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The Joint Action of Some Broadleaf Herbicides on Potato (*Solanum tuberosum* L.) Weeds and Photosynthetic Performance of Potato

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Abstract: Herbicide mixtures are a modern weed management practice as they reduce herbicide application. This study aimes to evaluate the effect of metribuzin, halosulfuron and flumioxazin applied individually and as mixtures (metribuzin:halosulfuron and metribuzin:flumioxazin) on *Chenopodium album*, *Amaranthus retroflexus,* and potatoes on biomass and chlorophyll-*a* fluorescence in 21 experiments. The individual herbicide experimental design was a randomized completely block design with seven doses and three replications. The factorial experiments were performed on the basis of randomized completely block design in three replications for binary mixture experiments. Flumioxazin was very potent in controlling *C. album*, *A. retroflexus*, and injured potatoes with a 50% effective dose (ED_{50}) of 1.21, 0.54, and 12.23 g ai·ha^{-1}, respectively. Both mixtures of metribuzin:halosulfuron and metribuzin:flumioxazin generally showed an antagonistic effect on both weeds and potato in 12 independent experiments. Metribuzin, halosulfuron, and flumioxazin significantly decreased photosystem II activity by decreasing the maximum quantum efficiency (F_v/F_m). The metribuzin:halosulfuron mixtures almost followed the Additive Dose Model for F_v/F_m, whilst there was an antagonistic effect for the metribuzin:flumioxazin that was closely related to biomass. The results indicated that mixtures were generally antagonistic, and the endpoint choice is pivotal when assessing the joint action of mixtures.

Keywords: antagonism; binary mixture; isobole model; maximum fluorescence

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1. Introduction

In Iran, the most important weeds in potatoes are *Amaranthus* spp., *Chenopodium album* L., *Portulaca oleracea* L., *Polygonum* spp., *Setaria* spp., *Echinochloa crus gali*, *Hordeum leporinum*, and *Lolium* spp. [1]. The purpose of using herbicides is to prevent competition between with weeds and crops [2]. This relies on the fact that potato growers have few available herbicide options in Iran (i.e., metribuzin and paraquat) [3]. Metribuzin is one of the principal herbicides used to control mono and dicotyledonous weeds in potato crops [4]; but it does not provide season-long control. Halosulfuron is a systemic sulfonylurea herbicide [5]. Previous studies demonstrated that halosulfuron pre-emergence at 110 g ha^{-1} provided more than 95% control of redroot pigweed (*Amaranthus retroflexus* L.) and common lambsquarters (*Chenopodium album*) 45 days after treatment [6]. Flumioxazin is an N-phenyl phthalimide herbicide that inhibits protoporphyrinogen oxidase (PPO) [7].

Flumioxazin has both preemergence and postemergence activity [8]. Flumioxazin applied pre-emergence at 35 and 70 g ai·ha^{-1} provided excellent control of *A. retroflexus*, *C. album*, common purslane (*Portulaca oleracea* L.), and barnyardgrass (*Echinochloa crus-galli* [L.] P. Beauv.) [8].

Repeated applications of herbicides over the last five decades have led to the spread of herbicide-resistant weeds. The herbicide mixtures have received a great deal of attention in recent decades. The isobole method can be used to calculate the joint action of herbicides. The most famous principle is to employ the reference models of the Additive Dose Model (ADM) or the Multiplicative Survival Model (MSM) [9]. In the ADM, it is assumed that the two identical modes of action of herbicides do not interfere at the binding site. On the other hand, when non-identical modes of action of herbicides are combined, their reference model can follow MSM. The ADM model is simple and useful for the end-user of mixtures [10]. Both models are valid, but MSM is difficult to interpret as the isoboles do not have a district form as does the ADM isobole. In fact, the MSM is based upon binomial responses (dead/alive, affected/not affected) [11]. If the effectiveness of a mixture is lower than what is seen in the straight-line ADM isobole at ED_{50} for example, the efficacy of the herbicide ratios is displaced to the right of the ADM isobole, which is antagonistic. If the ratios efficacy are displaced to the left of the isoboles, the mixture is synergistic [12].

A simple, rapid, and non-destructive method to evaluate stress is chlorophyll *a* fluorescence measurement. It is utilized for investigating plant reactions to herbicide in a short period [13].

The objective of this study was to examine whether single and binary mixtures of metribuzin:halosulfuron and metribuzin:flumioxazin deviate from the ADM on potato weed and to compare the endpoint of chlorophyll *a* fluorescence measurements with biomass measurements. We hypothesize that *C. album* and *A. retroflexus* will exhibit different responses to the herbicides from different families in individual and mixture-applied. On the other hand, the effect of mixtures of metribuzin:halosulfuron and metribuzin:flumio xazin would predict the synergistic effect on *C. album*, *A. retroflexus*, and potato.

2. Materials and Methods

2.1. Dose-Response Experiments for Individual Herbicide

Seeds of *C. album* and *A. retroflexus* were collected from mature plants in the potato research field of Mohaghegh Ardabili University, Iran (longitude 48°20′, 38°15′; altitude 1350 m). *C. album* and *A. retroflexus* seeds were kept in H_2SO_4 (98%) for two minutes, and then they were washed to break their dormancy, according to [14]. Twenty weed seeds were planted 0.5 cm deep in 2 L plastic pots. The pots of weeds filled a clay loam soil, sand, and peat (1:1:1 *v*/*v*). One potato tuber (cv. Agria) was planted at 4 cm depth in 23 cm pots containing a mixture of clay loam soil, sand, and peat with 1:1:1 *v*/*v*.

Plastic pots were kept in controlled conditions: photoperiod (16:8 light:dark) and temperatures (22 ± 2 °C: 15 ± 1 °C day:night). At the two-leaf stage, the weed seedlings were thinned to four per pot. Subsequently, formulated metribuzin (Sencor, WP 70%) was obtained from Bayer, Persian AG, Tehran, Iran, halosulfuron (Sempra, WG 75%) from Nufarm, the Iranian Research Institute of Plant Protection, Tehran, Iran, and flumioxazin (Pledge, WP 50%) was obtained from the Sumitomo Chemical Company, and the Iranian Research Institute of Plant Protection, Tehran, Iran. The stock solutions and dilution series of metribuzin, halosulfuron, and flumioxazin were prepared, and the dilution series was applied on the day of the experiments. The experimental design was a randomized completely block layout with seven doses for each herbicide, and three replications. The doses of metribuzin were 0, 15.62, 31.25, 62.5, 125, 250, 500, 1000; for halosulfuron they were 0, 1.56, 3.125, 6.25, 12.5, 25, 50, 100; and for flumioxazin they were 0, 3.90625, 7.8125, 15.625, 31.25, 62.5, 125, 250 g ai·ha^{-1}, respectively. Plants with two to four true leaves were used for herbicides treatments. Herbicides were applied by a CO_2-pressurized backpack sprayer fitted with 8002 vs. flat fan nozzles at 300 kPa, and a spray volume of 200 L ha^{-1}. The weeds in each pot were harvested three weeks after application of herbicides, and fresh weight was measured. Crop tolerance is determined by assessment of the visible injury. Percentage of injury to the potato was measured, with 0 representing no crop injury and 100 representing the death of the potato plants. Visible injury of post-treatment was assessed three weeks after treatment. The individual herbicides experiment was carried out once.

The dose-response data were analyzed using the R program (Version 3.6.1) with the drc package (Version 3.6.1). The log-logistic regression of fresh weight of *C.album*, *A. retroflexus,* and potato on dose was fitted with a log-logistic regression using a three parameters log-logistic model [15]:

$$y = \frac{D}{1 + \exp(b(\log(z) - \log(ED_{50}))} \tag{1}$$

where y is fresh weight at the z-th dose, D is the upper limit where the dose is zero, ED_{50} denotes the dose required for reducing fresh weight by half and bis proportional to the slopes of the curves around ED_{50}. The dose-response fit the data reasonably well, assessed by graphical analysis of residuals and tested for lack of fit.

2.2. Dose-Response Experiments for Mixtures Herbicide

After the determination of individual metribuzin, halosulfuron, and flumioxazin ED_{50} by Equation (1), the various mixture ratios were calculated based on the relative potency of the individual herbicides applied alone to ensure that the ratios were evenly distributed along the ADM isoboles [12]. The biological exchange rate in a single application, i.e., the relative potency (r), between the herbicides was calculated as follows:

$$r \ = \ ED_{50A} / ED_{50B} \tag{2}$$

Mixtures ratios used were: (100:0), (95:5), (87:13), (69:31) and (0:100)% for metribuzin:halo sulfuron and for metribuzin:flumioxazin (100:0), (98:2), (95:5), (87: 13) and (0:100)% for *C. album*, respectively. Mixtures ratios were varied on *A. retroflexus* and were calculated (100:0), (97:3), (91:9), (77:23) and (0:100)% for metribuzin:halosulfuron and for metribuzin:flumioxazin, (100:0), (98:2), (95:5), (86:14) and (0:100)%, respectively. The experimental design was a so-called ray design [16]. Treatment was prepared using distilled water as a solvent, and volumes were raised to 0.5 L per mixture ratio of herbicides. Procedures reported by [17] were followed whenever product mixing was required. Since mixes of products constituted many of the treatments in this study, a specific mixing order was followed according to the pesticide formulations in each treatment, as follows: WG (wet granule) formulations were added first, if present, followed by WP (wettable powder) type of formulations. The dose-response mixture of metribuzin:halosulfuron and metribuzin:flumioxazin experiments were carried out as individual herbicides, and the experiments were independently repeated twice for each mixture. Percentage of injury to the potato plant was measured, with 0 representing no crop injury and 100 representing the death of the potato plants. Visible injury of post-treatment was assessed three weeks after treatment. The ED_{50} of any mixture ratio of metribuzin:halosulfuron and metribuzin:flumioxazin was calculated by Equation (1).

The metribuzin:halosulfuron and metribuzin:flumioxazin mixture at any ratio follows ADM at equivalent doses of z_m which is obtained by [18]:

$$Z_1 = r.Z_2 = z_m \ = z_1 \ + r.z_2 \tag{3}$$

Z_1 and Z_2 are the ED_{50} of herbicides 1 and 2 when applied singly, and z_1 and z_2 are the ED_{50} herbicide 1 and 2 in a mixture with the same biological response. The relative potency (r) between herbicide 1 and 2 is r = Z_1/Z_2 according to Equation (2).

In addition, in the ADM model, the ED_{50} dose of mixtures calculated by the ED_{50} values of singly herbicides as well as their different mixture ratios in the mixtures:

$$ED_{50amix} = ED_{50A}/(\alpha + (1-\alpha)(ED_{50A}/ED_{50B}) \quad (4)$$

where ED_{50A} is the herbicide 1 ED_{50} value, α is the herbicide 1 ratio in the mixture, and r is the relative potency according to Equation (2).

In an ED_{50} isobologram (Figures 1–5), the X and Y axes are the dose axes of each individual herbicide in a mixture, e.g., metribuzin:halosulfuron. Thus, if metribuzin is the dose of the X-axis and halosulfuron is the dose on the Y-axis, and the mixtures are plotted; likewise, the mixture points represent the isobole points. The points on the graph represent the combination of the two herbicides that are iso-effective for a given response (ED_{50}). The solid lines for each ED_{50} point are the confidence intervals for the mixtures. If the herbicides in a mixture do not interact, the points will form a straight-line relationship as indicated in Figures 1–6. When herbicides are less effective than expected from their response curves, also denoted antagonistic action, larger amounts of each herbicide are required to produce the same effect as that of the herbicides applied singly. Consequently, the mixture points will fall above and to the right of the zero-interaction line.

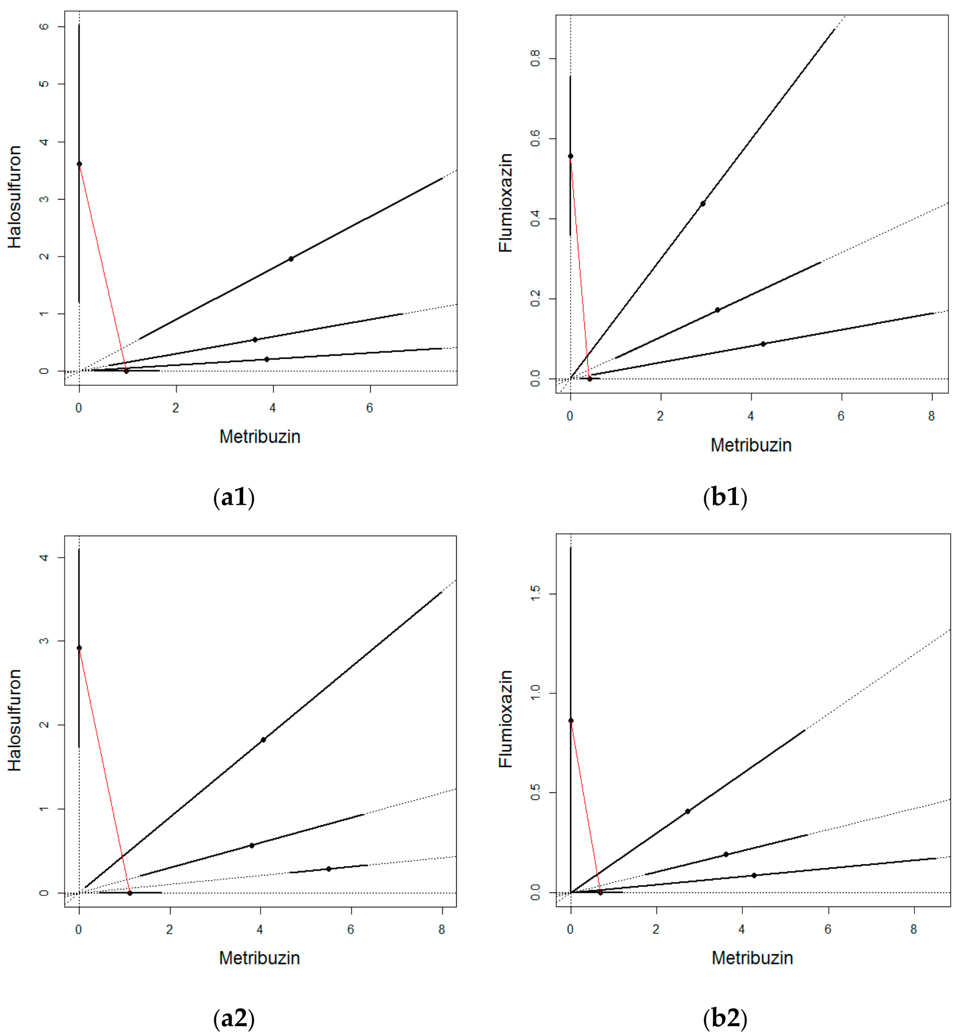

Figure 1. ED_{50} isobologram showing the interactions of metribuzin:halosulfuron (**a**) and metribuzin:flumioxazin (**b**) on fresh weight *C. album* in first experiment (**1**) and second experiment (**2**). The straight line of the isobologram indicates additivity. The lines around the mixture points have 95% confidence intervals.

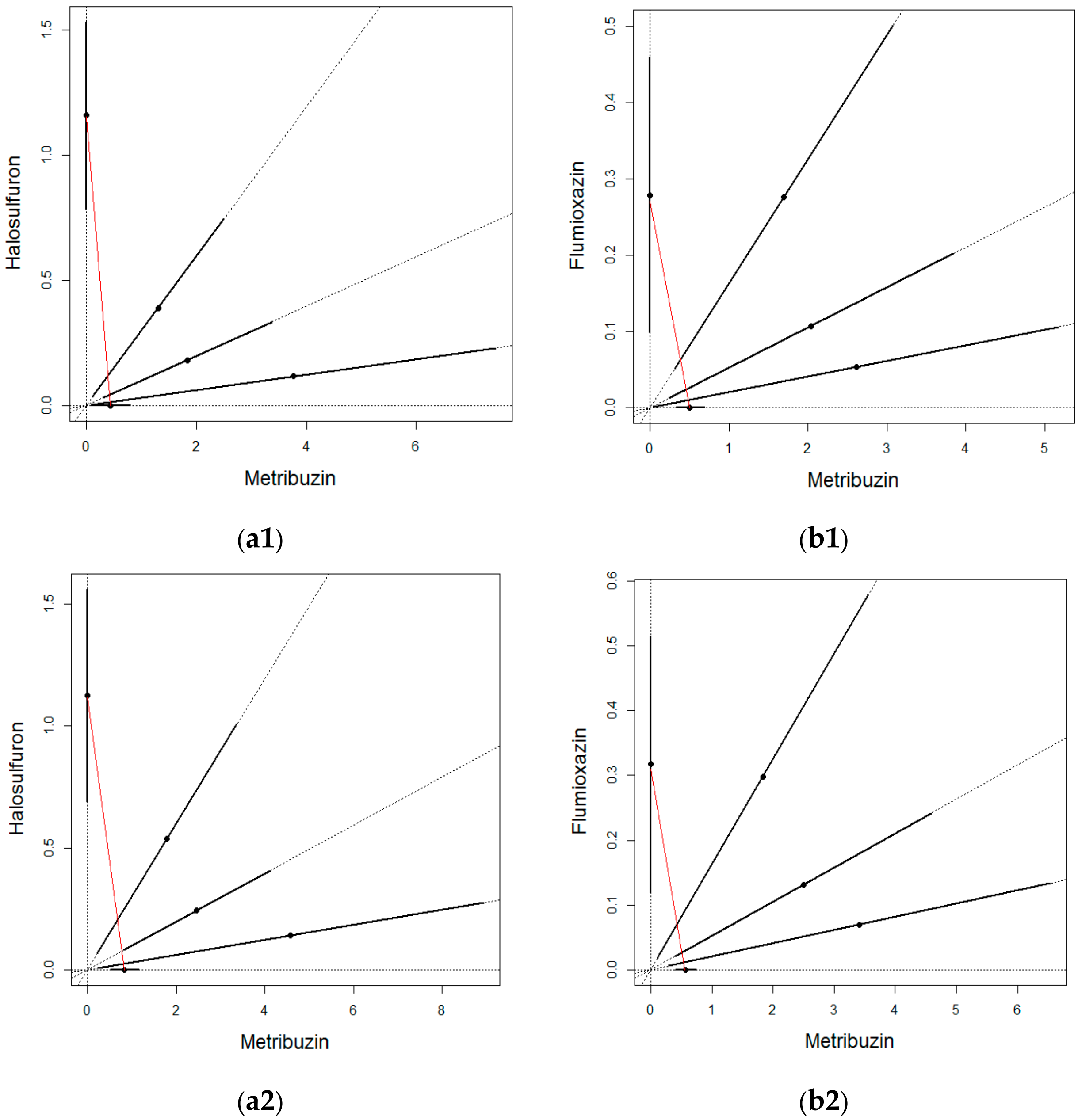

Figure 2. ED_{50} isobologram showing the interactions of metribuzin:halosulfuron (**a**) and metribuzin:flumioxazin (**b**) on fresh weight *A. retroflexus* in the first experiment (**1**) and second experiment (**2**). The straight line of the isobologram indicates additivity. The lines around the mixture points are 95% confidence intervals.

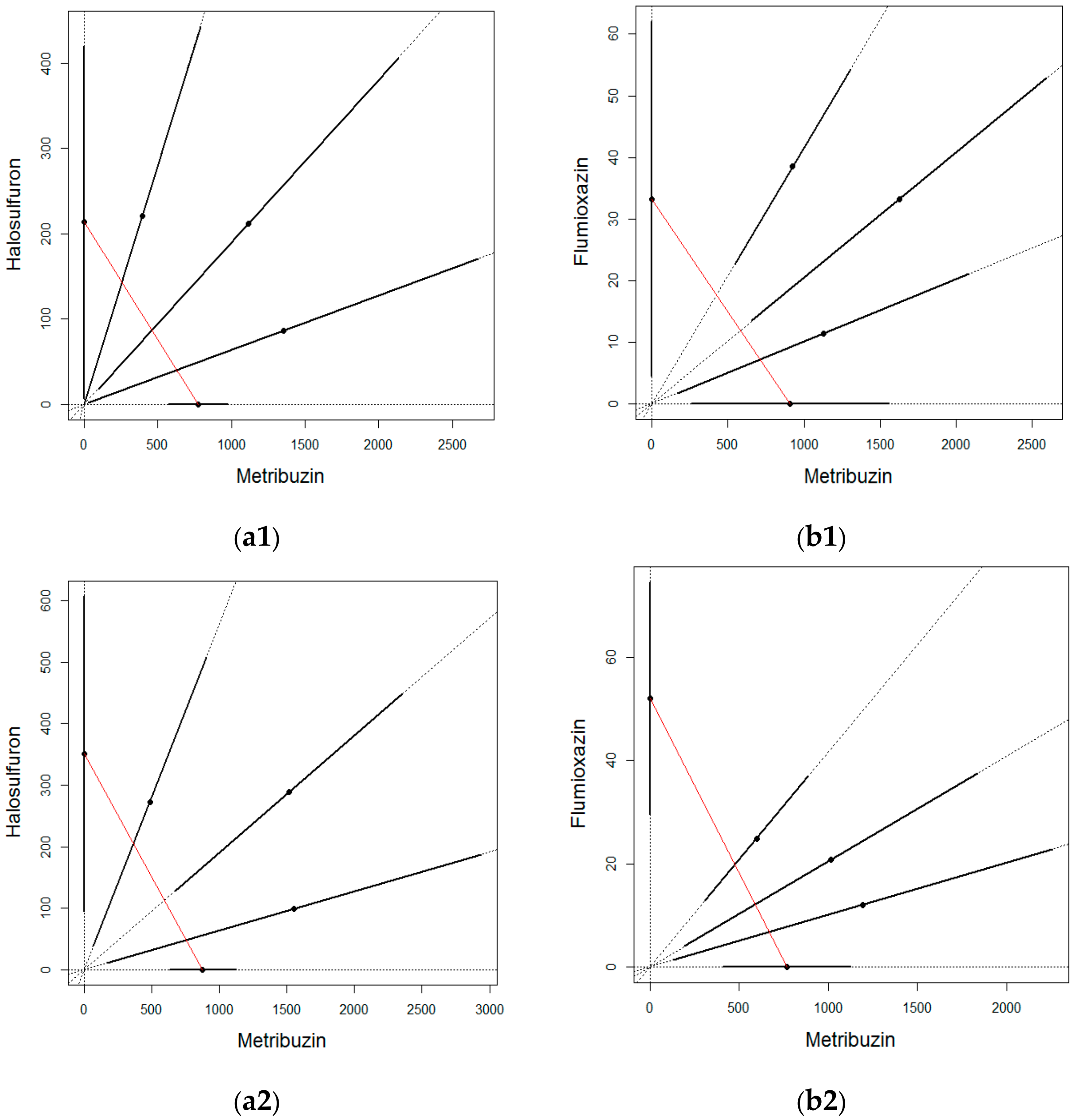

Figure 3. ED_{50} isobologram show the interactions of metribuzin:halosulfuron (**a**) and metribuzin:flumioxazin (**b**) on potato fresh weight in first experiment (**1**) and second experiment (**2**). The straight line of isobologram indicates additivity. The lines around the mixture points are 95% confidence intervals.

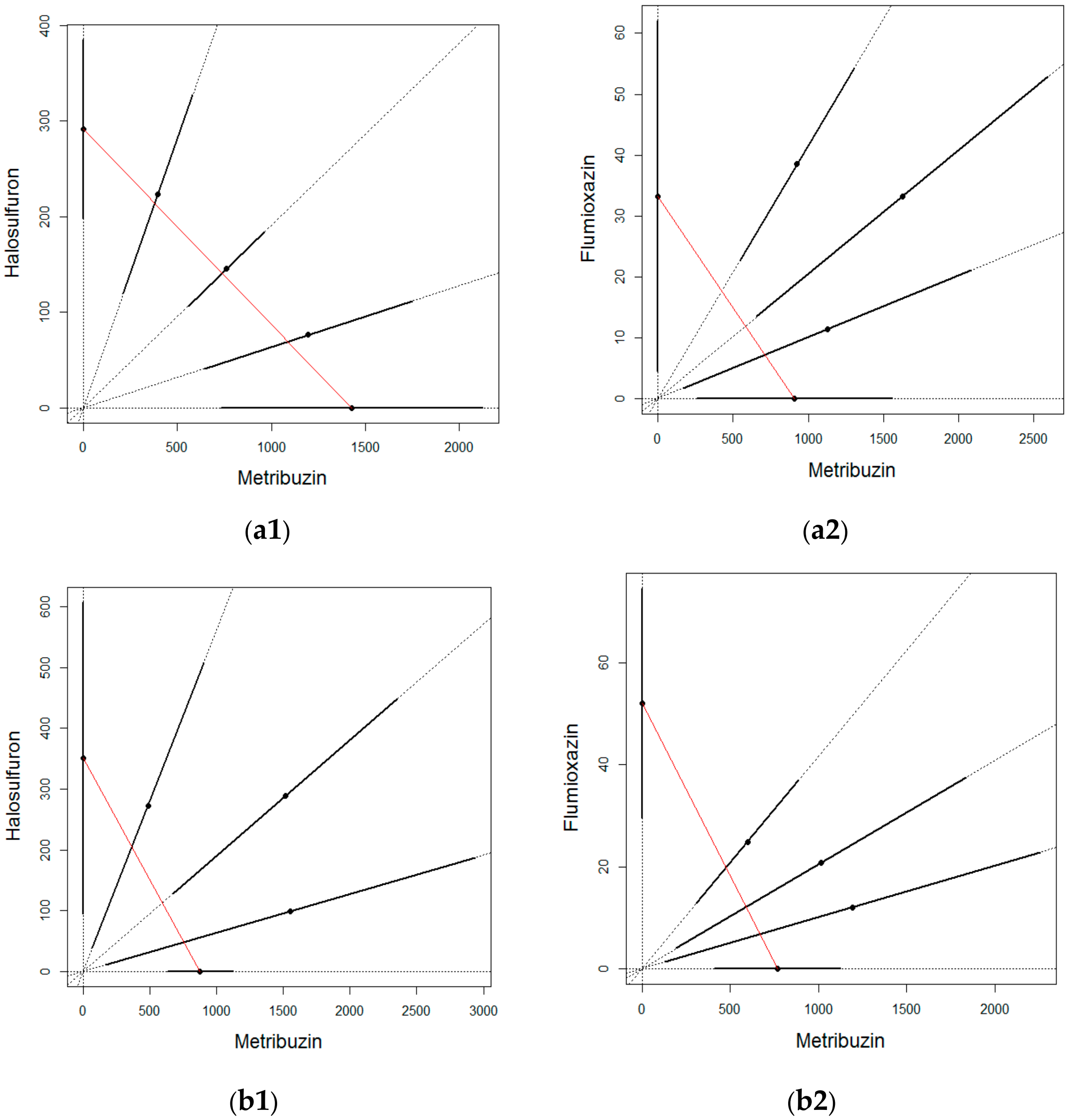

Figure 4. ED_{50} isobologram showing the interactions of metribuzin:halosulfuron on F_v/F_m of potato in 4 h (**1**) and 8 h (**2**) in the first (**a**) and second experiment (**b**). The straight line of isobologram indicates additivity. The lines around the mixture points are 95% confidence intervals.

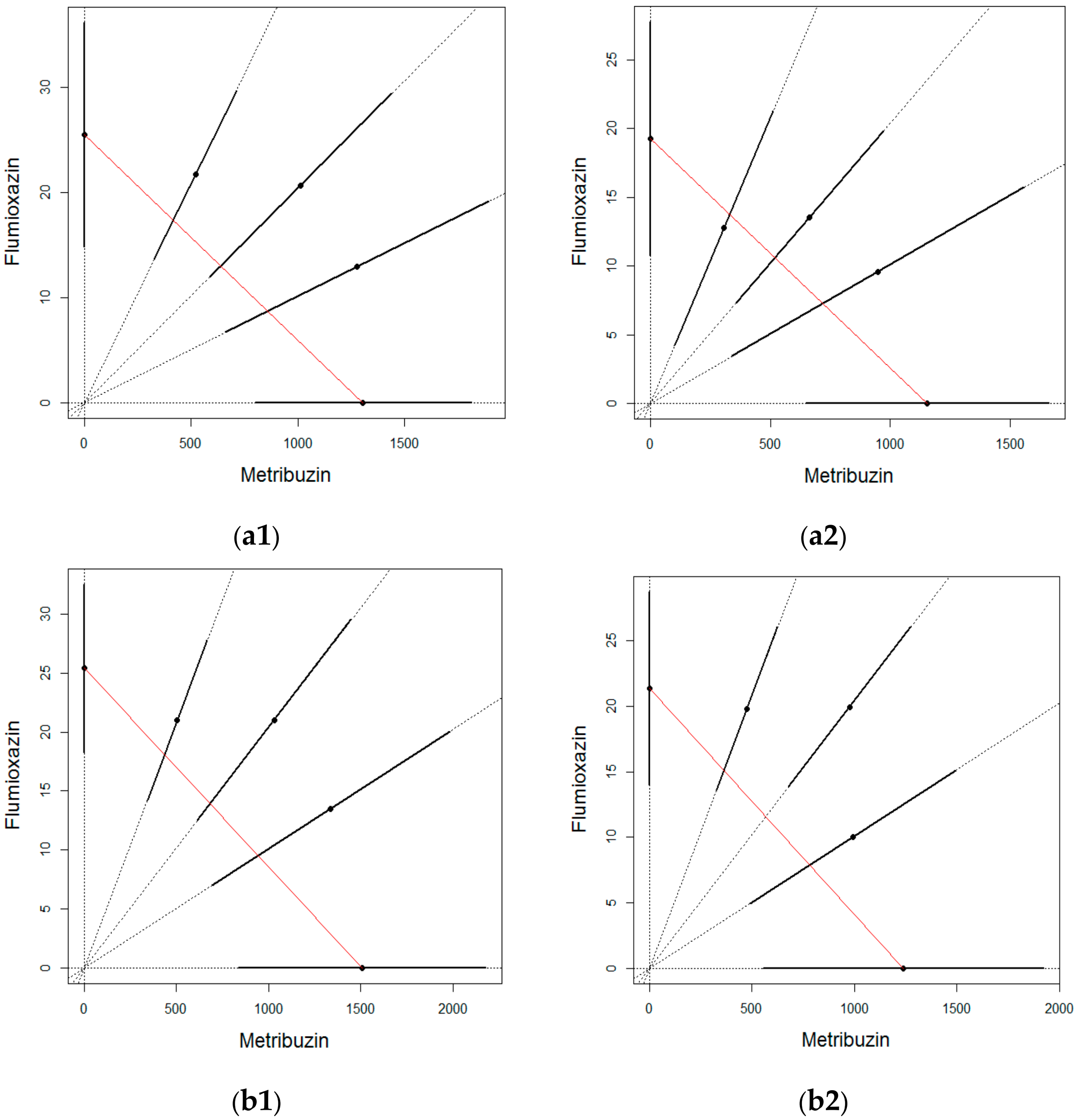

Figure 5. *ED*$_{50}$ isobologram showing the interactions of metribuzin:flumioxazin on F_v/F_m of potato in 4 h (**1**) and 8 h (**2**) in first (**a**) and second experiment (**b**). The straight line of isobologram indicates additivity. The lines around the mixture points are 95% confidence intervals.

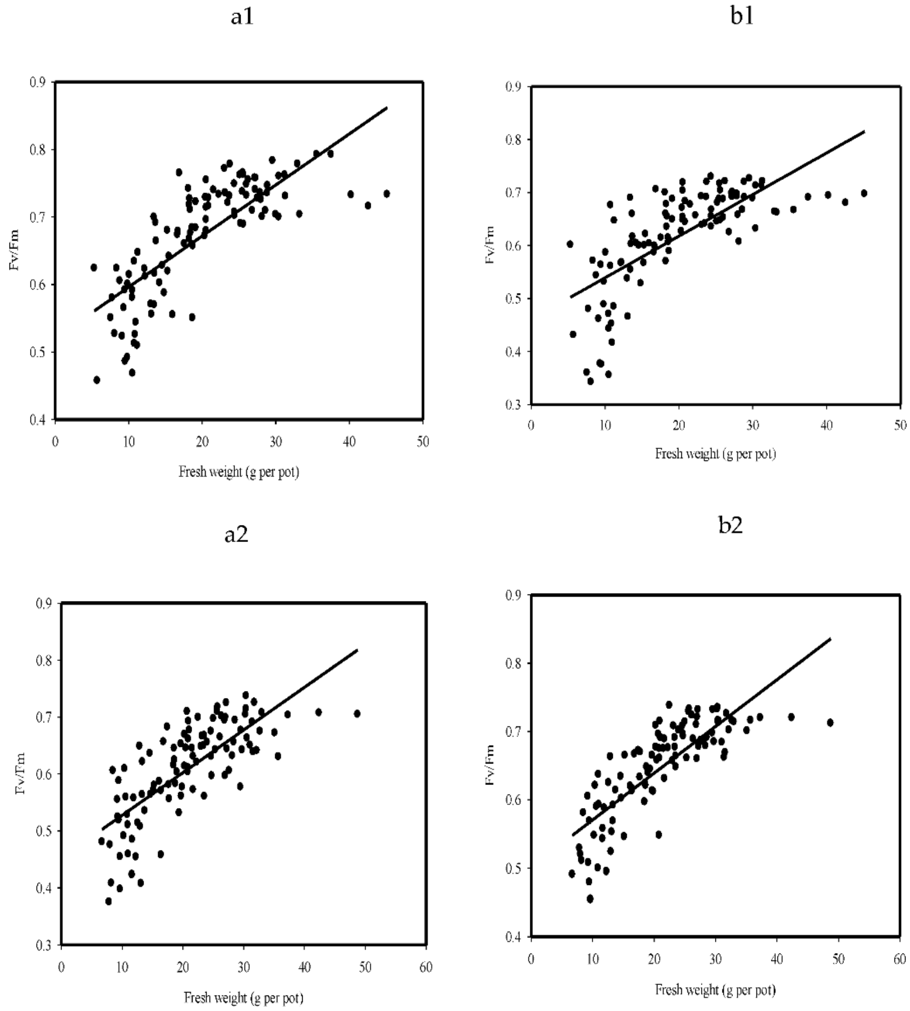

Figure 6. Linear regression of the maximum quantum photosystem II (F_v/F_m) on Fresh weight, 4 days (**a1**,**a2**) and 8 (**b1**,**b2**) days after treatment in potato and fresh weight 3 weeks after treatment for metribuzin:halosulfuron in first (**a**) and second experiment (**b**). Each data point on the graph represents one replication of potato with indifferent mixture herbicide.

2.3. *Individual and Mixture Herbicides on Potato Chlorophyll a Fluorescence*

Several experiments were carried out on potatoes in the greenhouse of the University of Mohaghegh Ardabili (longitude 48°20′, 38°15′; altitude 1350 m) in 2019 and 2020. The Individual experiment was set up as a randomized completely block design with three replications. One tuber of potato (cv. Agria) was planted 4 cm deep in 10 cm pots in each pot. The soil contained a mixture of clay loam soil, sand, and peat (1:1:1 *v*/*v*). Plastic pots were kept in controlled conditions: photoperiod (16:8 light:dark) and temperature (22 ± 2 °C: 15 ± 1 °C day:night). In potato, the metribuzin doses were 15.625, 31.25, 62.5, 125, 250, 500, 1000 g ai·ha^{-1}, halosulfuron 1.5625, 3.125, 6.25, 12.5, 25, 50, 100 and flumioxazin 3.90625, 7.8125, 15.625, 31.25, 62.5, 125, 250 g ai·ha^{-1}, respectively. Chlorophyll *a* fluorescence (ChlF) was measured using an Opti-Sciences (OS30p+) portable system (USA) 1, 2, 4, and 8 days after herbicide treatment (3 weeks after emergence) on the middle region of fully developed leaves. Before monitoring ChlF signals, dark-adapted leaves (30 min.) were exposed to saturated white light to estimate F_v/F_m, The quantum efficacy of the photosystem II. The mixture ratios were used (100:0), (94:6), (84:16), (64:36) and (0:100)% for metribuzin:halosulfuron and (100:0), (99:1), (98:2), (96:4) and (0:100)% for metribuzin:flumioxazin. The experiment of each mixture was independently repeated twice. The mixtures of metribuzin:halosulfuron and metribuzin:flumioxazin were carried out as an individual herbicide to assess ChlF parameters.

F_v/F_m of potato data resulting from metribuzin was fitted by Equation (1). While F_v/F_m of potato data resulted in halosulfuron, flumioxazin, and binary mixtures of metribuzin:halosulfuron and metribuzin:flumioxazin, and was fitted with a log-logistic regression four parameters log-logistic models [12]:

$$y = \frac{D - C}{1 + \exp(b(\log(z) - \log(ED_{50}))} \quad (5)$$

where y is F_v/F_m at the z-th dose, D and C is the upper and lower limit where the dose is zero and at an infinite dose of herbicide. ED_{50} denotes the dose required for reducing F_v/F_m by half and b is proportional to the slopes of the curves around ED_{50}. In addition, linear regression analyses were performed across all three replications of all treatments in two experiments using SigmaPlot (version 11.0) to determine the relationship between potato-fresh weight and F_v/F_m at 4 and 8 days after treatment in potato.

3. Results

Incompatibility of physico-chemicals such as inactive and insoluble compounds reduce herbicide mixture efficacy in the tank mixture. To evaluate the physico-chemical incompatibility of metribuzin:halosulfuron and metribuzin:flumioxazin, one experiment tested possible physico-chemical incompatibility issues upon mixing several metribuzin: halosulfuron and metribuzin:flumioxazin ratios. There was no physico-chemical incompatibility observed for metribuzin:halosulfuron and metribuzin:flumioxazin for different doses and different mixture ratios.

3.1. Potato Injury for Individual and Mixtures Herbicides

Injury of potato ranged from 0 to 2.5, 0 to 12.5, and 10 to 86% for metribuzin, halosulfuron, and flumioxazin in three weeks after application, respectively (data not shown). The order of specific injury of herbicides was ranked as flumioxazin > halosulfuron > metribuzin. Potato injury from metribuzin included chlorosis and yellow spotting of foliage. Visual injury symptoms of halosulfuron consisted of potato stunting, yellow spotting of foliage, and leaf margin necrosis. Yellow spotting resulted from halosulfuron contact. Reddening and intensive necrosis of treated leaves and stunted growth of potato resulted from flumioxazin application.

Metribuzin:halosulfuron mixture injury on potato ranged from 0 to 23.33 and from 0 to 26.66% after post application in first and second experiments, respectively. Injury by metribuzin:flumioxazin mixture was from 0 to 33.33 and from 0 to 28.33% in first and second experiments, respectively. The potato injury of (0:100) percentage mixture ratio of metribuzin:halosulfuron and metribuzin:flumioxain were higher than other mixture ratios. Visible symptoms of metribuzin:halosulfuron were yellow spotting of foliage and growth stunting of potato, while injury of metribuzin:flumioxazin included stunting of the foliage and browning and necrosis of the stem and leaves.

3.2. Dose-Response Analyses Herbicide Mixtures on C. album and A. retroflexus

The regressions demonstrated that the singly of metribuzin, halosulfuron, and flumioxazin doses were well described by a three-parameter log-logistic model judged by analysis of residuals and a test for lack of fit on *C. album* ($p > 0.05$) (Table 1). The ranking of ED_{50} for the three herbicides was metribuzin> halosulfuron> flumioxazin. The regression fits for *A. retroflexus* fresh weight were reasonable (Table 2). The test for lack of fit for metribuzin was significant, however, the analyses of residuals did not cause any alarm. The ED_{50} for the herbicides clearly showed that this weed species was much more sensitive to the herbicides than was *C. album*. (Tables 1 and 2).

Table 1. Estimated sigmoidal parameters for metribuzin, halosulfuron and flumioxazin on *C. album*. Standard errors in parentheses.

Herbicide	*d*	ED_{50}	Lack of Fit
Metribuzin	3.04 (0.15)	20.51 (7.24)	0.46
Halosulfuron	3.57 (0.16)	7.98 (3.44)	0.94
Flumioxazin	2.82 (0.07)	1.21 (0.38)	0.73

Table 2. Estimated sigmoidal parameters for metribuzin, halosulfuron and flumioxazin on *A. retroflexus.* Standard errors in parentheses.

Herbicide	*d*	ED_{50}	Lack of Fit
Metribuzin	2.88 (0.11)	9.37 (2.78)	0.001
Halosulfuron	2.75 (0.06)	1.14 (0.21)	0.06
Flumioxazin	1.51 (0.03)	0.54 (0.26)	0.53

The mixture dose-response curves described the response well. It is noticed in Figures 1 and 2 that the confidence intervals were significant. Still, the general trend of two independent experiments was, in all instances, an antagonistic effect for *C. album* and *A. retroflexus*. The binary mixture of metribuzin:halosulfuron and metribuzin:flumioxazin showed an antagonistic effect relative to the ADM reference model; the mixture ratios were displaced to the right of the ADM isobole for *C.album* and *A. retroflexsus.* (Figures 1 and 2). It means the mixtures would require higher doses of the tested mixture to get the same ED_{50} than applying the herbicides singly.

3.3. The Effect of Herbicide Mixtures on Potato Biomass and Maximum Quantum Efficiency (F_v/F_m) of Potato

For the individual herbicides, the ED_{50} values for potato biomass were much higher for metribuzin than for halosulfuron and flumioxazin (Table 3). The ranking of the herbicides was the same as in *C. album* and *A. retroflexus* (Tables 1–3). Table 4 showed the same ED_{50} ranking of the potency of the individual herbicides of the F_v/F_m dose-response curves. F_v/F_m values of the herbicide treatments were not affected until 4 days after treatment in all mixtures. The interesting issue of the F_v/F_m parameter is that the theoretical maximum is 0.83. Consequently, we have a reference with which we can compare the upper limit, d, in the log-logistic curve. In Table 4 the upper limit is between 0.63 to 0.76 and thus close to the theoretical value of completely non-stressed plants. The results of metribuzin:halosulfuron and metribuzin:flumioxazin on potato biomass showed the same pattern for the independently replicated experiment that the antagonistic effect was clear as for the weeds in Figures 1–3. In Figure 4, the metribuzin:halosulfuron of F_v/F_m seemed to follow the ADM model. All observations were placed outside of the ADM model except for one observation of metribuzin:halosulfuron on days 8 and 4 in the first and second experiments, respectively. For metribuzin:flumioxazin, the ED_{50} isobologram in Figure 5 shows the same picture in both experiments except for one observation on day 8 in the first experiment. Generally, the joint action of the metribuzin:flumioxazin acted antagonistically in relation to the ADM reference model.

Table 3. Estimated sigmoidal parameters for metribuzin, halosulfuron and flumioxazin on potato. Standard errors in parentheses.

Herbicide	*d*	ED_{50}	Lack of Fit
Metribuzin	36.58 (1.82)	872.24 (165.79)	0.79
Halosulfuron	39.89 (1.70)	165.81 (74.69)	0.92
Flumioxazin	36.95 (1.62)	12.23 (2.14)	0.08

Table 4. Estimated sigmoidal parameters for metribuzin, halosulfuron, and flumioxazin on F_v/F_m values of potato on days 4 and 8 days after treatment. Standard errors in parentheses.

Herbicide	*c*	*d*	ED_{50}	Lack of Fit
Metribuzin 4 days	-	0.67 (0.03)	212.44 (38.91)	0.95
Metribuzin 8 days	-	0.63 (0.03)	137.71 (23.07)	0.32
Halosulfuron 4 days	0.27 (0.12)	0.67 (0.02)	32.94 (17.71)	0.94
Halosulfuron 8 days	0.40 (0.04)	0.68 (0.01)	20.62 (6.04)	0.60
Flumioxazin 4 days	0.36 (0.07)	0.76 (0.04)	34.13 (13.93)	0.88
Flumioxazin 8 days	0.23 (0.06)	0.67 (0.05)	22.16 (7.93)	0.99

3.4. Relationship between Biomass and F_v/F_m

Figures 6 and 7 show the regression of potato fresh weight and potato F_v/F_m on days 4 and 8 after treatment. In mertriuzin:halosulfuron and metribuzin:flumioxazin mixtures, the regression slopes were positive in the first and second experiments. The regression slope of the two mixtures was almost similar in both experiments (Table 5).

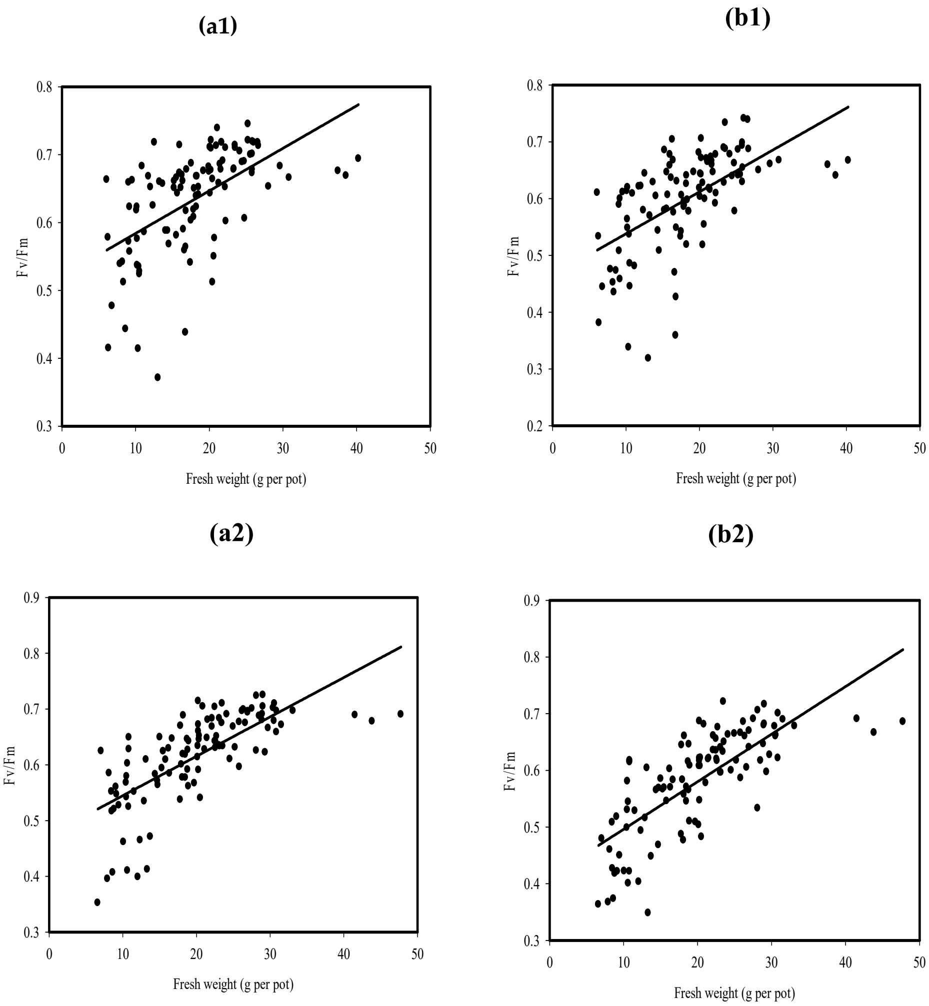

Figure 7. Linear regression of the maximum quantum photosystem II (F_v/F_m) on Fresh weight, 4 days (**a1**,**a2**) and 8 (**b1**,**b2**) days after treatment in potato and fresh weight 3 weeks after treatment for metribuzin:flumioxain in first (**a**) and second experiment (**b**). Each data point on the graph represents one replication of potato with indifferent mixture herbicide.

Table 5. Estimated linear regression parameters for F_v/Fm on biomass (Figures 6 and 7) metribuzin:halosulfuron and metribuzin: flumioxazin on days 4 and 8 after treatment for the two independent experiments. Standard errors in parentheses.

The First Experiment 4 Days after Treatment			The First Experiment 8 Days after Treatment	
Mixture Type	**Slope**	**Intercept**	**Slope**	**Intercept**
Metribuzin:halosulfuron	0.007 (0.0006)	0.52 (0.013)	0.007 (0.0008)	0.46 (0.016)
Metribuzin:flumioxazin	0.006 (0.0009)	0.52 (0.017)	0.007 (0.001)	0.46 (0.019)
The second experiment 4 days after treatment			The second experiment 8 days after treatment	
Mixture type	slope	intercept	slope	intercept
Metribuzin:halosulfuron	0.006 (0.0005)	0.50 (0.011)	0.007 (0.0008)	0.45 (0.017)
Metribuzin:flumioxazin	0.007 (0.0007)	0.47 (0.015)	0.008 (0.0007)	0.41 (0.015)

4. Discussion

Metribuzin, halosulfuron, and flumioxazin caused visible injury in potatoes three weeks after herbicides application. The order of herbicide causing injury of herbicides was ranked as flumioxazin > halosulfuron > metribuzin. Visible injury symptoms of metribuzin included chlorosis and yellow spotting of foliage. The results of our study are in line with the findings of [19]. According to the results of [19], vein discoloration or chlorosis was a symptom observed after metribuzin applications. Injury of potato was minimal for metribuzin. It should also be noted that less injury of potato might correlate with the potato tolerance. Metabolism of metribuzin causes potato tolerance [20]. Potato stunting, yellow spotting of foliage, and leaf margin necrosis resulted from halosulfuron. Reference [21] demonstrated post-application of halosulfuron resulted in 7 to 20% of stunting of potato. Injury of flumioxazin consisted of reddening and intensive necrosis of treated leaves and stunted growth of the potato. The potato injury was greatest for flumioxazin. The potential of flumioxazin injury has also been reported in different crops [22,23]. Our results are in line with [24]. According to the results of [24] flumioxazin caused greater phytotoxicity on potatoes, and the symptoms of flumioxazin were leaves and stem browning and necrosis. Necrosis of leaves and stunted growth of sugarcane were reported by [25]. Metribuzin:flumioxazin caused greater potato injury than metribuzin:halosulfuron. Stem and leaf browning and necrosis were observed in the metribuzin:flumioxazin mixture. The greater growth stunting resulted from metribuzin:flumioxazin. Injury symptoms of metribuzin:halosulfuron were yellow spotting of foliage and growth stunting of potato. There is no published research on metribuzin:halosulfuron and metribuzin:flumioxazin in potato injury.

Metribuzin, halosulfuron, and flumioxazin decreased weeds' biomass. Tables 1 and 2 show the dose-response curve parameters (d, ED_{50}) of the individual herbicides of metribuzin, halosulfuron, and flumioxazin on *C. album* and *A. retroflexus*. The order of performance was ranked as flumioxazin > halosulfuron > metribuzin in the two species (Tables 1 and 2). The present study also supports the previous finding. The post-emergence ED_{50} of metribuzin was 79 g ai·ha^{-1} on *C.album*, and 77 g ai·ha^{-1} on *A. retroflexus* in the potato field [4]. Several studies have reported good weed control with metribuzin, halosulfuron, and flumioxazin. Although research has demonstrated the excellent effect of metribuzin, halosulfuron, and flumioxazin singly on potato weeds, previous research has mainly focused on the analysis of the variance of herbicides on weeds. However, there are no halosulfuron and flumioxazin ED_{50} studies on potato weeds. Metribuzin post at 420 and 560 g ha^{-1} provided greater than 92% control of *C. album* and *A. retroflexus* [26]. Halosulfuron provides an additional herbicide option for the control of annual broadleaf weeds, including *C. album* and *A. retroflexus* [27]. Flumioxazin applied pre-emergence at 35 and 70 g ai·ha^{-1} provided excellent broadleaf weed control [8]. In previous research, flumioxazin at 35 to 105 g ha^{-1} controlled *C. album* and *A. retroflexus* [8].

Herbicides efficiency was affected by weed species. The *C. album* produced much more biomass (d) than *A. retroflexus* and was more tolerant to metribuzin, indicating its ineffectiveness at low doses. The different responses of the two species suggest that there are 66% polar components in the leaf surface of *C. album*, in comparison with 55% for *A. retroflexus* [28]. In addition, the hair covering on the abaxial side of the *C. album* leaves and crystalline structure of hair led to less herbicide retention and penetration into the tissue of the plant while the *A. retroflexus* leaf surface has smooth cuticular [29]. Consequently, higher amounts of herbicide are required to be absorbed, transferred, and reach the target in the photosynthetic system in *C. album*. The angle of the spray droplet was 76° on the *C. album* leaves in comparison with 54° on *A. retroflexus* [28]. Weak herbicide performance of *C. album* was due to the leaf's surface lower wettability. The reduction of herbicide retention and absorption into the plant tissue leads to lower wettability [30].

The responses of potato biomass to metribuzin, halosulfuron and flumioxazin doses were slightly different from those of *C. album* and *A. retroflexus.* The estimated ED_{50} was higher to *C. album* and *A. retroflexus*; but the ranking of the herbicides was the same, with flumioxazin and halosulfuron providing the highest level of efficiency on both weed species (Tables 1–3). The high performance of halosulfuron and flumioxazin could be correlated to the translocation of herbicides. Several researchers reported the high translocation of halosulfuron in other crops. The ^{14}C-halosulfuron translocation in treated leaves of corn (*Zea mays* L.) was 96% [31]. In contrast, the ^{14}C-metribuzin translocation was limited in wild oat (*Avena Sterilis* L.) [32].

Choice of appropriate rates and tank-mix partner(s) is critical for weed control. Numerous studies have demonstrated the mixture of herbicides has good potential for weed control. Our results showed that mixtures of metribuzin:halosulfuron and metribuzin:flumio xazin generally showed an antagonistic effect in *C. album*, *A. retroflexus* and potato (Figures 1–4). Generally, metribuzin:flumioxazin provided higher performance than metribuzin:halosulfu ron on both weeds. The highest efficiency of metribuzin:flumioxazin could be attributed to chemical properties of herbicides. High Log $_{\mathrm{Kow}}$ of flumioxazin (PubChem CID: 92425) led to an increase in lipophilic properties and thus increased penetration of herbicide to the cuticle of leaves in weeds and potato.

No references were found on joint acion of metribuzin:halosulfuron and metribuzin:flu mioxazin on weeds and potato. Previous studies with mixtures of halosulfuron and diverse groups of herbicides have revealed excellent control of *C. album* with pendimethalin applied at rates of 35:1080 g ai·ha^{-1} [33]. Flumioxazin applied alone as a preemergence resulted in poor annual grass control that was improved by tank mixtures [34]. Similarly, flumioxazin two-three-way combinations improved control of *C. album* and *A.retroflexus* [35]. The results of our study were not in line with the finding of [36]. According to the results, the photosystem II and different herbicides group mixtures followed synergistic effects by multiplicative survival model (MSM) on grass and broadleaf weeds.

Chlorophyll fluorescence measurement is a nondestructive, easy, and rapid assessment method for stress evaluation making it possible to assess plant response to herbicides in a short time [13]. F_v/F_m indicates the maximum quantum efficiency of photosystem II [37]. It is a stress indicator and describes the potential yield of a photochemical reaction. Our results provided ED_{50} values of F_v/F_m with the decreasing order of performance: metribuzin> flumioxazin> halosulfuron on 4 and 8 days after treatment (Table 4). The estimated ED_{50} values of F_v/F_m demonstrated was not significant difference flumioxazin and halosulfuron. The metribuzin:halosulfuron mixtures almost followed the Additive Dose model, whilst there was an antagonistic effect for the metribuzin:flumioxazin mixtures (Figures 4 and 5).

In Table 4 the upper limit is between 0.63 to 0.76 and thus close to the theoretical value of non-stressed plants. Our results were similar to previous studies e.g., [38], that reported the maximum quantum efficiency of photosystem II (PSII) as close to 0.8. A lower value indicates damaged PSII reaction centers, typically following the application of a PSII inhibiting herbicide. F_v/F_m is widely considered to be a sensitive indicator of plant photosynthetic performance, with healthy samples typically achieving a maximum F_v/F_m value of approx. 0.85 [39]. The results of our study were in line with the finding of [38] that reported the common mode of death for plants exposed to photosynthesis, ACCase, PDS, HPPD, and ESPS inhibiting herbicides is the generation of Reactive Oxygen Species (ROS).

5. Conclusions

Our present findings show that the ranking of ED50 for the three herbicides was metribuzin > halosulfuron > flumioxazin on *C. album*, *A. retroflexus* and potato biomass. The results demonstrated with increasing metribuzin, halosulfuron, and flumioxazin doses the F_v/F_m values decreased in potatoes. According to our results, both mixtures of metribuzin:halosulfuron and metribuzin:flumioxazin generally showed an antagonistic effect in both weeds and potatoes in the first and second experiments. The joint action of the metribuzin:flumioxazin acted as an antagonistic effect relative to the ADM reference model whilst the metribuzin:halosulfuron mixtures almost followed the Additive Dose model on F_v/F_m. The potato biomass was completely related to maximum quantum effi iency. Potato growers have few available herbicide options in Iran (i.e., metribuzin and paraquat). As halosulfuron provides various modes of action for weed control in potatoes, it could be useful in potato weeds due to its low use rate and high-performance comparison to metribuzin, as a recommended herbicide in Iran. Halosulfuron has less environmental risk. The studies of [40] the half-life of metribuzin in soil range from 75–120 days, while [41] indicates the half-lives of halosulfuron from 7–98 days. Our results show that F_v/F_m is a proper parameter for evaluating the effect of singly and mixture herbicides shortly after application. The measurement of chlorophyll *a* fluorescence parameters, such as F_v/F_m, three weeks before biomass measurement, can save time and awareness of the physiological stresses caused by herbicides.

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