (150 g/L)	0.55 L/ha
Lumax	Mesotrione (125.5 g/L) + S-metolachlor (375 g/L) + terbuthylazine (37 g/L)	4.5 L/ha
U46 Combi Fluid	2. 4, D (360 g/L) + MCPA (315 g/L)	1.5 L/ha
Bromicide MA	Bromoxynil (200 g/L) + MCPA (200 g/L)	1.5 L/ha
Bromicide MA + Cruze	Bromoxynil (200 g/L) + MCPA (200 g/L) + Nicosulfuron (40 g/L)	1.5 + 0.5 L/ha

To conduct the experiment, a field with a significant history of weed infestation was selected in the spring of the year 2021. After preparing the land and seedbed, ready-made plots were planted. Each experimental plot had dimensions of approximately 3 × 8 meters (4 rows, spaced 0.75 centimeters apart, with a length of 8 meters). Each plot was divided into two parts in length, in which the upper part of the plot was left unsprayed and considered as the control, while the lower part was treated with the herbicides. Spraying of the herbicides was carried out using a backpack sprayer equipped with a flat fan nozzle at a pressure of 2 to 2.5 bars, calibrated based on 300 liters of water per hectare. Throughout the growth period, all weeds present in the control plot were manually removed. Thirty days after spraying, a 50 by 75 cm quadrat (i.e., half a meter in length, one row) was randomly thrown in the sprayed and unsprayed sections of each plot, and all weeds present in each quadrat were counted by species. Then, to determine the dry weight of weeds, they were placed in an oven at a temperature of 72 °C for 48 hours. The percentage reduction in weed density in each plot compared to the control plot (unsprayed section) was calculated based on the following equation.

$$\% \text{ Reduction in Density} = [(\text{Unsprayed} - \text{Sprayed}) / \text{Unsprayed}] \times 100$$

Where Unsprayed and Sprayed represent the number of weeds counted in the quadrats in the unsprayed and sprayed sections, respectively. To calculate the percentage reduction in dry matter of weeds, the above equation was used, with the difference that Unsprayed and Sprayed represented the dry weight of weeds in the unsprayed and sprayed quadrats, respectively.

Leaf chlorophyll was measured by a non-destructive method with a SPAD-502 chlorophyll-meter (Konica Minolta). It should be noted that SPAD does not specify chlorophyll content; rather, it is an estimation of chlorophyll concentration. SPAD has a strong correlation with leaf chlorophyll content (Hoel and Solhaug 1998).

To measure the proline content and catalase activity, three biological replications from the most recently fully developed leaves of three different plants were acquired for each duplicate section in each plot. Proline content of the fresh leaves was measured using the procedure described by Bates *et al*. (1973). Catalase activity was measured by the method of Karo and Mishra (1976).

At physiological maturity, ears from two rows of the center of each plot (sprayed and unsprayed sections) were harvested by hand and air-dried to measure grain yield. The data were analyzed using SAS 9.4 software. Arcsine transformation was used on percent weed control data when needed to mitigate the skewness of the data and meet the requirements of normality for analysis. The means were compared using Duncan's multiple range test at a significant level of 5%, using the same software.

Results and Discussion

The spectrum of broadleaf weed species in the corn field is shown in Table 2. Only the data related to the dominant weeds in each replication were separately subjected to statistical analysis.

Effect of herbicide treatments on the density and dry weight of weeds

The results of the analysis of variance showed that there was a significant difference between the herbicide treatments in terms of the percentage reduction in density and dry weight of redroot pigweed and common lambsquarters (data not shown). Based on the results obtained from the density of redroot pigweed in this experiment, it can be indicated that controlling this weed with treatments of

1.2, 1.5, 1.8, and 2 liters of Amaize-ing, Adengo, Lomax, U46 Combo Fluid, Bromicide MA, and Bromicide MA + Cruze was satisfactory, with over 90% effectiveness (Table 3). But using 1 liter of commercialized Amaize-ing showed poor efficiency in controlling this weed (Table 3).

Table 2. The composition of weed species in the corn field.

Common name	Scientific name	Presence
Redroot pigweed	*Amaranthus retroflexus* L.	+++
Velvetleaf	*Abutilon theophrasti* L.	+
Common lambsquarters	*Chenopodium album* L.	+++
Jimsonweed	*Datura stramonium* L.	+
Common Purslane	*Portulaca oleracea* L.	+
Ground cherries	*Physalis divaricata* L.	+

+++: Dominant presence; +: low presence; -: No presence

The results indicated that the herbicide treatments of 1.2, 1.5, 1.8, and 2 liters of Amaize-ing, Adengo, Lumax, U46 combi fluid, Bromicide MA, and Bromicide MA + Cruze significantly reduced weed density of common lambsquarters by over 90% (Table 3). Observing the percentage reduction in weed density, it can be seen that the effectiveness of 1 liter of Amaize-ing commercial product in controlling this weed was also weak, resulting in a 72.38% reduction in weed density (Table 3).

Among the applied treatments, the best result in controlling the dry weight of redroot pigweed was achieved by the treatments of 1.2, 1.5, 1.8, and 2 liters of Amaize-ing, Adengo, Lumax, U46 combi fluid, Bromicide MA, and Bromicide MA + Cruze. However, the treatment of 1 liter of Amaize-ing herbicide was not able to effectively reduce the dry weight of this weed (Table 3). Also, the results indicated that the treatments of 1.2, 1.5, 1.8, and 2 liters of Amaize-ing, Adengo, Lumax, U46 combi fluid, Bromicide MA, and Bromicide MA + Cruze were able to reduce the dry weight of common lambsquarters by over 90% (Table 3). But the effect of 1 liter of Amaize-ing herbicide in controlling this weed was significantly lower than other concentrations (Table 3).

In general, the results showed that the application of Amaize-ing herbicide in the early stages of growth had a greater inhibitory effect on weed density and dry weight due to the greater sensitivity of weed seedlings to herbicides. In such conditions, due to the prevention of early-season interference of weeds with corn, their competitive effects on the crop were minimized, and the application of even lower amounts of herbicides (even less than 2 liters per hectare) had positive results in increasing corn yield. But based on the evaluation of herbicide efficiency, it can be said that the use of Amaize-ing herbicide at the concentration of 1 liter per hectare as a pre-emergence application after corn planting showed smaller effect in weed control, and therefore, is not recommended.

Table 3. Comparison of the effect of herbicides on the percent reduction in density and dry weight of weeds as compared to the non-spraying control conditions.

Treatment	Density reduction (%)		Dry weight reduction (%)	
	Redroot pigweed	**Common lambsquarters**	**Redroot pigweed**	**Common lambsquarters**
Amaize-ing 1 L	67.95b	72.38b	66.74b	70.07b
Amaize-ing 1.2 L	93.88a	92.26a	95.33a	92.95a
Amaize-ing 1.5 L	94.79a	94.09a	95.61a	95.44a
Amaize-ing 1.8 L	100a	100a	100a	100a
Amaize-ing 2 L	100a	100a	100a	100a
Adango 0.55 L	91.42a	92.85a	92.22a	92.81a
Lumax 4.5 L	100a	100a	100a	100a
U46 Combi Fluid 1.5 L	100a	100a	100a	100a
Bromicide MA 1.5 L	100a	100a	100a	100a
Bromicide MA 1.5 L+ Cruze 0.5 L	100a	100a	100a	100a

The means with similar letters are not significantly different (Duncan's Multiple Range Test at $p \leq 0.05$)

Effect of herbicide treatments on corn traits

The results of the analysis of variance showed a significant difference between treatments in terms of corn grain yield, SPAD, leaf weight, proline, and Catalase (data not shown). The highest corn grain yield was associated with treatments of 1.8 and 2 liters of Amaize-ing, Bromicide MA + Cruze, and Bromicide MA, which did not show a statistically significant difference from the control (hand weeding) (Table 4). The use of these herbicides resulted in a 20 to 30% increase in corn yield (Table 4). The results showed that the application of 1, 1.2, and 1.5 liters of Amaize-ing, had the lowest efficiency in controlling the existing weeds, which resulted in the lowest corn yield. Baghestani *et al*. (2007) concluded that chemical control of weeds can lead to increased corn yield compared to uncontrolled weeds. Nurse *et al*. (2006) also reported that weed control can increase crop yield compared to uncontrolled weeds. These results align with the findings of this study.

Regardless of the weed-free treatment (hand weeding), the highest corn chlorophyll content (SPAD) was associated with treatments of Adengo, Lumax, U46 Combi Fluid, Bromicide MA, Bromicide MA + Cruze, and 1 and 1.2 liters of Amaize-ing (Table 4). The results of corn leaf weight showed that the treatments Adengo, Lumax, U46 Combi Fluid, Bromicide MA, Bromicide MA + Cruze, and 1 liter of Amaize-ing were placed in the same statistical group as the control (hand weeding) treatment (Table 4).

Based on the analysis of variance, the proline content and activity of catalase enzyme were significantly affected by herbicide treatments (data not shown). In terms of proline, all treatments of the Amaize-ing herbicide resulted in an increase in the proline content compared to the other treatments, including the hand weeding treatment (Table 4). Among the treatments, Adengo, Lomax, U46 Combifluid, Bromicide MA, and Bromicide MA + Cruze had the lowest activity of catalase enzyme, but increasing the concentration of Amaize-ing increased the activity of catalase enzyme in corn (Table 4). In an experiment, both the interference of weeds and the use of herbicides in plants stimulated the synthesis of antioxidant molecules, including proline, in response to stress (Harre *et al.* 2018). In the study of Grigoryuk *et al.* (2016), the herbicide treatments increased the catalase activity in the root and stem of corn, which was consistent with the results of the present study. The study of Hassannejad and Porheidar Ghafarbi (2018) showed that by increasing clodinafop-propagrgyl (TOPIK) herbicide, the maximum fluorescence (Fm), variable fluorescence (Fv), efficiency, and/or activity of water-splitting complex at the donor side of photosystem II (Fv/F0), and maximum photochemical efficiency of photosystem II (Fv/Fm) decreased, but minimum fluorescence (F0) increased. In another experiment, by examining the Topik, Titos, Equip, Mister, Lumax, Bromicide, and Oltima herbicides on the photosynthetic efficiency of three corn cultivars (CC-260, 400, 704), it was found that Topik, Titos, Equip, Mister, and Oltima herbicides did not affect the chlorophyll fluorescence (Porheidar Ghafarbi *et al*. 2017). Hassannejad *et al*. (2020) indicated that the application of nicosulfuron did not affect most of the chlorophyll a fluorescence (ChlF) characteristics. But, the application of Bentazon significantly decreased PSII activity due to increasing F0 and decreasing Fm, Fv, Fv/F0, and especially Fv/Fm.

The results of this experiment indicated that treatments with a higher percentage of weed density and dry weight control led to an increase in leaf weight and photosynthetic capacity, ultimately resulting in better corn grain yield. Since old herbicides are widely used in Iran for controlling corn weeds, this study aimed to replace these herbicides with new ones to eliminate some of the problems associated with herbicide use, such as risks in subsequent crops and environmental pollution. Overall, all applied herbicides showed a significant effect on weed control compared to the uncontrolled weeds. In total, Amaize-ing herbicide, at concentrations of 1.8 to 2 liters per hectare, had a desirable effect in controlling weeds and producing higher corn grain yield. Although the use of 2 liters of Amaize-ing had higher efficiency in weed control compared to the concentration of 1.8 liters, its use is not recommended due to the lack of statistical difference. Therefore, the use of 1.8 liters of Amaize-ing herbicide is recommended for corn, especially in terms of sustainable weed management and chemical control hazards.

The Amaize-ing herbicide is an electron transfer inhibitor in the target site receptor of photosystem II and belongs to the triazine chemical family. It is mainly absorbed through the roots.

Table 4. Comparison of the effect of herbicide treatment of weeds on corn traits.

Herbicide treatment	Grain Yield (t/ha)	Percentage grain yield compared to the uncontrolled weeds	SPAD	Leaf weight (g)	Proline content (µmol/g fresh weight)	Catalase (Dansitometric activity)
Amaize-ing 1 L	5.94e	87.4e	53.83abc	2.539abc	73.60ab	595.87de
Amaize-ing 1.2 L	6.91d	110.2cd	53.11abcd	2.216bc	73.62ab	602.09c
Amaize-ing 1.5 L	6.93d	113.1cd	52.13bcd	1.185bc	73.95ab	602.32c
Amaize-ing 1.8 L	7.60ab	120.8abcd	51.38cd	2.117bc	74.33a	610.95b
Amaize-ing 2 L	7.77ab	122.4abc	48.75d	1.812c	74.53a	614.32a
Adango 0.55 L	7.09c	112.2cd	55.83ab	2.808ab	71.03d	592.06e
Lumax 4.5 L	7.01c	102.3de	55.03abc	2.581abc	71.77cd	595.13d
U46 Combi Fluid 1.5 L	7.03c	107.0d	55.76ab	2.858ab	71.82cd	559.71g
Bromicide MA 1.5 L	7.81a	127.8ab	54.18abc	2.623abc	72.63bc	590.84e
Bromicide MA 1.5 L+ Cruze 0.5 L	7.88a	130.8a	53.82abc	2.613abc	72.90bc	573.48f
Hand weeding	8.03a	133.4a	56.5 a	3.291a	69.28e	409.93h

The means with similar letters in each column are not significantly different (Duncan's Multiple Range Test at $p \leq 0.05$)

This herbicide controls several broadleaf weeds in corn fields, such as redroot pigweed (*Amaranthus hybridus*), common purslane (*Portulaca oleracea*), and hairy nightshade (*Solanum sarrachoides*) (Anonymous 2021). The tolerance of corn to this chemical family is due to its binding with glutathione. It has also been registered for weed control in citrus, grape, apple orchards, fallow lands, and industrial areas in different quantities worldwide (Anonymous 2021). Also, based on the results of this experiment and the need to reduce herbicide use to minimize undesirable environmental effects, the use of Adengo herbicide at a rate of 1.8 liters per hectare is recommended for weed control in corn fields of Moghan, Iran. It is evident that the use of this herbicide, compared to other herbicides at higher recommended rates, particularly Lumax at a rate of 4.5 liters per hectare, is preferred. In general, the herbicide U46 combi fluid (2,4-D + MCPA) has been previously registered as effective for controlling broadleaf weeds in corn fields and can provide a wider spectrum of control when used in combination. Additionally, the mixture of two herbicides, Nicosulfuron and Bromoxynil + MCPA, had a desirable effect on weed control. It has also been reported that the mixture of Bromoxynil + MCPA and Nicosulfuron in corn fields increases the herbicidal spectrum and effectively controls dominant weeds in the field. One solution to broaden the spectrum of Nicosulfuron herbicide in controlling broadleaf weeds is to mix it with other broadleaf herbicides (Dobbels and Kapusta, 1993; Baghestani *et al*. 2013). Bromicide MA herbicide is a combination of Bromoxynil and MCPA herbicides that act differently from Nicosulfuron and successfully control a wide range of broadleaf weeds. It appears that the mixture of these two herbicides not only enhances the herbicidal spectrum but may also reduce the required dosage for achieving the desired control level compared to the recommended doses of each herbicide alone (Dobbels and Kapusta 1993; Mamnoei and Baghestani 2014; Bahari *et al*. 2011).

Conclusion

Based on the results of this experiment, Amaize-ing herbicide ((terbuthylazine, 50% SC)) at a rate of 1.8 liters of commercial product in pre-emergence (after corn sowing and before weed emergence), Adengo in early post-emergence (between the first and second irrigation) can effectively control broadleaf weeds in corn fields without negatively affecting corn plants and can enhance the yield of this crop.

Conflict of Interest

The authors have no conflict of interest concerning this article.

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