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Effect of drought stress on herbicide performance and photosynthetic activity of *Avena sterilis* subsp. *ludoviciana* (winter wild oat) and *Hordeum spontaneum* (wild barley)

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Abstract

The severity of drought is increasing owing to global climate change. Knowledge about the influence of drought on weeds and herbicide performance is necessary for optimising herbicide applications. Therefore, dose-response trials were conducted to investigate the influence of drought stress on the efficacy of four herbicides. Drought-stressed and non-stressed *Hordeum spontaneum* seedlings were exposed to nine doses of sulfosulfuron and sulfosulfuron + metsulfuron-methyl. Clodinafop-propargyl and mesosulfuron-methyl + iodosulfuron-methyl sodium were applied to drought-stressed and non-stressed seedlings of *Avena sterilis* subsp. *ludoviciana*. Leaf greenness index (SPAD), total chlorophyll content, stomatal conductance and photosynthesis rate of both species were measured. The efficacy of herbicides on *H. spontaneum* was not influenced by drought stress. In contrast, the performance of herbicides on *A. sterilis* subsp. *ludoviciana* was reduced under drought stress, where the ED_{50} increased by 1.5-fold. The low doses (<2.2 g a.i. ha^{-1}) of mesosulfuron-methyl + iodosulfuron-methyl-sodium caused hormesis on *A. sterilis* subsp. *ludoviciana*. Total chlorophyll content and SPAD index of both species increased due to drought stress. The stomatal conductance and photosynthesis rate of *A. sterilis* subsp. *ludoviciana* decreased under drought stress, while *H. spontaneum* was not influenced. The lack of detrimental effect of drought stress on sulfosulfuron and sulfosulfuron + metsulfuron-methyl performance might be attributed to similar stomatal conductance and photosynthesis rate of the drought-stressed and non-stressed *H. spontaneum*. The performance reduction in herbicides on drought-stressed *A. sterilis* subsp. *ludoviciana* might be owing to the reduction in stomatal conductance and photosynthesis rate. Using adjuvants, diverse formulations and early application of herbicides were suggested for future research to hamper the negative effect of drought on herbicides.

KEYWORDS

Chlorophyll content, global climate change, herbicide efficacy, photosynthesis rate, stomatal conductance, water deficit stress

1 | INTRODUCTION

Caused by natural forces (solar activity, volcanic eruptions, etc.) and human activities (deforestation, fossil fuels burning, etc.), global climate change is one of the most important environmental challenges the world faces today. Global climate changes most often relate to a rise in concentration of atmospheric greenhouse gases, especially CO_2 that may lead to high global temperature and drought (Jugulam et al., 2018). As a side effect of climate change, drought stress is not a new environmental problem, particularly in the world's semiarid and arid zones. Unfortunately, the severity, duration and extent of drought have been projected to be increased by several studies in several parts of the globe owing to global change of climate (Dewes et al., 2017). Detrimental influences of drought conditions on the productivity of crops have been confirmed previously. For example, morphophysiological influences of drought on crops were reviewed by Fahad et al. (2017). Drought stress can also affect weed management (Varanasi et al., 2016). Generally, it is accepted that under drought conditions, the weed-crop competition for water increases in favour of some weeds leading to a high level of weed control (Patterson, 1995).

The efficacy of herbicides is influenced by many environmental factors, for example drought stress (Varanasi et al., 2016). These factors modify physicochemical properties of herbicides and plant characteristics (e.g. anatomy, morphology and physiology), leading to change in herbicides' performance due to change in absorption, translocation and sequestration of herbicides in plants. Effect of these factors on the performance of herbicides might be pronounced due to global climate change. Therefore, knowledge about the influence of global climate change items such as drought stress on weeds and herbicide performance is necessary for optimising herbicide applications and effective weed management (Varanasi et al., 2016). Efficacy of herbicides on weeds affected by drought stress may result in poor herbicide performance and weed control, high production costs and probably more environmental pollution (Oyarzabal, 1991). Previous studies reported the reduced efficacy of some herbicides, such as fenoxaprop, under drought-stress conditions (Xie et al., 1993). The neutral effect of drought stress on some herbicides such as imazamethabenz has also been reported (Xie et al., 1996). Accordingly, the detrimental effect of drought stress cannot be generalised to all herbicides' efficacy on all weeds. Effects of environmental factors that is soil moisture, relative humidity, light and temperature on performance of herbicides may differ among herbicides having different and/or similar sites of action (Varanasi et al., 2016). It means it is required to evaluate the performance of herbicides on drought-stressed weed species. Moreover, when a reduction in herbicide performance occurred, it is highly needed to measure the extent of decrease and fix the herbicide rates needed to obtain an appropriate weed control.

Drought conditions also can influence many plant physiological traits, especially photosynthesis capacity. Knowledge about biological, ecological and physiological aspects of weeds grown under different environmental situations, especially drought-stress conditions, may help to develop appropriate weed management strategies and optimise herbicide application. Stomatal conductance, photosynthesis rate and leaf chlorophyll content of plants are affected by drought-stress conditions (Cornic, 2000; Flexas et al., 2002; Schütz and Fangmeier, 2001). Drought stress is more problematic in semiarid to very arid regions globally, including The Middle East and Southwest Asia, and future drought might worsen in the mentioned areas (Barlow et al., 2016). According to our knowledge, drought stress is a very serious problem in agricultural production of the mentioned areas, especially in dryland farms.

Hordeum spontaneum [C. Koch] Thell (wild barley) is one of the most troublesome grasses, widely distributed, and economically important weeds in the winter fields of Western Asia. It can cause up to 38% yield loss in winter wheat (*Triticum aestivum* L.) when grown at a density of 80 plants/m^2 (Hamidi, 2006). It is a semi-native to Southwest Asia, and it stems from the eastern parts of Mediterranean seacoasts to the semi-deserts in Afghanistan (Jakob et al., 2014). *Avena sterilis* subsp. *ludoviciana* (Durieu.) Gillet & Magne (winter wild oat) is also one of the utmost persistent and economically prominent weeds in agroecosystems of many countries worldwide, such as Australia, India, Iran and the USA. For example, it was estimated that winter wheat grain yield could be reduced between 17% and 62% by *A. sterilis* subsp. *ludoviciana* (Balyan et al., 1991). A range of herbicides, including sulfosulfuron, sulfosulfuron + metsulfuron-methyl, benzoylprop-ethyl, mesosulfuron + iodosulfuron and clodinafop-propargyl are used to control these two grass-weed species in cereal fields (Zand et al., 2017). Little knowledge is available about the mentioned herbicides' performance on these weeds grown under drought-stress situations.

The present research objectives were to evaluate the effect of drought stress, as one of the negative effects of global climate change: (a) on the performance of the commonly used herbicides, including sulfosulfuron sulfosulfuron + metsulfuron-methyl, mesosulfuron-methyl + iodosulfuron-methyl sodium and clodinafop-propargyl for controlling *H. spontaneum* and *A. sterilis* subsp. *ludoviciana*, and (b) on physiological traits of the mentioned weeds in the greenhouse conditions.

2 | MATERIALS AND METHODS

2.1 | Plant material and growing seeds

In July 2017, seeds of *H. spontaneum* and *A. sterilis* subsp. *ludoviciana* were harvested within the experimental field of Tabiat Modares University, Iran (35°44′ 25.941″N, 51°9′ 56.747″E, 1,265 metre above sea level). The seeds of both grass species were kept at room temperature until the initiation on 19 November 2017 and 12 January 2018. A dormancy breaking cold treatment was carried out to promote the germination of seeds, allowing uniform seedlings in the whole plant dose-response assays. In this aim, the seeds of both weed species were placed in glass Petri-dishes (9 cm diameter) comprising one filter paper (Whatman No. 1) and were soaked with distilled water, 10 ml per Petri-dish. The seeds of *A. sterilis* subsp. *ludoviciana* were dehulled before the seed dormancy releasing process. Then, three water irrigated Petri-dishes were placed in a closed polythene bag to avoid loss of water by evaporation. Then, the bags were placed in a dark refrigerator at 4°C for 7 days. The germinated seeds (i.e. having a visible radicle) were used in greenhouse experiments immediately.

2.2 | Dose-response experiments

Ten similar germinated seeds of each weed species were chosen and sown in dark plastic bags (10-cm diameter with a volume of 2 L). A mixture of field soil (sand 58%, clay 22% and silt 20%, pH 7.39, bulk density (P_b) 1.43 g/cm^3, specific density (P_s) 2.6 g/m^3, field capacity (FC) 24% by volume, permanent wilting point (PWP) 14.3% by vol.) and peat (1:1 v/v) was used to fill the bags. After seedling emergence, five similar seedlings were kept per bag to provide a similar experimental condition throughout the experiments.

The plants were grown under two irrigation (watering) regimes. Half of the plants were exposed to an irrigation regime to maintain 90% field capacity (FC, considered as non-drought-stressed). In comparison, the rest of the plants were irrigated to only reach 60% FC (considered as drought stressed). The non-drought-stressed weeds were irrigated to keep the soil's water content to 90% of FC during the period of experiments, while the drought-stressed weeds were irrigated similar to the non-drought-stressed weeds. Still, for two weeks, the water soil content was retained to be FC 60% from one week before until one week after applying herbicides. Fourteen days of drought-stress treatment was chosen because it was stated that environmental factors one to two weeks before and after using herbicides can affect absorption of foliar-applied herbicides (Monaco et al., 2002). The irrigation regimes were established on maximum allowable depletion (MAD) (see equation 1). The volumetric content of soil water was measured using a TRIME-FM TDR (IMKO Micromodultechnik, Ettlingen, Germany) as described by Mokhtassi-Bidgoli et al. (2013). The TDR was calibrated in a preliminary experiment where the values provided by the TDR device were read and then transformed to volumetric content of soil water. The proportion of MAD of ASW in the bags was assessed with the Equation 1:

$$\mathrm{MAD} = \mathrm{FC} - \theta / \mathrm{FC\text{ - }PWP} \tag{1}$$

where FC is field capacity, θ is the volumetric soil water content in plastic bag container and PWP represents the volumetric soil water content at permanent wilting point. The amount of needed water was calculated according to the previously defined MAD (Equation 1) and equations 2 and 3:

$$\mathrm{ASW} = \mathrm{FC\text{ - }PWP} \tag{2}$$

$$V_\mathrm{d} = \mathrm{MAD} \times \mathrm{ASW} \times R_z / 100 \tag{3}$$

where ASW is 9.7 cm/m soil depth, V_d is the amount water (mm) for irrigation, R_z is the length of the plastic bag container filled by soil (200 mm). The required volume of water was measured and added to the plastic bags with a laboratory volumetric beaker container.

At the two- to the three-leaf growth stage (BBCH 12-13), the seedlings of *H. spontaneum* were sprayed with sulfosulfuron and sulfosulfuron + metsulfuron-methyl, while *A. sterilis* subsp. *ludoviciana* seedlings were exposed to mesosulfuron-methyl + iodosulfuron-methyl sodium and clodinafop-propargyl (Table 1). Nine doses of the herbicides were applied, where untreated plant (i.e. zero dose of herbicide) was the control treatment. The herbicide solutions were applied using a knapsack sprayer carrying an even nozzle (8001E), providing 283.4 L/ha (250 kPa pressure, 5 km/hr speed). The nozzle was located 50 cm on top of the weed canopy. The aboveground parts of plants were harvested four weeks after herbicide treatment to measure dry weight. The harvested plants were dried at 80°C for 72 hr to measure the dry weight.

The plastic bags were arranged based on a completely randomised design (CRD). There were four bags (replications) per treatment. All the experiments were carried out twice in a greenhouse with a 25/17 ± 3°C and 16/8-hr day/night cycle with a supplemental light (457 µmol m^{-2} s^{-1}).

2.3 | Plant physiological experiments

To evaluate physiological responses (i.e. leaf greenness, total chlorophyll content, stomatal conductance and photosynthesis rate) of *H. spontaneum* and *A. sterilis* subsp. *ludoviciana* under the above-mentioned drought conditions (FC 60% and FC 90%), two separate experiments were carried out for each weed species. Notice, plants

TABLE 1 Herbicides names, recommended field doses and nine doses applied in two successive experiments

Active ingredient	Formulation	Doses (g a.i. ha^{-1})[a]	Trade name	Company name
Sulfosulfuron	WG 75%	0, 1.9, 3.4, 6.2, 11.1, **20**, 35.9, 64.6, 116.3	Sulfosulfuron Golsam®	Golsam Gorgan Chemicals CO.
Sulfosulfuron + metsulfuron-methyl	WG 75% + 5%	0, 3.4, 6.2, 11.1, 20, **36**, 64.8, 116.6, 210	UPL Total®	UPL Limited CO.
Mesosulfuron-methyl + iodosulfuron-methyl sodium	OD 1% + 0.2%	0, 1.7, 3.1, 5.5, 10, **18**, 32.4, 58.3, 105	Atlantis®	Bayer Parsian AG CO.
Clodinafop-propargyl	EC 8%	0, 8, 13.6, 24.8, 46.4, **80**, 144, 259.2, 466.4	Topik®	Meli Agrochemical CO.

[a] Recommended field dose (g a.i. ha^{-1}) of each herbicide was written in bold.

were not treated with herbicides. The experiments were conducted based on a CRD, and there were four replications per bag (treatment). Similar to the previous experiment, the weeds were exposed to either the drought-stress (FC 60%) or the non-drought-stress conditions (FC 90%) seven days before the two- to the three-leaf growth stage (BBCH 12–13). The above-mentioned physiological traits of weeds were quantified seven days after the outset of the irrigation treatments. It is worth noting that applying herbicides and measuring the physiological properties were carried out at the same plant growth stage as in the dose-response experiments.

The total chlorophyll content of both weed species was quantified based on a method explained by Hiscox and Israelstam (1979). Ten mg fresh leaf material of each bag (treatment) was harvested at BBCH 12-13 and were stored at −80°C. We cut each leaf sample into smaller pieces and kept in tubes comprising 3 ml dimethyl sulfoxide (DMSO). The samples were incubated in a water bath for 65 for 45 min. Immediately after the temperature reduction in samples, the absorbance was measured at 648 and 664 nm wavelength through a spectrophotometer (Cary 100, Varian, Australia). The plant leaf greenness was also recorded by a Chlorophyll Meter (SPAD-502 plus, Osaka, Japan). Stomatal conductance and photosynthetic rate of the weeds were evaluated concurrently by a portable photosynthesis systems (LI-COR 6400, Lincoln, USA). The conditions of the measuring chamber were adjusted as follows: reference CO_2 350 µmol/mol, temperature of leaf 30°C and PAR 1,400 $\mu\text{mol m}^{-2}\text{ s}^{-1}$. The infrared gas analyser (IRGA) was matched by hand, and concentrations of reference H_2O and CO_2 were constant before reading the data. The newest completely expanded leaf of weed was selected to measure photosynthetic rate, stomatal conductance and leaf greenness index.

2.4 | Statistical analysis

2.4.1 | Dose-response experiments

A two-step analytical method was applied to analyse the dose-response data, as mentioned elsewhere (Jiang and Kopp-Schneider, 2015). At first, the dry weight (Y) was regressed against increasing dose (x) of herbicides (g a.i. ha^{-1}) using one of the following equations (equations 4 and 5) to estimate parameters.

$$Y = c + \frac{d - c}{1 + \exp\left[b\left(\log(x) - \log\left(ED_{50}\right)\right)\right]} \quad (4)$$

$$Y = c + \frac{d - c + fx}{1 + \exp\left[b\left(\log(x) - \log(e)\right)\right]} \quad (5)$$

The equation 4, a four-parameter log-logistic model described by Streibig et al. (1993), was used for the majority of the dose-response data. In the equation 4, c value represents the lower value of the curve at the highest dose of herbicide, and d is the upper value of response in the absence of herbicide, that is zero dose of herbicide or the control treatment. ED_{50} is the effective rate of herbicide (g a.i. ha^{-1}) reducing the dry weight of weed by 50% (mid-point between the d and c values), and b value is the curve's steepness around the ED_{50}. Whenever a hormesis effect (i.e. low doses increasing Y) was observed, the Brain-Cousens model, a five-parameter (equation 5), was fitted to the data (Brain and Cousens, 1989) . The f parameter shows the rate of increase (the degree of hormesis effect) at small doses, and e parameter represents the curve's inflection point. In the equation 5, the c, d and b parameters are similar to equation 4. As the parameter e is not a biologically meaningful parameter, that is not equal to the ED_{50}, the ED_{50} was estimated after fitting equation 5. The equation 5 was only fitted to data of mesosulfuron-methyl + iodosulfuron-methyl sodium. The goodness of fit for the used models was tested using *F*-test.

At step two, a linear mixed model was applied to analyse the estimated parameters, that is b, c, d and ED_{50}, obtained from the equations 1 and 2 at the first step, where the experiments and irrigation treatments were included in the model as random and fixed effect respectively. Then, the estimated parameters of non-drought-stressed and drought-stressed weeds were compared using pairwise comparisons. The add-on packages *drc* (Ritz et al., 2015a), *lme4* (Bates et al., 2015) and *multcomp* (Hothorn et al., 2008) in the R statistical software were used for statistical analysis and making plots (R Core Team, 2013).

2.4.2 | Plant physiological experiments

A *t*-test (at the 0.05 probability level) was used to compare the influences of irrigation treatments on each weed species' physiological traits. Equality of variances and normality of residuals were tested, and it was shown that data met the assumptions for *t*-test. The base package *stats* and the add-on package *agricolae* (Mendiburu, 2014) were used for statistical analysis and making plots, respectively, in the R statistical software (R Core Team, 2013).

3 | RESULTS

3.1 | Dose-response experiments

3.1.1 | Sulfosulfuron

The equation 4 was fitted to the data of both experiments (i.e. *p*-value of the lack-of-fit test was between 0.48 and 0.87). It meant that the regression model reasonably described the dry weights of *H. spontaneum* with increasing sulfosulfuron doses. However, a significant difference was observed between the experiments leading to fit the model separately. After extracting the parameters, the linear mixed model averaged the parameters. The averaged parameters (averaged over the two experiments) of non-drought-stressed and drought-stressed weeds are presented in Table 2. In the absence of herbicide (i.e. *d* parameter or the upper limit of the

TABLE 2 The averaged parameters obtained by equation 4 or equation 5 over the experiments using a linear mixed model analysis method. The standard errors are presented in parentheses

Species	Herbicide	Drought treatment (FC %)[a]	Slope (*b*)	Lower limit (*c*)	Upper limit (*d*)	Hormosis (*f*)	*ED*$_{50}$ (g a.i. ha^{-1})
Hordeum spontaneum	Sulfosulfuron	60	1.1 (0.82)ns	1.20 (0.38)ns	5.5 (0.49)**	–	24.4 (11.04)ns
		90	1.2 (0.82)	3.0 (0.59)	7.7 (0.64)	–	12.2 (10.52)
	Sulfosulfuron + metsulfuron-methyl	60	2.0 (0.26)ns	2.1 (1.12)ns	5.8 (0.29)**	–	10.7 (7.69)ns
		90	1.9 (0.40)	2.7 (1.12)	7.0 (0.28)	–	9.6 (7.69)
Avena sterilis subsp. *ludoviciana*	Clodinafop-propargyl	60	7.2 (5.53)ns	0.9 (0.04)ns	3.6 (0.29)**	–	13.4 (0.80)**
		90	2.7 (0.52)	0.8 (0.04)	5.2 (0.53)	–	8.9 (1.08)
	Mesosulfuron-methyl + iodosulfuron-methyl sodium	60	2.8 (0.27)***	1.0 (0.34)***	3.5 (0.32)**	1.2 (0.38)ns	8.1 (0.71)*
		90	2.0 (0.26)	0.75 (0.34)	4.6 (0.32)	1.1 (0.39)	5.8 (0.66)

Abbreviation: ns, Non-significant.

[a] FC, field capacity; FC 60%, drought-stress condition, FC 90% non-drought-stress (normal) conditions.

***, **, and * represent significant at 0.001, 0.01 and 0.05 level respectively.

curve), dry weight of the drought-stressed (5.5 ± 0.49) plants was significantly lower than that of the non-drought-stressed plants (7.7 ± 0.64). In contrast to the *d* parameter, no significant difference was observed between the non-drought-stressed and the drought-stressed plants considering the other parameters, including *b*, *c* and *ED*$_{50}$ (Table 2).

3.1.2 | Sulfosulfuron + metsulfuron-methyl

The *ED*$_{50}$ of sulfosulfuron + metsulfuron-methyl on *H. spontaneum* was similar for the non-drought-stressed and the drought-stressed weeds (Table 2). The *ED*$_{50}$ did not differ from 1.00, suggesting that sulfosulfuron + metsulfuron-methyl is not an effective herbicide to control *H. spontaneum*. The dry weight of non-drought-stressed weeds (7.0 ± 0.28) was significantly higher than that of the drought-stressed weeds (5.7 ± 0.29) when sulfosulfuron + metsulfuron-methyl was not used, that is the *d* parameter (Table 2). In the presence of infinitely large doses of sulfosulfuron + metsulfuron-methyl (i.e. *c* parameter), both drought-stressed and non-drought-stressed plants produced similar dry weight.

3.1.3 | Clodinafop-propargyl

The equation 4 was fitted to the first experiment's data, while it was impossible to fit this equation for the second experiment due to model convergence failure. Accordingly, the estimated parameters from experiment one are only presented here (Table 2). As it was expected and similar to the previous herbicides, the *d* parameter of the drought-stressed weeds (3.6 ± 0.29) was significantly lower than that of the non-drought-stressed weeds (5.2 ± 0.53) in the absence of clodinafop-propargyl (Table 2). However, no significant difference was found in the lower limit *c* parameter between the non-drought-stressed and the drought-stressed *A. sterilis* subsp. *ludoviciana*. A significant difference was detected in the *ED*$_{50}$ between the irrigation regimes, where the *ED*$_{50}$ was 13.4 ± 0.80 and 8.9 ± 1.08 for the non-drought-stressed and the drought-stressed weeds respectively.

3.1.4 | Mesosulfuron-methyl + iodosulfuron-methyl sodium

The low doses (<2.2 g a.i. ha^{-1}) of mesosulfuron-methyl + iodosulfuron-methyl sodium stimulated the growth of the non-drought-stressed and the drought-stressed plants, meaning that a hormesis effect (i.e. *f* parameter) was observed in *A. sterilis* subsp. *Ludoviciana*. To make it illustrative, the relative dry weight of both non-drought-stressed and drought-stressed weeds to mesosulfuron-methyl + iodosulfuron-methyl was plotted over two experiments (Figure 1). It is worth noting that the observed hormesis effect was statistically similar for both non-drought-stressed and drought-stressed plants (Table 2 and Figure 1).

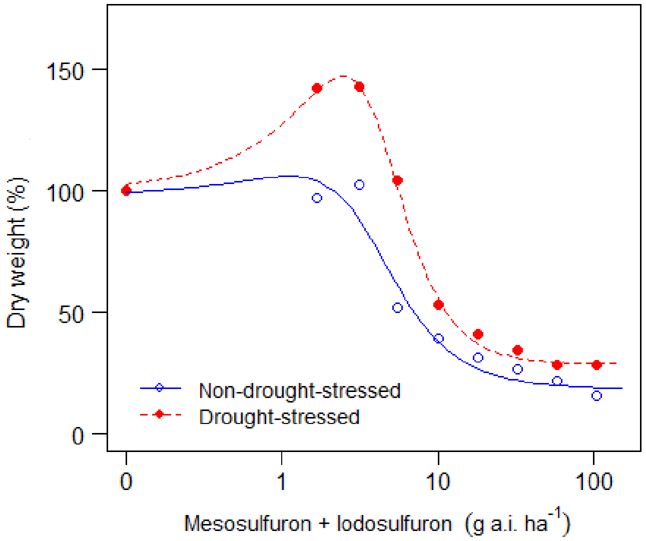

FIGURE 1 Response of non-drought-stressed (FC 90%) and drought-stressed (FC 60%) *A. sterilis* subsp. *ludoviciana* to mesosulfuron-methyl + iodosulfuron-methyl sodium. The parameters estimated by the five-parameter Brain-Cousens model (equation 5) are presented in Table 2. Notice, the low doses of mesosulfuron-methyl + iodosulfuron-methyl sodium caused a hormesis effect, that is an increase in the responses of plants

As with the other experiments, the *d* parameter of the drought-stressed plants (3.5 ± 0.32) was reduced significantly compared with the non-drought-stressed plants (4.6 ± 0.32). A significant difference was also observed for the *c* parameter, meaning that at the highest concentration of mesosulfuron-methyl + iodosulfuron-methyl sodium, the dry weight of the non-drought-stressed was lower than that of the drought-stressed weeds. Also, the ED_{50} of mesosulfuron-methyl + iodosulfuron-methyl sodium on *A. sterilis* subsp. *ludoviciana* increased significantly ($p < 0.05$) due to drought-stress conditions, as observed for clodinafop-propargyl.

3.2 | Plant physiological experiments

Leaf greenness index and total chlorophyll content of both weed species increased significantly under the drought-stress conditions (Figure 2). The leaf chlorophyll content of the non-drought-stressed *H. spontaneum* and *A. sterilis* subsp. *ludoviciana* was 1.9- and 1.5-fold lower than that of the corresponding drought-stressed weeds respectively (Figure 2A and B). Similarly, the leaf greenness of the non-drought-stressed *H. spontaneum* and *A. sterilis* subsp. *ludoviciana* was 1.2- and 1.7-fold lower than that of the corresponding drought-stressed weeds respectively (Figure 2c and d).

The stomatal conductance and photosynthesis rate of *A. sterilis* subsp. *ludoviciana* were decreased significantly under drought conditions (Figure 3a and c). At the same time, no significant difference was detected between the non-drought-stressed and drought-stressed *H. spontaneum* plants (Figure 3b and d).

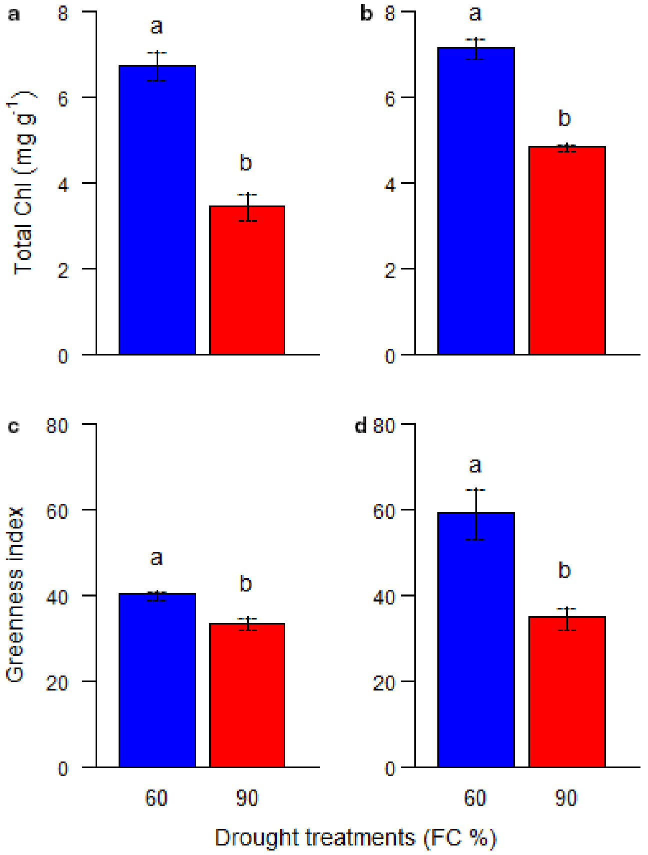

FIGURE 2 Effect of non-drought-stress (FC 90%) and drought-stress (FC 60%) treatments on the total chlorophyll content (a and b) and the leaf greenness index (c and d) of *Avena sterilis* subsp. *ludoviciana* (a and c) and *Hordeum spontaneum* (b and d). Differences between treatments (significant at $p < 0.05$ level) are shown by dissimilar letters on columns

4 | DISCUSSION

4.1 | Dose-response experiments

The results concluded that drought-stress conditions did not influence the performance of sulfosulfuron for controlling *H. spontaneum*. The neutral influence of drought on the efficacy of some herbicides has been reported. For example, it has been stated that the efficacy of imazamethabenz on *Avena fatua* L. (wild oat) was not influenced by drought (Xie et al., 1996). It might be worth noting that similar to Asadi-Sabzi et al. (2020), we also found that sulfosulfuron herbicide cannot be used as a suitable chemical option for controlling *H. spontaneum* as the ED_{50} was similar to the recommended field dose (20 g a.i. ha^{-1}).

As with the results for sulfosulfuron, the efficacy of sulfosulfuron + metsulfuron-methyl on *H. spontaneum* was not influenced by drought-stress treatment. In accordance with our results, it was shown that drought stress had limited or no detrimental effect on the efficacy of imazamethabenz to control several *A. fatua* L. populations (Xie et al., 1993, 1997). Previous studies have reported that the influence of drought on the efficacy of herbicides differed among

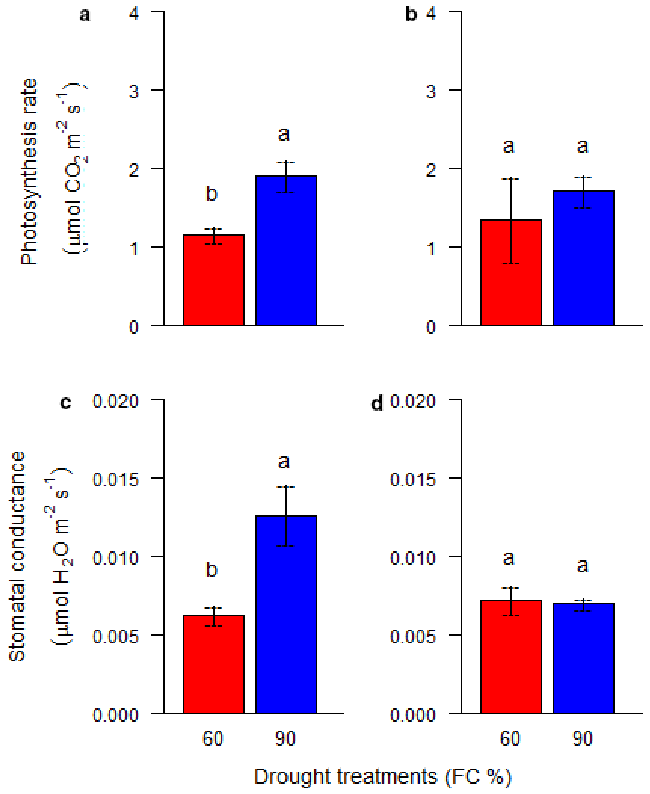

FIGURE 3 Effect of non-drought-stress (FC 90%) and drought-stress (FC 60%) treatments on the photosynthesis rate (a and b) and the stomatal conductance (c and d) of *Avena sterilis* subsp. *ludoviciana* (a and c) and *Hordeum spontaneum* (b and d). Differences between treatments (significant at $p < 0.05$ level) are shown by dissimilar letters on columns

herbicides, as the performance of fenoxaprop-ethyl was decreased by water deficit but not that of imazamethabenz-methyl (Xie et al., 1996). Accordingly, it might be needed to determine the negative effects of drought stress on the performance of herbicides. It is worth noting that we have tested a series of herbicide doses, while Xie et al. (1993, 1996) tested only one or two doses of the herbicides, suggesting that the present results were robust.

As was mentioned in the results section, due to model convergence failure in the second experiment of clodinafop-propargyl, the results from the first experiment are only presented in Table 2. The difference between the two experiments is not uncommon, as it was attributed to various responses in biological experiments (Ritz et al., 2015b). So, dose-response curves are rarely similar (Ritz et al., 2006). As the ED_{50} of drought-stressed plants was greater than the non-drought-stressed plants, it can be concluded that the efficacy of clodinafop-propargyl on *A. sterilis* subsp. *ludoviciana* was reduced by drought. Xie et al. (1993) showed that the response of five *A. fatua* populations to fenoxaprop was significantly different under drought-stress conditions. The efficacy of fenoxaprop on two out of the five studied *A. fatua* populations was reduced under short-term drought conditions, that is 12 days (Xie et al., 1993). Based on the differences in ED_{50} between the two irrigation regimes, it might be expected that herbicide performance would be decreased whenever clodinafop-propargyl is applied under the drought-stress conditions.

The hormesis effect of mesosulfuron-methyl + iodosulfuron-methyl sodium on *A. sterilis* subsp. *Ludoviciana* was a contrast result to the results of the other herbicides used in this study. The hormesis effect of mesosulfuron-methyl + iodosulfuron-methyl sodium on *Tripleurospermum perforatum* (Mérat) M. Lainz (scentless false mayweed), has also been reported (Belz and Sinkkonen, 2016). As it was reviewed by Velini et al. (2017), herbicides causing a hormesis effect can be used as a beneficial molecule such as plant growth regulator. However, it is worth noting that the hormesis effect of mesosulfuron-methyl + iodosulfuron-methyl sodium might increase the evolution of weed resistance, especially under the drought condition, due to growth stimulation resulting in a higher fitness of plants. Recently, Belz (2018) demonstrated that the hormetic effects of metamitron, a PSII inhibitor herbicide, enhanced reproductive fitness of a PSII-TSR *Chenopodium album* L. (common lambsquarters.) population, meaning that intensify herbicide resistance evolution. Accordingly, the observed secondary effect of mesosulfuron-methyl + iodosulfuron-methyl sodium on *A. sterilis* subsp. *ludoviciana* is remained to be tested in detail in the future studies.

Importantly, drought-stress conditions had a significant negative effect on the performance of mesosulfuron-methyl + iodosulfuron-methyl sodium at the ED_{50}. Similar to the present results for mesosulfuron-methyl + iodosulfuron-methyl sodium, reduced performance of some herbicides such as fenoxaprop, diclofop, clodinafop-propargyl, flamprop and mesosulfuron-methyl on *A. fatua* and *A. sterilis* subsp. *ludoviciana* was reported previously, where the inadequate number of doses for the studied herbicides did not allow to calculate the ED_{50} (Aghabeigi and Khodadadi, 2017; Xie et al., 1993, 1997). Based on the differences in the ED_{50} between the two irrigation regimes, as it was suggested for clodinafop-propargyl, care must be taken whenever mesosulfuron-methyl + iodosulfuron-methyl sodium are applied under the drought-stressed field conditions. Otherwise, the performance of mesosulfuron-methyl + iodosulfuron-methyl sodium might be reduced, but the hormesis effect may favour the growth of *A. sterilis* subsp. *ludoviciana* leading to intensifying crop damage and herbicide resistance evolution. However, further researches in field conditions should consider this issue to be sure about this conclusion.

Our results indicated that the influence of drought on the efficacy of herbicides varied with type of herbicide (sulfosulfuron, sulfosulfuron + metsulfuron-methyl, mesosulfuron-methyl + iodosulfuron-methyl sodium and clodinafop-propargyl) and weed species (*H. spontaneum* and *A. sterilis* subsp. *ludoviciana*). According to our knowledge, previous experiments have also shown that the performance reduction caused by drought conditions differed based on herbicide formulation (Xie et al., 1994), type of herbicide (Xie et al., 1996), weed population and level of drought (Aghabeigi and Khodadadi, 2017; Xie et al., 1993). Therefore, it might be necessary to evaluate the effects (i.e. negative, neutral or even positive effects) of drought stress on the efficacy of herbicide individually for controlling different weed species independently, owing to the variation of weed species, differences in modes of action, chemical structure, physicochemical properties and formulation of herbicides.

4.2 | Plant physiological experiments

Different results have been reported considering the effect of drought stress on greenness index and total chlorophyll content of plants grown in different environments. For instance, it has been reported that chlorophyll content decreases under drought conditions (Schütz and Fangmeier, 2001). However, in agreement with our results, the chlorophyll content of leaves and leaf greenness index has been reported to be increased (Rahbarian et al., 2011). The increase in chlorophyll content was ascribed to a reduction in leaf area index (Rahbarian et al., 2011). Moreover, increased pigment levels might be associated with limited growth and cell division of drought-stressed plants. A reverse relation between leaf area and leaf greenness index and leaf water content has also been detected (Marenco et al., 2009). It was stated that due to a tendency of leaf area reduction in drought-stressed plants, the performance of foliar-applied herbicides is reduced (Ziska and Dukes, 2011).

In contrast to the leaf greenness index results and total chlorophyll content, consistent results were not found for the stomatal conductance and photosynthesis rate across the weed species. Almost all plant physiology traits, especially photosynthesis capacity, are adversely affected by drought stress, depending on the deficiency and plant species or crop varieties. For example, a dramatic decrease in photosynthesis rate and stomatal conductance of pigweed (*Amaranthus retroflexus*) has been reported due to water deficit (Lovelli et al., 2010). The results of *H. spontaneum*, photosynthesis rate and stomatal conductance of prickly lettuce (*Lactuca serriola*) did not reduce under drought-stress conditions (water holding capacity (WHC) 75%) in comparison with well-watered conditions (WHC 100%) (Chadha et al., 2019). The lack of detrimental effect of the drought conditions on stomatal conductance and photosynthesis rate of *H. spontaneum* might be due to its ability to tolerate different abiotic stresses because *H. spontaneum* is an abiotic-stresses tolerant weed species (Wang et al., 2018). Nevertheless, it is expected that under higher drought-stress levels, that is <FC 60% *H. spontaneum* physiological traits might be affected negatively.

The results showed that chemical control of *H. spontaneum* using sulfosulfuron and sulfosulfuron + metsulfuron-methyl was not influenced by a drought-stress condition (60% of FC). The efficacy of both mesosulfuron-methyl + iodosulfuron-methyl sodium and clodinafop-propargyl on *A. sterilis* subsp. *ludoviciana* was reduced under the drought conditions. So that, the average ED_{50} for mesosulfuron-methyl + iodosulfuron-methyl sodium and clodinafop-propargyl on the non-drought-stressed *A. sterilis* subsp. *ludoviciana* was *c.* 1.5-fold lower than that for the drought-stressed weeds. Actually, it might be expected that the negative effect of drought condition on the performance of mesosulfuron-methyl + iodosulfuron-methyl sodium and clodinafop-propargyl may be pronounced under field conditions, as plants growing in greenhouse are usually more sensitive to herbicides than those in field conditions. Accordingly, care must be taken whenever these two herbicides are applied under drought-stress field conditions.

Generally, it is agreed that weeds can be killed by herbicides when they grow actively because all physiological processes, including photosynthesis, are taken place at full speed. Environmental parameter reducing photosynthetic activity modify herbicide performance because other plant metabolic pathways (e.g. amino acid synthesis), absorption and translocation of herbicides could also decelerate or cease, due to photosynthesis reduction (Varanasi et al., 2016; Ziska and Dukes, 2011). Accordingly, it is stated that decreased stomatal conductance and photosynthesis can alter the efficacy of herbicides (Varanasi et al., 2016). Hence, the diverse responses of the studied weeds to herbicides might be due to the variation of the physiological properties. The reduced performance of mesosulfuron-methyl + iodosulfuron-methyl sodium and clodinafop-propargyl, at the ED_{50} level, could have been attributed to the reduced stomatal conductance and photosynthesis reduction in the drought-stressed *A. sterilis* subsp. *ludoviciana*.

The lack of negative effect of drought-stress conditions on sulfosulfuron and sulfosulfuron + metsulfuron-methyl performance might be due to the physiological traits of *H. spontaneum*, as the non-drought-stressed and the drought-stressed weeds showed similar photosynthesis activity and stomatal conductance. Under drought conditions photosynthesis rate of *H. spontaneum* did not reduce dramatically, so it might allow other plant metabolic pathways such as amino acid synthesis to normally occur in drought-stressed cells, and consequently, herbicides perform well. Measuring the activity of the target enzymes, for example ALS enzyme and determining the thickness of cuticle may provide complementary evidence for the lack of adverse influence of drought condition on the performance of herbicides. Moreover, measuring the absorption and translocation of herbicides can be useful. However, Xie et al. (1996) suggested that the alterations in herbicide translocation and absorption might not be the main physiological processes related to different whole-plant response of *A. fatua* to fenoxaprop-ethyl and imazamefhabenz-methyl under drought stress.

5 | CONCLUSIONS

The influence of drought stress (FC 60%) on the performance of sulfosulfuron and sulfosulfuron + metsulfuron-methyl herbicide for controlling *H. spontaneum* was not significant in the studied sandy clay loam soil. In contrast, the performance of clodinafop-propargyl and mesosulfuron-methyl + iodosulfuron-methyl sodium on *A. sterilis* subsp. *ludoviciana* was reduced under the drought-stress condition. It might be expected that under higher drought-stress levels, that is <FC 60% in the heavier soils the performance of herbicides, especially mesosulfuron-methyl + iodosulfuron-methyl sodium and clodinafop-propargyl on *H. spontaneum*, may be influenced more obvious, especially under field conditions. Therefore, it is suggested to test this hypothesis in the future studies. To avoid the potential

efficacy reduction of mesosulfuron-methyl + iodosulfuron-methyl sodium and clodinafop-propargyl on the drought-stressed *A. sterilis* subsp. *ludoviciana*, it is also recommended that farmers irrigate the field if necessary before application of the herbicides. Following regional weather predictions before applying, herbicides may assist farmers in arid areas. Using adjuvants, diverse formulations of herbicides, and early application of herbicides might also overcome the potential performance reduction in mesosulfuron-methyl + iodosulfuron-methyl sodium and clodinafop-propargyl due to drought stress. However, further studies are needed to asses these recommended tactics, especially under field conditions.

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CONFLICT OF INTEREST

No conflicts of interest have been declared.

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