Evaluating the effects of hand weeding and selective herbicides on weeds control and maize (*Zea mays* L.) traits

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Abstract. An experiment was performed with a randomized complete block design and four replications at the Agricultural and Natural Resources Research Center of Moghan during 2021-2022. The herbicides included Amaize-ing (terbuthylazine, 50% SC), 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), Bromicide MA (Bromoxynil + MCPA 40% EC) + Cruze (Nicosulfuron 4% SC). The results of the analysis of variance showed a significant difference between treatments in terms of weeds density, weeds dry weight, maize grain yield, chlorophyll index (SPAD), leaf weight, proline, and catalase enzyme activity. The use of 1.8 and 2 L. ha-1 treatments of Amaize-ing, Adengo, Lumax, U46 Combi Fluid, Bromicid MA and Bromicid MA + Cruze herbicides had a favorable effect on weeds and resulted in a 20 to 30% increase in maize yield for these herbicides. The results showed that the highest chlorophyll content and leaf weight of maize was associated with treatments of Adengo, Lumax, U46 Combi Fluid, Bromicide MA and Bromicide MA + Cruze. But the Amaize-ing herbicide had a lower effect on leaf weight of maize. Also, increasing the dose of Amaize-ing increased the proline content and activity of catalase enzyme in maize.

1 Introduction

Maize ranks first globally in terms of yield and production, and it is the second most cultivated crop after wheat in terms of acreage [20]. Among various methods of weed control, the use of herbicides plays a crucial role in weed management due to their effectiveness and cost efficiency [4]. Currently, it is widely accepted that the use of herbicides has been

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successful [10]**,** and this factor has led to increased production in major crop plants, and every year, new compounds are proposed for chemical control of weeds [7]**.** New compounds may include new active ingredients or previous active ingredients that have been blended together in new formulations with optimal ratios [18, 21, 25]. Among the herbicides used in Iran for weed control in maize, we can mention U46 Combi Fluid, Eradican, Bromoxynil + MCPA, Foramsulfuron, Nicosulfuron, Rimsulfuron, Nicosulfuron + Rimsulfuron, Mesotrione + S-metolachlor + Terbuthylazine and Topramezone [27]. Some of these herbicides have been used in Iranian maize fields for years, and in addition to the environmental risks, there is also a high risk of weed resistance to some of them (such as Nicosulfuron) [8]. Therefore, the limited mechanism of action of recommended herbicides for maize fields and the risks associated with consecutive use of herbicides with similar mechanisms of action, are the main reasons for introducing and registering herbicides with a wide control spectrum [12], especially with diverse target sites, for weed management in Iranian maize fields.

Despite the inherent selectivity mechanisms that facilitate crop production [11], herbicides can cause some phytotoxicity to crop plants and reduce shoot dry weight, plant height, and alterations in plant metabolism by generating reactive ogygen species (ROS) [3]. Most of the perturbations caused by herbicide treatment in plants are related to ROS generation and consequent oxidative stress [3]. The mode of action for some herbicides is the generation of ROS in plants as the secondary effect after the specific target site was sufficiently inhibited.

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

2 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

Treatments	Commercial name	Common name	Dosage
1	Amaize-ing	Terbuthylazine (500 g.L)	1 L. ha^{-1}
2	Amaize-ing	Terbuthylazine	1.2 L. ha^{-1}
3	Amaize-ing	Terbuthylazine	1.5 L. ha^{-1}
4	Amaize-ing	Terbuthylazine	1.8 L. ha^{-1}
5	Amaize-ing	Terbuthylazine	2 L. ha^{-1}
6	Adango	Isoxaflutole (225 g. L^{-1})+ Thiencarbazone (90 g. L^{-1})+ Ciprosulfamide safener (150 g. L^{-1})	0.55 L. ha^{-1}
7	Lumax	Mesotrione (125.5 g. L^{-1}) + S-metolachlor (375 g. L^{-1}) + terbuthylazine (37 g. L^{-1})	4.5 L. ha^{-1}
8	U46 Combi Fluid	2. 4, D (360 g. L^{-1}) + MCPA (315 g. L^{-1})	1.5 L. ha^{-1}
9	Bromicide MA	Bromoxynil (200 g. L^{-1}) + MCPA (200 g. L^{-1})	1.5 L. ha^{-1}
10	Bromicide MA + Cruze	Bromoxynil (200 g. L^{-1}) + MCPA (200 g. L^{-1}) + Nicosulfuron (40 g. L^{-1})	1.5+0.5 L. ha^{-1}
11	Hand weeding	-	-

In order 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 cultivated. Each experimental plot had dimensions of approximately 3x8 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 herbicide [4]. Spraying of the herbicide 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 a water amount of 300 liters 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 ^{O}C for 48 hours. The reduction was calculated by dividing the weed density/biomass in the treated half by the weed density/biomass in the untreated half and multiplying by 100.

In this equation, No Spray and Spray 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 No Spray and Spray represent the dry weight of weeds in the unsprayed and sprayed quadrats, respectively).

During the harvest, the yield of each section of the plot (at least from an equivalent area of two square meters) was separately harvested (sprayed and unsprayed sections), and the yield for each plot was calculated.

Leaf chlorophyll was estimated 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 [5].

To measure the proline content and catalase activity, three biological replications from the most recent fully developed leaves of three different plants were acquired for each duplicate sections in each plot. Proline content of fresh leaves was measured using the procedure described by Bates *et al*. [14]. Catalase activity was measured by the method of Karo and Mishra [17].

At physiological maturity, ears from two rows of the center of each plot 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 the Duncan's multiple range test at a significant level of 5%, using the same software.

3 Results and Discussion

The spectrum of broadleaf weed species in the maize field is shown in Table 1. Only the data related to the dominant weeds in each replication were separately subjected to statistical analysis.

Table 2. The weed species composition in the maize 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 +++, inferior presence +, no presence -

3.1 Effect of treatments on 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 concluded that controlling this weed with treatments of 1.2, 1.5, 1.8, 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 efficacy in controlling this weed (Table 3).

The results indicated that the herbicide treatments of 1.2, 1.5, 1.8, 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 weak, resulting in a 72% reduction in weed density (Table 3).

The results showed that among the applied treatments, the best efficacy in controlling the dry weight of redroot pigweed was achieved by the treatments of 1.2, 1.5, 1.8, 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 commercial product was not able to effectively reduce the dry weight of the mentioned weed (Table 3).

The results obtained for the percentage reduction in dry weight of common lambsquarters indicate that the treatments of 1.2, 1.5, 1.8, 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 efficacy of 1 liter of Amaize-ing commercial product in controlling this weed was significantly lower (Table 3).

In general, the results showed that the application of doses of Amaize-ing herbicide in the early stages of growth, due to the greater sensitivity of weed seedlings to herbicides, had a greater inhibitory effect on weed density and dry weight. In such conditions, due to the prevention of early-season interference of weeds with maize, their competitive effects on the crop were minimized, and the application of even lower amounts of herbicides (even less than 2 liters of commercial product per hectare) had very positive results in increasing crop yield. But based on the evaluation of herbicide efficacy, it can be said that the use of Amaize-ing herbicide at doses ranging from 1 to 1.2 liters per hectare as a pre-emergence application after maize planting showed lower efficacy in weed control, so their use is not recommended.

Table 3. Mean comparison of chemical control on the percent reduction in density and dry weight of weeds (compared to the control of no spraying)

Treatments	Density reduction percentage		Dry weight reduction percentage	
	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 1.5L	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 letter did not show significant differences (Duncan $P≤0.05$)				

3.2 Effect of treatments on maize traits

The results of the analysis of variance showed a significant difference between treatments in terms of maize grain yield, SPAD, leaf weight, proline and Catalase (data not shown). The results of the comparison of mean values showed that the highest maize 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 compared to the control (hand weeding) (Table 4). The use of these herbicides resulted in a 20 to 30% increase in maize yield (Table 4). The results showed that the application of 1, 1.2, and 1.5 liters of Amaize-ing, the lowest efficiency in controlling the existing weeds resulted in the least increase in maize yield. Baghestani *et al*. [16] concluded that chemical control of weeds can lead to increased maize yield compared to the uncontrolled weeds. Nurs *et al*. [23] also reported that weed control can increase crop yield compared to the uncontrolled weeds. These results align with the findings of this study.

The results of mean comparisons showed that regardless of the weed free treatment (hand weeding), the highest maize chlorophyll content (SPAD) was associated with treatments of Adengo, Lumax, U46 Combi Fluid, Bromicide MA, and Bromicide MA + Cruze (Table 4). The results of maize leaf weight showed that regardless of the weed free treatment (hand weeding), the all treatments were placed in the same statistical group as the control with hand weeding (Table 4). The treatments of Amaize-ing herbicide have a lower effect on leaf weight of maize, without a significant difference from each other, which can be attributed to effective weed control in competition with maize plants.

The results of analysis of variance showed that the content of proline and activity of catalase enzyme were significantly affected by treatment herbicides (data not shown). In terms of proline, all treatments of Amaize-ing herbicide resulted in an increase in maize content of proline compared to the other treatments (Table 4). The results showed that among the applied treatments, Adengo, Lomax, U46 Combifluid, Bromicide MA and Bromicide MA + Cruze had the lower activity of catalase enzyme but increasing the dose of herbicide Amaize-ing increased the activity of catalase enzyme in maize (Table 4). In an experiment, it was reported that both the interference of weeds and the use of herbicides in plants stimulate the synthesis of antioxidant molecules, including proline, in response to stress [22]. In a study on shallot plant (*Allium stipitatum*), they stated that the highest content of proline was observed in the treatment without weed control [19]. The study of Grigoryuk et al. [9] by evaluating the herbicide treatments on the catalase enzyme activity in the root and stem of maize showed that the herbicide increased the catalase enzyme activity in both organs studied in the maize plant which was consistent with the results of the present study. The study of Hassannejad and Porheidar Ghafarbi [24] showed that increasing of clodinafop-propagrgyl (TOPIK) herbicide, the maximum fluorescence (Fm), variable fluorescence (Fv), efficiency and/or activity of water-splitting complex at 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 Topik, Titos, Equip, Mister, Lumax, Bromicide and Oltima herbicide on the photosynthetic efficiency of three maize cultivars (CC-260, 400, 704), found that Topik, Titos, Equip, Mister and Oltima herbicides had no effect on the chlorophyll fluorescence [27].

Table 4. Mean comparison of the effect of chemical control of the weeds on maize traits.

	Grain yield t. ha^{-1}	Spad	Leaf weight (g)	Proline content (µmol/g fresh weight)	Catalase (Dansitometric activity)
Amaize-ing 1 L	5.94e	53.825 abc	2.539 abc	73.600 ab	595.871 de
Amaize-ing 1.2 L	6.91d	53.114 a-d	2.216 bc	73.617 ab	602.088 c
Amaize-ing 1.5 L	6.93d	52.125 bcd	1.185 bc	73.951 ab	602.317 c
Amaize-ing 1.8 L	7.60ab	51.375 cd	2.117 bc	74.329 a	610.953 b
Amaize-ing 2 L	7.77ab	48.75 d	1.812 c	74.530 a	614.320 a
Adango 0.55 L	7.09c	55.825 ab	2.808 ab	71.033 d	592.058 e
Lumax 4.5 L	7.01c	55.025 abc	2.581 abc	71.767 cd	595. 129 d
U46 Combi Fluid 1.5 L	7.03c	55.764 ab	2.858 ab	71.817 cd	559.713 g
Bromicide MA 1.5 L	7.81a	54.175 abc	2.623 abc	72.633. bc	590.835 e
Bromicide MA 1.5 L+ Cruze 0.5 L	7.88a	53.821 abc	2.613 abc	72.896 bc	573.483 f
Hand weeding	8.03a	56.5 a	3.291 a	69.283 e	409.931 h
The means with similar letters in each column are not significantly different Duncan $p\leq 0.05$)					

The results of this experiment indicated that treatments that had a higher percentage of weed density and dry weight control shifted the competitive conditions towards the cultivated crop, leading to an increase in leaf weight and photosynthetic capacity, ultimately resulting in increased maize grain yield. Since old herbicides are widely used in Iran for controlling maize 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. Based on the results of expriment, it can be concluded that Amaize-ing herbicide, at doses ranging from 1.8 to 2 liters per hectare, had a desirable efficacy in controlling weeds and increasing maize grain yield, without significant differences compared to other registered herbicides for grain yield. Although the use of 2 liters of Amaize-ing herbicide showed higher efficacy in weed control compared to a dose of 1.8 liters, due to the lack of statistical difference, its use is not recommended. Therefore, based on the results of this experiment, the use of 1.8 liters of Amaize-ing herbicide is recommended for maize, especially in terms of sustainable weed management and chemical control hazards.

Amaizeing 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. This herbicide controls several broadleaf weeds in maize fields, such as redroot pigweed (*Amaranthus hybridus*), common purslane (*Portulaca oleracea*), and hairy nightshade (*Solanum sarrachoides*) [1]. The tolerance of maize 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 [1]. Also based on the results of expriment and the need to reduce herbicide consumption to minimize undesirable environmental effects, the use of Adengo herbicide at a rate of 0.55 liters per hectare is recommended for weed control in maize fields. 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 maize 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 maize 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 [2, 15]. Bromicide MA herbicide is a combination of Bromoxynil and MCPA herbicides that acts differently from Nicosulfuron and successfully controls 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 [2, 6, 13].

4 Conclusion

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

Additionally, Adengo herbicide, when applied in the early post-emergence stage (typically between the first and second irrigation), also demonstrates excellent control of broadleaf weeds. The combination of these herbicides offers a robust solution for weed management in maize, promoting healthier crop growth. Not only do these herbicides help in maintaining weed-free conditions, but they also contribute to the enhancement of maize yield by preventing competition for resources such as water, nutrients, and light, which are crucial for optimal maize development. The results suggest that using these herbicides as part of an integrated weed management strategy can lead to significant improvements in maize productivity while ensuring the sustainability of the crop's growth and minimizing any potential adverse effects on the environment

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