SBCPD
<http://www.sbcpd.org>

Planta Daninha

Journal of The Brazilian Weed Science Society

ISSN 0100-8358 (print)
1806-9681 (online)

Research Article

Effects of light-dependent herbicides on growth and physiology of *Salvia officinalis*

Mina T. Jervekani[a], Hassan Karimmojeni[a]*, and Jamshid Razmjoo[a]

[a] Department of Agronomy and Plant Breeding, College of Agriculture, Isfahan University of Technology, Isfahan, 84156-83111, Iran.

INFORMATION ARTICLE

Received: May 11, 2019
Accepted: May 13, 2019

Keywords:
bentazon
metribuzin
oxadiargyl
oxyfluorfen
phenmedipham+desmedipham.

***Corresponding author:**
<kmojeni@cc.iut.ac.ir>

Jervekani MT, Karimmojeni H, Razmjoo J. Effects of light-dependent herbicides on growth and physiology of *Salvia officinalis*. Planta Daninha. 2020;38:e020223839. https://doi.org/10.1590/S0100-83582020380100073

Conflict of Interest:
The authors declare that there is no conflict of interest regarding the publication of this manuscript.

HIGHLIGHTS

- There is little information on chemical weed control of *Salvia officinalis.*
- The effects of some photosynthetic inhibitor herbicides were tested on *S. officinalis*.
- Phenmedipham+desmedipham and bentazon herbicides may be suitable for weeds control in *S. officinalis.*

ABSTRACT

Background: *Salvia officinalis* a medicinal plant which is severely affected by weeds competition.
Objective: The objective of this study was to determine the growth and physiological responses of *S. officinalis* to some light-dependent herbicides.
Methods: A factorial experiment was conducted to determine the effects of oxadiargyl (T), bentazon (B), oxyfluorfen (O), metribuzin (M), phenmedipham+desmedipham (P) at 0.75 (1), 1 (2) and 1.25 (3) rates on growth and physiological parameters of *S. officinalis*.
Results: All herbicides initially caused visual injury to *S. officinalis*. SPAD values were decreased by all herbicide treatments except for low rate of phenmedipham+desmedipham and oxadiargyl. The relative leaf water content (RLWC) decreased following herbicide treatments except in P1, P2, B1, and B2. Membrane stability index decreased by herbicide treatments however there were no differences among control and P1, P2, B1, B2, O1, O3, M3, T1, T2, and T3. All rates of oxyfluorfen and oxadiargyl, P1, B1 and B2 had no marked effect on the maximum quantum efficiency of PSII (Fv/Fm). Plant growth was not affected by herbicide treatments probably due to the recovery of the plants at the end of the experiment excepting for metribuzin. Essential oil content increased as the herbicide rate increased based on herbicide type.
Conclusions: The results showed that *S. officinalis* had the ability to recover over time depending on the herbicide type and rate. Results showed that phenmedipham+desmedipham and bentazon are suitable and metribuzin unsuitable herbicides for weeds selective control in *S. officinalis.*

1 INTRODUCTION

Common sage (*Salvia officinalis* L.) is a perennial woody sub-shrub native to the Mediterranean region but is now cultivated in many parts of the world mainly for use in food, pharmaceutical and perfume industries (Bettaieb et al., 2009). As a medicinal plant, *S. officinalis* has an important role in traditional medicine. The World Health Organization has estimated that 80% of the global population relies chiefly on traditional medicines for health care and 51% of all drug preparations in industrialized

countries are derived from plants (Zuin and Vilegas, 2000).

Weed infestation is one of the main limiting factors that reduce yield, growth, and quality of medicinal plants such as common sage (Carrubba, 2017). Weed management in the initial growth stage is important because weeds grow much faster than perennial crops (Carrubba and Militello, 2013). Different non-chemical methods including physical control (mechanical weeding, flame control), cultural controls (stale or false seedbed technique) and biological control may be sufficient to provide the desired level for weed control. Chemical control (herbicides) represent a simple, fast and cheap method that can be used to provide weed control (Carrubba and Militello, 2013). Herbicides while a useful method for farmers, can have adverse effects on the crop and environment (El-Keltawi and Croteau, 1987; Galhano et al., 2009). Indiscriminate use of non-selective herbicides may lead to adverse effects on the environment and various organisms at different levels of the food chain (Baig et al., 2012). Herbicides lead to disruption in various physiological and biochemical process such as photosynthesis and growth and development of plants, so that the selection of selective herbicides with the lowest impact on the crop and environment is important (Baig et al., 2012).

As the most important pigment, chlorophyll is an indicator of crop health and productivity that is affected by herbicides application (Ruttanaprasert et al., 2012). The light energy absorbed by chlorophyll is used in two ways: I, photochemical energy (photosynthesis) and II, non-photochemical energy (excess energy is released as, heat and chlorophyll fluorescence) (Dayan and Zaccaro, 2012). The amount of chlorophyll fluorescence (1-2% of total light absorption) represents the thylakoid membrane integrity, efficiency of electron transport from photosystem II to photosystem I and CO_2 fixation that is affected by light-dependent herbicides, such as the inhibitors of photosystem II (phenmedipham +desmedipham, metribuzin, bentazon) and protoporphyrinogen oxidase (oxyfluorfen, oxadiargyl) (Maxwell and Johnson, 2000). Light-dependent herbicides inhibit PSII through block the flow of electron by competing for the binding of plastoquinone, and generating reactive oxygen species by reacting with molecular oxygen (Dayan and Zaccaro, 2012). Symptoms from herbicides that interfere with PS II such as chlorosis (yellowing) and necrosis (tissue death) slowly evolve over several days. The chlorophyll destroys through photooxidation and cause chlorosis, then membrane destroys through lipid peroxidation and causes necrosis (Hess, 2000). So, measurement of chlorophyll and chlorophyll fluorescence parameters as a sensitive indicator can be used for assessing herbicide efficacy for detecting the herbicides damage to photosynthetic apparatus (Ali and Honermeier, 2016; Dayan and Zaccaro, 2012; Maxwell and Johnson, 2000).

A few studies have investigated the application of the herbicides for weed control in medicinal plants such as *Coriandrum sativum* L., *Menthapiperita* L., *Menthaarvensis* var. *piperascens Malinv*, *Silybum marianum Gaertn*., fennel *Foeniculum vulgare* Mill., *Salvia officinalis* L, *Satureja officinalis* L., and *Thymus officinalis* L., (Ali and Honermeier, 2016; Carrubba and Militello, 2013; El-Keltawi and Croteau, 1987; Qasem and Foy, 2006). Oxadiazon provided good weed control and increased the shoot dry matter of marjoram (*Origanum syriacum*) (Qasem and Foy, 2006). The effects of broadleaved and grass weed herbicides application in *Valeriana officinalis* showed that oxyfluorfen and bentazon caused severe damage to *V. officinalis*, while oxadiargyl, oxadiazon, sethoxydim, and haloxyfop-R (methyl ester) did not injure the crop (Monjezi et al., 2015). Application of desmedipham and phenmedipham after the four-leaf-pair stage of calendula (*Calendula officinalis* L.) controlled several weed species and avoid injury to calendula (Forcella et al., 2012).

There is little information on the tolerance of common sage to the application of herbicides. The correlation between growth and essential oil production in *S. officinalis* following foliar application of pre and post-emergence herbicides under controlled environmental conditions was investigated (El-Keltawi and Croteau, 1987). The authors showed linuron application at 200 and 400 ppm reduced the sage growth and oil yield (El-Keltawi and Croteau, 1987).

For the chemical weed control, the information regarding to tolerance and sensitivity of crop and weed to herbicides is required, so the aim of this study was to investigate the physiological and growth responses of *S. officinalis* to the photosynthetic inhibitor herbicides oxadiargyl, bentazon, oxyfluorfen, metribuzin, phenmedipham+desmedipham to identify appropriate herbicide for commercial production..

2 Materials and Methods

2.1 Plant material and growth condition

The experiment was conducted in a greenhouse at the Isfahan University of Technology (32’43”E, 51’31”N), Iran, during May-August, 2015.

Salvia officinalis seeds (Isfahan accession) were purchased commercially. At first, the seeds were sown in the outdoor sandy bed in March 2015 and the seedlings were then transplanted at 4-6 leaf stage to 10 cm pots containing a potting mix (manure, sand and field soil at a ratio of 1:1:2). Pots were watered every other day. The plants were thinned to two plants per pots before application of herbicides. The plants were grown at a day/night cycle of 14/10 h, at 25/20 ºC, and at a light intensity of 12000 LUX. Plants were fed with NPK fertilizer (20-20-20+TE (trace elements), 1 g L^{-1}) two times to avoid any nutrient deficiencies during the growth period.

2.2 Experimental design and herbicides treatment

Treatments included five broadleaf herbicides applied post-emergence at three different rates: 0.75X (1), 1X (2), and 1.25X (3) where X is the recommended rate (Table 1). The herbicides were produced commercially in Iran. Untreated plants (control) were sprayed with distilled water. Five herbicidal treatments with three rates along with a control (16 treatments) were arranged in a factorial arrangement based on a randomized complete block design (RCBD) with three replications. Herbicides were applied at the 8-10 leaf stages by pressure sprayer (1 Lit).

2.3 SPAD values

Chlorophyll density was estimated by using the chlorophyll meter SPAD-502 Plus on three fully expanded leaves per pots and reported as a SPAD value. Chlorophyll densities and SPAD values were closely correlated and corresponded to the amount of chlorophyll present in the sample leaf (Ruttanaprasert et al., 2012).

2.4 Chlorophyll fluorescence

Chlorophyll fluorescence was measured on three fully expanded leaves per pots after a dark acclimation period of 30 min using a portable chl fluorometer (Opti-Sciences, Inc.,Hudson, NH, USA). Maximum fluorescence (Fm), minimum fluorescence (Fo) and Fv/Fm (maximal efficiency of photosystem II) were recorded that the difference between maximum fluorescence and minimum fluorescence is Fv, or variable fluorescence.(Kopsell et al., 2011).

2.5 Relative leaf water content (RLWC)

Relative leaf water content (RLWC) was measured when leaf injury symptoms became visible as described by Askari and Ehsanzadeh (2015). Leaf fresh matter (FM) was measured quickly then floated in distilled water for 12 h in dark and then measured the leaves as turgor matter (TM). Leaf dry matter (DM) was measured after drying (at 70 ºC for 48 h. RLWC) and was calculated using the formula:

$$RLWC\% = \frac{(FM - DM)}{(TM - DM)} X\ 100$$

FM = Fresh matter; *TM* = Turgor matter; *DM* = Dry matter.

2.6 Membrane stability index (MSI)

Membrane stability index (MSI) was also measured as described by Siaram et al (2005). Leaf tissue (0.1 g) was floated on 10 mL distilled water at two temperatures, at 40 ºC for 30 min in a water bath and measured the EC then 100 ºC for 10 min respectively. Finally, the electrical conductivity of the samples was measured with an EC meter (Model Cyberscan, Singapore) MSI was calculated using the following formula:

$$MSI = 1 - \left(\frac{EC_{40\ ^\circ C}}{EC_{100\ ^\circ C}}\right) X\ 100$$

$EC_{40\ ^\circ C}$ = Electrical conductivity at 40 ºC; $EC_{100\ ^\circ C}$ = Electrical conductivity 100 ºC.

2.7 Visual symptoms (V.S)

After herbicides application, phytotoxic symptoms (cessation of growth, chlorosis, necrosis of tips and edges of leaves and death of the whole plant) were

Table 1 - Herbicides and rates used in the experiment

Herbicide	Trade name	Chemical class	Chemical formula	Treatment abbreviation	Proportion of labeled use rate		
					0.75x(1)	1x(2) (g a.i. ha^{-1})	1.25x(3)
Phenmedipham +Desmedipham	Betanal compact	Phenylcarbamate	$C_{32}H_{32}N_4O_8$	P	616.5	822 (3 L ha^{-1}, 27.4% EC)	1027.5
Bentazon	Basagran	Benzothiadiazole	$C_{10}H_{12}N_2O_3S$	B	720.0	960 (2 L ha^{-1}, 48% SL)	1200.0
Oxyfluorfen	Goal	Diphenyl ether	$C_{15}H_{11}ClF_3NO_4$	O	360.0	480 (2 L ha^{-1}, 24% EC)	600.0
Metribuzin	Sencor	Triazinone	$C_8H_{14}N_4OS$	M	525.0	700 (kg ha^{-1}, 70% WP)	875.0
Oxadiargyl	Top star	Oxadiazole	$C_{15}H_{14}Cl_2N_2O_3$	T	675.0	900 (3 L ha^{-1}, 30% EC)	1125.0
untreated(control)	-	distill water	-	C	-	-	-

assessed according to the scale proposed by the EWRC (Sofiatti et al., 2012) 10, 20 and 30 days after treatment (DAT). Visual ratings were done using a 0-100% scale, zero means no visible damage to the plant and a score of 100 means plant death (Dear et al., 2006).

2.8 Plant growth and biomass

At the end of the experiment (30 DAT), plant height was recorded and plants were harvested and dried under shade (without direct sunlight) at 35-40 ºC for 1-2 weeks, then shoot and root dry matter were measured.

2.9 Essential oil content

Essential oil content was measured by Clevenger methods (1928) using Clevenger's apparatus (Clevenger, 1928). Nine folded of the dry weight of leaves, distilled water was added, distillation process continued for 3 h at 100 ºC. The essential oil phase was separated and kept in darkness at -20 ºC (Govahi et al., 2015). Essential oil content percentage was calculated by the following formula:

$$\text{Essentialoil\%} = \left(\frac{Essentialoilweight}{Dryweightleaves}\right) X\ 100$$

2.10 Statistical analysis

Data were subjected to analysis of variance (ANOVA), using SAS statistical software (version 9.1) and means were compared using the least significant difference (LSD) at a probability level of 0.05.

3 Results and Discussion

3.1 Chlorophyll content (SPAD value)

An analysis of variance showed that herbicidal treatments had a significant effect on SPAD value but there were no differences between the control and the average of treatments (Table 2). Herbicides and herbicide rate had a significant effect on SPAD value but the interaction was not significant (Table 2). There were no differences between rate reduction of phenmedipham+desmedipham (P1) and oxadiargyl (T1) but other herbicides significantly reduced the SPAD value compared to the control (Table 3).

Table 2 - Main results of ANOVA on chlorophyll content (SPAD value), chlorophyll fluorescence parameters (Fv/Fm, Fo, Fm), relative leaf water content (RLWC), membrane stability index (MSI), visual symptoms (at 10 days after treatment (DAT), 20DAT and 30DAT), Shoot dry matter (SDM), Root dry matter (RDM) and Height of S. officinalis treated with different herbicides. df – degrees of freedom; s.o.v-source of variation

S.O.V	Df	Mean Square												
		SPAD	Fv/Fm	Fo	Fm	RLWC (%)	MSI (%)	V.S.10DAT (%)	V.S.20DAT (%)	V.S.30DAT (%)	SDM (g pot^{-1})	RDM (g pot^{-1})	Height (cm)	Essential oil (%)
Rep	2	25.57*	0.0016^{ns}	279.38^{ns}	822.52^{ns}	30.40^{ns}	19.46^{ns}	9.89^{ns}	18.13^{ns}	4.18^{ns}	0.028^{ns}	0.290^{ns}	9.13^{ns}	0.94 ^{ns}
Treatment	15	41.12**	0.0700**	3622.32**	8078.24**	46.77**	346.60**	196.92**	1671.86**	1769.24**	0.534^{ns}	0.330*	65.17**	1.67**
Control vs Treatment	1	7.37^{ns}	0.0480*	2677.68*	241.51^{ns}	124.04**	267.39^{ns}	4.27^{ns}	205.51**	177.50**	0.440^{ns}	0.188^{ns}	9.22^{ns}	1.67**
Herbicide	4	75.80**	0.2020**	8176.51**	22715.91**	74.45**	536.09**	599.50**	2521.04**	4839.10**	0.660^{ns}	0.630*	192.46**	5.14**
Dose	2	50.12**	0.0560**	2487.21**	499.87^{ns}	67.78*	139.09^{ns}	88.02**	338.57**	819.95**	0.628^{ns}	0.459^{ns}	1.22^{ns}	0.45*
Herbicide*Dose	8	5.45^{ns}	0.0072^{ns}	1454.72**	3644.29*	7.38^{ns}	287.70*	20.86*	166.19**	622.22**	0.100^{ns}	0.078^{ns}	14.14^{ns}	0.43**
Error	30	6.92	0.0097	407.81	1501.34	12.56	99.04	7.39	6.78	15.38	0.260	0.150	14.65	0.12
CV(%)	-	8.85	14.78	21.42	12.74	4.35	13.29	33.34	22	29.53	21.51	30.9	17.97	23.88

^{ns}, *, ** differences non-significant (P>0.05), significant (0.01<P<0.05) or highly significant (P<0.01), respectively.

Table 3 - Means comparison of chlorophyll content (SPAD value), and chlorophyll fluorescence parameters (Fo, Fm), Shoot dry matter (SDM), Root dry matter (RDM) and Height of *S. officinalis* affected by herbicidal treatments: (P) Phenmedipham+Desmedipham, (B) Bentazone, (O) Oxyfluorfen, (M) Metribuzin, (T) Oxadiargyl (Top star), (C) Control, (1) low rate, (2) recommended rate and (3) high rate

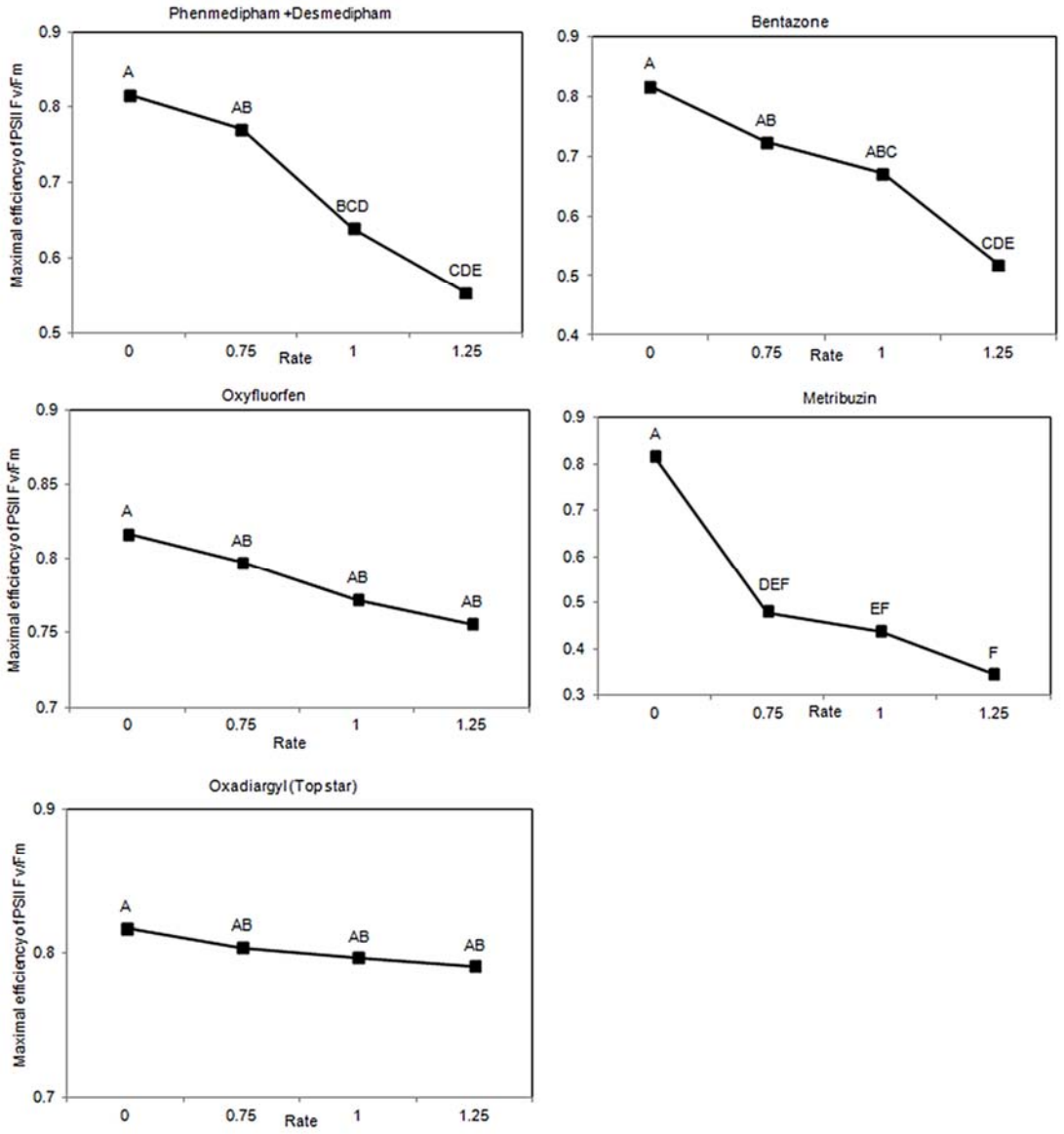
Treatment	SPAD	Fo	Fm	V.S.10DAT (%)	V.S.20DAT (%)	V.S.30DAT (%)
P 1	32.88 abc	87 efg	373.17 a	1.3 gh	1.1 hi	1.0 e
P 2	28.53 cd	120.83 bcd	329.33 abc	2.3 fgh	1.6 ghi	1.6 de
P 3	26.53 de	157.50 a	361.33 ab	3.0 e-h	3.0 fgh	2.6 de
B1	31.45 bc	88.00 d-g	316.50 a-d	5.5 d-g	5.6 ef	4.0 de
B 2	30.81 bcd	105.17 de	325.00 abc	4.0 e-h	4.0 e-h	2.3 de
B 3	29.05 cd	140.17 abc	370.00 a	6.0 def	6.0 e	3.0 de
O1	30.88 bcd	55.17 g	277.83 cd	4.3 e-h	5.0 ef	3.8 de
O2	30.21 bcd	64.67 g	280.83 cd	10.0 cd	10.0 d	5.6 de
O3	29.95 cd	71.33 fg	291.17 cd	10.6 c	12.0 d	8.1 d
M1	26.65 de	116.50 cde	253.17 cd	18.3 b	26.6 c	31.6 c
M2	24.21 ef	153.17 ab	270.50 cd	20.0 b	38.3 b	41.6 b
M3	22.05 f	99.50 def	160.33 e	30.0 a	61.6 a	93.3 a
T1	34.58 ab	65.00 g	332.17 abc	3.3 e-h	3.5 e-h	3.5 de
T2	29.63 cd	64.17 g	312.67 a-d	4.6 efg	4.5 efg	4.1 de
T3	31.25 bc	65.33 g	312.67 a-d	7.0 cde	6.16 e	5.8 de
C	37.01 a	54.67 g	297.00 bcd	0.0 h	0.0 i	0.0 e
LSD*	4.38	33.67	64.61	4.53	2.9	6.53

* Fishers Least Significant Difference (LSD).

Visual symptoms recorded 10 DAT also confirmed these results (Table 3). With increasing herbicide rates, SPAD values decreased, for example with phenmedipham+desmedipham the SPAD value was decreased from 32.88 to 26.53 (Table 3). A lower SPAD value indicated that the herbicides interfered with photosynthesis, which was expected as the mechanism of action of the applied herbicides is inhibition of photosystem II. In control plants of mung bean (*Phaseolus aureus* Roxb) chloroplasts were normal and the grana and thylakoids were intact but in metsulfuron-treated plants, chloroplasts were disorganized and swollen (with a large number of starch grains), and the chlorophyll concentration decreased (Kaushik, 2006). Seven DAT herbicide application reduced SPAD values in Brassica species (*B. napus* and *B. rapa*), but they had recovered 28 DAT (Jin et al., 2010). In contrast to our results, Kopsell et al (2011) reported that different sweet corn genotypes displayed different sensitivity to post-emergence herbicides but herbicides had no effect on photosynthesis (Kopsell et al., 2011).

3.2 Chlorophyll fluorescence

An analysis of variance showed that herbicidal treatments had a significant effect on maximal efficiency of PSII (Fv/Fm) and there were significant differences between the control and the average of treatments (Table 2). Herbicides and rates had significant effects on the maximum efficiency of PSII (Fv/Fm) but the interaction was not significant (Table 2). Herbicidal treatments caused significant stress in *S. officinalis* due to decline in maximum fluorescence (Fm) and an increase in minimum fluorescence (F0) which reflected a decrease in maximal efficiency of PSII (Fv/Fm) (Figure 1).



In all figure (0) is Control. Means are compared at the P ≤ 0.05 for significant difference according to the LSD test.

Figure 1 - Effect of five herbicides: Phenmedipham+Desmedipham, Bentazon, Oxyfluorfen, Metribuzin and Oxydiargyl (Top star) on Maximal efficiency of PSII (Fv/Fm) with three rates: (0.75) Low rate, (1) Recommended rate, (1.25) High rate.

Chlorophyll fluorescence measurement also revealed differences between control and herbicides but these reductions were minor. However, Fv/Fm was not affected by P1, B1, B2, O1, O2, O3, T1, T2 and T3 treatments (Figure 1). Therefore, these herbicides could be applied safely to *S. officinalis*. All herbicides had higher Fv/Fm values at low rates compared to higher rates (Figure 1). Minimum chlorophyll fluorescence observed in metribuzin suppressed photosynthesis and caused the most severe effects on *S. officinalis* (Figure 1). Oxyfluorfen and oxadiargyl (Top Star) had the lowest effects on Fv/Fm values and there were no differences with the control (Figure 1). The results indicated that *S. officinalis* had the potential to tolerate these photosynthesis inhibitor herbicides up to 75% of the recommended rates.

Bentazon caused a 80% reduction in chlorophyll fluorescence in cucumber (*Cucumis sativus*) within 3 hours after application and then stabilized at 50% and plants recovered within 15 h (Dayan and Zaccaro, 2012). In contrast, other herbicides such as pendimethalin and S-metolachlor had no effect on *Lactuca sativa* fluorescence parameters (Hussain et al., 2010). Pyridate herbicide decreased chlorophyll fluorescence up to 2 DAT, but the plants then recovered (Ali and Honermeier, 2016). The plant light use efficiency reduced by herbicidal treatments (metsulfuron-methyl, fluroxypyr and iso-propyl glyphosate with three concentrations) when the maximal efficiency of PSII (Fv/Fm) decreased, but this reduction was constant down more and more over time (Yang et al., 2012).

3.3 RLWC and MSI

An analysis of variance showed that herbicidal treatments had a significant effect on relative leaf water content (RLWC) and there were significant differences between the control and the average of treatments (Table 2). Herbicides and rates had significant effects on RLWC but the interaction was non-significant (Table 2). Also, analysis of variance showed that herbicidal treatments had a significant effect on membrane stability index (MSI) (Table 2). However, there were no differences between control and the average of treatments, while the interaction between herbicides and rates was significant (Table 2). Herbicide treatments P1, P2, B1, and B2 did not significantly affect RLWC in contrast to other treatments. The trend of RLWC was reduced by increasing the herbicide rates. The lowest RLWC (76.56 to 79.57) were observed with metribuzin (Figure 2).

Treatments including M1, M2, O2, P3 and B3, damaged membrane function in *S. officinalis* (Figure 3). RLWC and MSI were decreased in *S. officinalis* due to herbicide treatments that showed herbicide toxicity, by creating free radicals (data not shown) leading to increased permeability and membrane instability. Because of the critical role of cell membranes in metabolism regulation, the effects of different stresses on membrane stability index could be a suitable index for investigating the levels of membrane damage and the presence of oxidative stress (Esfandiari et al., 2011). Membrane electrolyte permeability induced by xenobiotic stresses leads to stability reduction (Garg and Kaur, 2013;

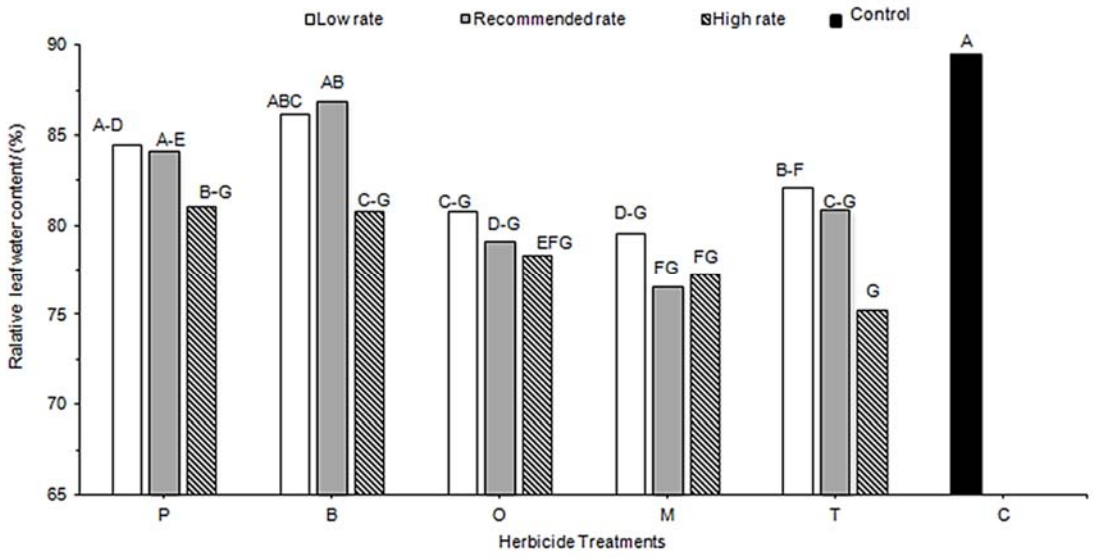


Figure 2 - Effect of herbicide treatments on Relative leaf water content. (P) Phenmedipham+Desmedipham, (B) Bentazone, (O) Oxyfluorfen, (M) Metribuzin, (T) Oxadiargyl (Top star), (C) is Control, Low rate (0.75), Recommended rate (1) and High rate (1.25).

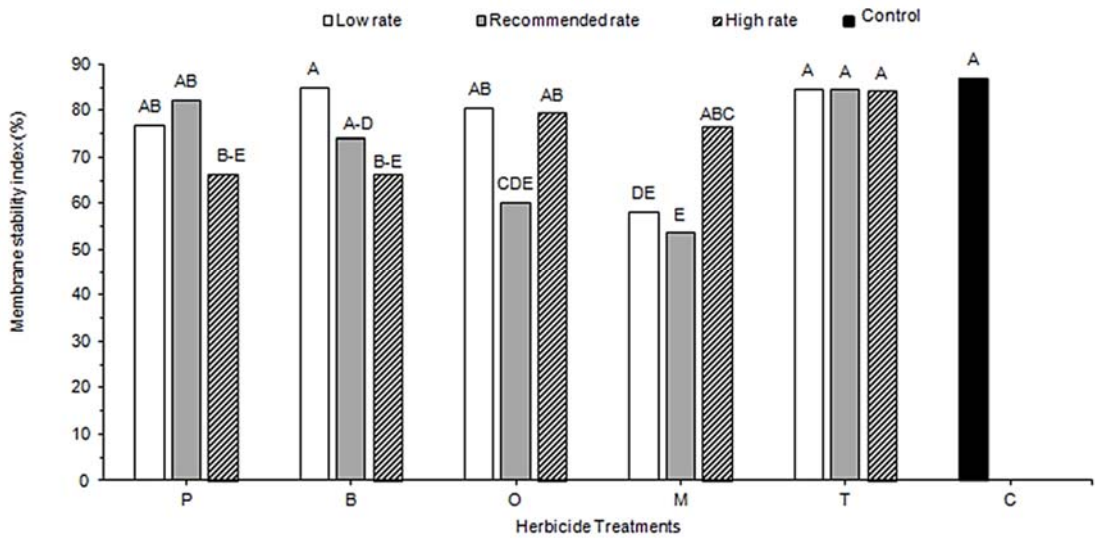


Figure 3 - Effect of herbicide treatments on MSI. (P) Phenmedipham+Desmedipham, (B) Bentazone, (O) Oxyfluorfen, (M) Metribuzin, (T) Oxadiargyl (Top star), (C) is Control, Low rate (0.75), Recommended rate (1) and High rate (1.25).

Singh et al., 2017). Herbicide stress due to imbalanced water and metabolite solution (e.g. amino acid) enhancement results in RLWC reduction (Ali et al., 2007). In another study, pendimethalin and S-metolachlor herbicides have no effect on RLWC in *Lactuca sativa* (Hussain et al., 2010).

3.4 Visual symptoms

Analysis of variance showed that herbicidal treatments had a significant effect on visual 20 and 30 DAT but not at 10 DAT (Table 2). The visual symptoms comprised of brown and spots on the leaves that would start from leaf margin and move towards the inside. These injury symptoms based on herbicide and rates increased until 10 DAT, after which some treatments showed signs of recovery until 30 DAT. The visual symptoms at all rates of phenmedipham+desmedipham, bentazon, and oxadiargyl increased up to 10 DAT and then decreased but for oxyfluorfen, the visual symptoms increased up to 20 DAT and then decreased (Table 3). Visual symptoms at all rates of metribuzin increased up to 30 DAT and caused the most damage to *S. officinalis* (Table 3).

Herbicide treatments that were visually not different from their control were, P1, P2, P3, B2, O1 and T1, at 10 DAT; P1, P2 and P3, at 20 DAT; and, P1, P2, P3, B1, B2, B3, O1, O3, T1, T2 and T3, at 30 DAT (Table 3). Metribuzin caused the highest (31.6 to 93.3%); while phenmedipham+desmedipham (1 to 2.6%), bentazon (2.3 to 4%), and oxadiargyl (3.5 to 5.8%), caused the lowest injury at all rates after 30 DAT (Table 3) where plant growth was comparable to that of the untreated (control).

Effects of metribuzin at all rates were irreversible and the crop did not recover. On the other hand, *S. officinalis* recovered from oxadiargyl, bentazon and oxyfluorfen injury by 30 DAT. Using the EWRC herbicide visual symptom score, scores under four suggest that the herbicide is selective because plants were able to recover from damage. Therefore, phenmedipham+desmedipham and bentazon could be classified as selective herbicides. Visual symptoms of protoporphyrinogen oxidase herbicides (oxadiargyl and oxyflourfen) were leaf cupping, crinkling, bronzing, and necrosis of the foliage that occur because of membrane peroxidation (Dayan and Zaccaro, 2012).

Similar to our results, Monjezi et al (2015) confirmed that bentazon caused only slight injury to valerian (*Valeriana officinalis*) that increased initially up to 20 DAT, and then decreased from 30 DAT. They also reported that oxyfluorfen injury increased up to 30 DAT, whereas in our study oxadiargyl injury increased up to 10 DAT (3.3 to 7%), and then was followed by recovery (Table 3). Oxadiargyl damage on potato increased up to 3 weeks after treatment (WAT), after which plants started to recover (Alebrahim et al., 2012). (Grichar et al., 2009) also reported that flufenacet tank mixed with metribuzin resulted in at least 72% reduction in sesame (*sesamum indicum*) stand in Texas (U.S.A).

Metribuzin damage on potato was increased over time under pre-emergence application, but at post-emergence application, potato recovered partially after 3 weeks, although the injury was not negligible (Alebrahim et al., 2012). In agreement with our results, Ali and Honermeier (2016) reported that

phenmedipham application was safe on *Cynara cardunculus* where injury symptoms increased until 14 DAT, followed by plant recovery. Pyridate, quizalofop-p and phenmedipham application had the greatest toxic effect on *Cynara cardunculus*, but no differences in yield were found (Ali and Honermeier, 2016). The difference in results found between our study and others, may be due to the different species, stage of plant growth, and herbicide rates used.

3.5 Plant growth and biomass

Analysis of variance showed that herbicide treatments had no significant effect on shoot dry mass (SDM) but they had a significant effect on root dry mass (RDM) and height (Table 2). There were no differences between control and the average of treatments for SDM, RDM, and height (Table 4). However, herbicides had no effect on SDM but they had a significant effect on RDM and height. Herbicide rate and the interaction between herbicides and rate had no significant effect on SDM, RDM, and height (Table 4). Herbicide treatments had no effect on shoot dry matter except for metribuzin (Table 4). Metribuzin resulted in at least a 49% reduction in SDM, 57% reduction in RDM and 51% reduction in height (Table 4). Herbicide treatments P1, B1, B2, B3, and O1 had no effect on root dry matter (Table 4).

Table 4 - Means comparison of chlorophyll content Shoot dry matter (SDM), Root dry matter (RDM) and Height of *S. officinalis* affected by herbicidal treatments: (P) Phenmedipham+Desmedipham, (B) Bentazone, (O) Oxyfluorfen, (M) Metribuzin, (T) Oxadiargyl (Top star), (C) Control, (1) low rate, (2) recommended rate and (3) high rate

Treatment	SDM (g pot^{-1})	RDM (g pot^{-1})	Height (cm)	Essential oil (%)
P1	2.41 bcd	1.70 abc	24.81 a-d	1.78 c
P2	2.58 a-d	1.01 def	27.16 a	2.19 abc
P3	2.29 bcd	0.94 ef	26.00 a-d	2.01 bc
B1	3.01 abc	1.79 ab	20.58 cd	2.02 abc
B2	2.55 bcd	1.60 a-e	22.11 a-d	2.18 abc
B3	2.32 bcd	1.64 a-d	21.41 a-d	1.10 de
O1	2.67 abc	1.49 a-e	27.08 ab	1.67 cd
O2	2.40 bcd	1.12 c-f	21.00 a-d	2.43 ab
O3	2.07 cd	1.12 c-f	20.28 cde	2.59 a
M1	1.99 cd	1.07 c-f	14.16 efg	0.56 ef
M2	1.73 d	1.04 c-f	13.28 fg	0.49 f
M3	1.94 cd	0.79 f	12.85 g	0.81 ef
T1	2.62 abc	1.17 b-f	19.66 def	0.34 f
T2	2.41 bcd	1.17 b-f	20.73 bcd	0.80 ef
T3	2.03 cd	1.043 c-f	23.00 a-d	0.89 ef
C	3.42 abc	1.84 a	26.66 abc	1.62 cd
LSD*	0.86	0.66	6.38	0.58

* Fishers Least Significant Difference (LSD).

Although *S. officinalis* plants were damaged by herbicide treatment at the early growth stage assessment, the shoot dry matter was not affected by herbicides at later growth stages since plants had started to recover (Table 4). *S. officinalis* treated with phenmedipham+desmedipham, and metribuzin, at recommended rates, produced highest (2.58 g) and lowest (1.73 g) shoot biomass, respectively. Nevertheless, most of the herbicidal treatment had no adverse effect on crop biomass. However, the efficacy of these herbicides on weeds in field conditions need to be investigated.

Plant growth is the result of the biochemical and physiological process and herbicides interfere with these process causing growth reduction (Follak and Hurle, 2003). Bentazon (1440 g a.i. ha^{-1}) was found to be an appropriate treatment for the control of broadleaf weeds in linseed (*Linum usitatissimum*), mainly because it maintained the yield at satisfactory levels (Karimmojeni et al., 2013). Leaf yield of *Cynara cardunculus* was affected by postemergence herbicides, and the type of herbicides used (Ali and Honermeier, 2016). In Australia, the persistence of sulfonylurea herbicide application in cereal crops affected crop dry matter production in the following growth season (Oldach et al., 2008).

Among the treatments, metribuzin resulted in the lowest height of *S. officinalis* relative to control treatments (Table 4) due to hormesis effect and plant recovery. It was reported that some herbicides, especially at low rate, can stimulate plant growth (Davies et al., 2003; Nelson et al., 2002). After *S. officinalis* recovery, plants may have low concentration of herbicide that released a hormetic effect.

3.6 Essential oil content

Analysis of variance showed essential oil content of *S. officinalis* was significantly affected by herbicidal treatments and their doses (Table 2). The highest essential oil content (5.28%) was observed at a higher dose of oxyfluorfen and the lowest (0.43%) was observed in lower dose of oxadiargyl (Table 4). With some exceptions, essential oil content generally increased as herbicide rate increase. Indeed, moderate stress induced by herbicide application at recommended doses increases essential oil content more than severe stress induced by higher rates of herbicides. High essential oil content under water stress could be due to high production of terpenes, because of low allocation of carbon to the growth (Govahi et al., 2015). Presumably, herbicides application in our experiment as an inhibitor of photosynthesis via disturbance in electron transport chain can produce toxic molecules, causing high essential oil content as a defense system in *S. officinalis*. Ali et al (2016) reported herbicides as xenobiotic stress affected phenolic compounds of

artichoke (*Cynara Cardunculus*) and Quizalofop-p application increased flavonoid content in artichoke leaves. Many researchers have highlighted that plants under environmental stress had high secondary metabolites content (Askari and Ehsanzadeh, 2015; Bettaieb et al., 2009; Govahi et al., 2015).

4 Conclusions

In this study, herbicides damage was related to herbicide type and rate. Herbicides reduced chlorophyll content and chlorophyll fluorescence. Chlorophyll fluorescence parameters showed that herbicides reduced maximum fluorescence (Fm) and increased minimum fluorescence (F0) that lead to a reduction in the maximal efficiency of PSII (Fv/Fm). RLWC and MSI decreased when plants were exposed to herbicides especially metribuzin. Generally, visual symptoms of damage increased until 10 DAT and then began to decrease. The plant growth data revealed that *S. officinalis* is tolerant to the majority of herbicide treatments when exposed to a short period of herbicidal stress and it was able to recover between 20 and 30 DAT. Overall, phenmedipham+ desmedipham and bentazon can be used as suitable herbicides for selective weed control in *S. officinalis* but metribuzin is an unsuitable herbicide for selective weed control in this crop.

5 Contributions

MTJ: experiments and data analysis and Manuscript preparation. HK and JR: Concept and experimental protocol for the study; Manuscript preparation.

6 Acknowledgements

This study was financially supported by the Isfahan University of Technology, Iran.

7 References

Alebrahim MT, Majd R, Rashed Mohassel MH, Wilcockson S, Baghestani MA, Ghorbani R, et al. Evaluating the efficacy of pre-and post-emergence herbicides for controlling *Amaranthus retroflexus* L. and *Chenopodium album* L. in potato. Crop Prot. 2012;42:345-50.

Ali B, Rani I, Hayat S, Ahmad A. Effect of 4-Cl-indole-3-acetic acid on the seed germination of *Cicer arietinum* exposed to cadmium. Acta Bot Croatica. 2007;66:57-65.

Ali S., Honermeier B. Post emergence herbicides influence the leaf yield, chlorophyll fluorescence and phenolic compounds of artichoke (*Cynara cardunculus* L.). Sci Hortic. 2016;203:216-223.

Askari E, Ehsanzadeh P. Drought stress mitigation by foliar application of salicylic acid and their interactive effects on physiological characteristics of fennel (*Foeniculum vulgare* Mill.) genotypes. Acta Physiol Plant. 2015;37:1-14.

Baig SA, Akhter NA, Ashfaq M, Asi MR, Ashfaq U. Imidacloprid residues in vegetables, soil and water in the southern Punjab, Pakistan. J Agric Tech. 2012;8:903-16.

Bettaieb I, Zakhama N, Aidi Wannes W, Kchouk ME, Marzouk B. Water deficit effects on *Salvia officinalis* fatty acids and essential oils composition. Sci Hortic. 2009;120:271-5.

Carrubba A. Weed and weeding effects on medicinal herbs. In: Medicinal Plants and Environmental Challenge. [S.l.]: Springer; 2017. p.295-327

Carrubba A, Militello M. Nonchemical weeding of medicinal and aromatic plants. Agron Sust Dev. 2013;33:551-61.

Clevenger J. Apparatus for the determination of volatile oil. J Pharm Sci. 1928;17:345-9.

Davies J, Honegger JL, Tencalla FG, Meregalli G, Brain P, Newman JR, et al. Herbicide risk assessment for non-target aquatic plants: sulfosulfuron–a case study. Pest Manag Sci. 2003;59:231-7.

Dayan FE, Zaccaro MLDM. Chlorophyll fluorescence as a marker for herbicide mechanisms of action. Pest Bioch Phys. 2012;102:189-97.

Dear BS, Sandral GA, Wilson BCD. Tolerance of perennial pasture grass seedlings to pre-and post-emergent grass herbicides. An Prod Sci. 2006;46:637-44.

El-Keltawi NE, Croteau R. Influence of herbicides and growth regulators on the growth and essential oil content of sage. Phytochemistry. 1987;26:675-9.

Esfandiari E, Enayati V, Abbasi A. Biochemical and physiological changes in response to salinity in two durum wheat (*Triticum turgidum* L.) genotypes. Not Bot Hort Agrobot Cluj-Napoca. 2011;39:165-70.

Follak S, Hurle K. Effect of airborne bromoxynil–octanoate and metribuzin on non-target plants. Environ Pollution. 2003;126:139-46.

Forcella F, Papiernik SP, Gesch R. Postemergence herbicides for calendula. Weed Technol. 2012;26:566-9.

Galhano V, Peixoto F, Gomes-Laranjo J, Fernandez-Valiente E. Differential effects of bentazon and molinate on *Anabaena cylindrica*, an autochthonous cyanobacterium of Portuguese rice field agro-ecosystems. Water Air Soil Pollut. 2009;197:211-22.

Garg N, Kaur H. Response of antioxidant enzymes, phytochelatins and glutathione production towards Cd and Zn stresses in *Cajanus cajan* (L.) Millsp. genotypes colonized by arbuscular mycorrhizal fungi. J Agron Crop Sci. 2013;199:118-33.

Govahi M, Ghalavand A, Nadjafi F, Sorooshzadeh A. Comparing different soil fertility systems in Sage (*Salvia officinalis*) under water deficiency. Ind Crops Prod. 2015;74:20-7.

Grichar WJ, Dotray, Langham DR. Sesame (*Sesamum indicum* L.) response to preemergence herbicides. Crop Prot. 2009;28:928-33.

Hess FD. Light-dependent herbicides: an overview. Weed Sci. 2000;48:160-70.

Hussain MI, Gonzalez L, Reigosa MJ. Phytotoxic effect of allelochemicals and herbicides on photosynthesis, growth and carbon isotope discrimination in *Lactuca sativa*. Allel J. 2010;26:157-74.

Jin ZL, Zhang F, Ahmed ZI, Rasheed M, Naeem MS, Ye QF, et al. Differential morphological and physiological responses of two oilseed Brassica species to a new herbicide ZJ0273 used in rapeseed fields. Pest Biochem Physiol. 2010;98:1-8.

Karimmojeni H, Pirbaloti A, Kudsk P, Kanani V, Ghafori A. Influence of postemergence herbicides on weed management in spring-sown linseed. Agron J. 2013;105:821-6.

Kaushik S. Phytotoxicity of selected herbicides to mung bean (*Phaseolus aureus* Roxb.). Environ Exp Bot. 2006;55:41-8.

Kopsell DA, Armel GR, Abney KR, Vargas JJ, Brosnan JT, Kopsell DE. Leaf tissue pigments and chlorophyll fluorescence parameters vary among sweet corn genotypes of differential herbicide sensitivity. Pest Biochem Physiol. 2011;99:194-9.

Maxwell K, Johnson GN. Chlorophyll fluorescence - a practical guide. J Exp Bot. 2000;51:659-68.

Monjezi N. Razmjo J, Karimmojeni H. Valerian (*Valeriana officinalis* L.) tolerance to some post-emergence herbicides. J Plant Prot Res. 2015;55:415-20.

Nelson KA, Renner KA, Hammerschmidt R. Effects of protoporphyrinogen oxidase inhibitors on soybean (*Glycine max* L.) response, *Sclerotinia sclerotiorum* disease development, and phytoalexin production by soybean 1. Weed Technol. 2002;16:353-9.

Oldach KH, Peck DM, Cheong J, Williams KJ, Nair RM. Identification of a chemically induced point mutation mediating herbicide tolerance in annual medics (*Medicago* spp.). Ann Botany. 2008;101:997-1005.

Qasem JR, Foy CL. Selective weed control in Syrian Marjoram (*Origanum syriacum*) with oxadiazon and oxyfluorfen herbicides 1. Weed Technol. 2006;20:670-6.

Ruttanaprasert R, Jogloy S, Vorasoot N, Patanothai A. Relationship between chlorophyll density and spad chlorophyll meter reading for Jerusalem Artichoke (*Helianthus tuberosus* L.). SABRAO J Breed Genet. 2012;44:149-62.

Singh H, Singh NB, Singh A, Hussain I, Yadav V. Physiological and biochemical roles of nitric oxide against toxicity produced by glyphosate herbicide in *Pisum sativum*. Russian J Plant Physiol. 2017;64:518-24.

Sofiatti V, Severino LS, Silva FMO, Silva, VNB, Brito GG. Pre and postemergence herbicides for weed control in castor crop. Ind Crops Prod. 2012;37:235-7.

Yang LQ, Liao FY, Zhao K. Effect of herbicides on the photosynthetic rate and chlorophyll fluorescence of *Solidago canadensis* L. Adv Mat Res. 2012;356:2785-90.

Zuin VG, Vilegas JH. Pesticide residues in medicinal plants and phytomedicines. Phytoth Res: I J Dev Pharm Toxic Eval Nat Prod Deriv. 2000;14:73-88.