Efficacy and Selectivity of Pre- and Post-emergence Herbicides in Chia (*Salvia hispanica* L.) under Mediterranean Semi-arid Conditions

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Abstract

In the present study, the selectivity and efficacy of several post and pre-emergence herbicides in chia (*Salvia hispanica* L.) were examined. Field experiments were conducted at two locations with different soil types and environmental conditions. Our results showed that the pre-emergence application of pendimethalin, oxyfluorfen and linuron reduced total weed density by 71-74%, 74-82% and 53-55%, respectively. Despite their high effectiveness against specific weed species, the above-mentioned herbicides had a negative effect on plant density and biomass yield. In addition, herbicides bentazon and flazasulfuron which applied post-emergence did not affect significantly plant height and biomass yield. In conclusion, the herbicides linuron, pendimethalin and oxyfluorfen do not seem to be a viable option for weed control in chia crop, whereas the post-emergence applied herbicides did not affect significantly plants' growth. Further evaluation of chia tolerance to herbicides is needed under different application rates in order to make safe suggestions for chemical control of weeds.

Keywords: biomass yield, herbicides selectivity, phytotoxicity, soil types, weed control

Introduction

Chia (*Salvia hispanica* L.) is a member of the Lamiaceae family, with Mesoamerica and Western Mexico being recognized as its center of genetic diversity (Cahill, 2004). *Salvia hispanica* is a diploid species with $2n=12$ chromosomes, which is the lowest chromosomes number among the species of *Salvia* genus (Estilai *et al.*, 1990). It is an annual herb with opposite leaves which is usually cultivated for its nutritious seeds (Ali *et al.*, 2012; Jamboonsri *et al.*, 2012; Porras-Loaiza *et al.*, 2014) and aromatic leaves (Ahmed *et al.*, 1994). In Europe, "chia seeds" are considered as a new food ingredient (Commission Implementing Decision 2013/50/EU). Chia is also cultivated as a forage crop, producing high-quality fodder (Peiretti and Gai, 2009; Bilalis *et al.*, 2016).

Chia seeds have a high nutritional value due to their high protein (18.5-22.3%), fat (21.5-32.7%) and fiber content (20.1-36.1%) (Porras-Loaiza *et al.*, 2014), while the main protein fractions of seeds are globulins (52-64.86%)

and glutelins (20.21%) (Sandoval-Oliveros and Paredes-López, 2013; Vázquez-Ovando *et al.*, 2010). Chia seed oil is also rich in omega-3 and omega-6 fatty acids, since it contains a high percentage of α -linolenic acid (59.9-63.2%) and linoleic acid (20%) (Ali *et al.*, 2012; Álvarez-Chávez *et al.*, 2008; Ayerza and Coates, 2011; Porras-Loaiza *et al.*, 2014). Moreover, omega-3/omega-6 fatty acid ratio is 3:1 and therefore considered of high nutritional value (Guil *et al.*, 1996; Porras-Loaiza *et al.*, 2014). In 2009, the European Commission authorized the use of chia seeds in bread products with a maximum content of 5% (Commission Decision 2009/827/EC). Tartary buckwheat (*Fagopyrum tataricum* (L.) Gaertn.) and chia seeds flours can be used as combined ingredients in gluten-free breads (Costantini *et al.*, 2014), since chia seeds do not contain gluten (Bueno *et al.*, 2010). Seeds are also considered a good source of phytosterols (Álvarez-Chávez *et al.*, 2008; Ciftci *et al.*, 2012) and phenolic compounds (i.e. myricetin, quercetin, kaempferol, chlorogenic acid) (Marineli *et al.*, 2014). Apart from seeds, leaves are used for aromatic and therapeutic purposes, with major essential oils compounds being β -

caryophyllene, globulol, γ -muurolene, β -pinene, α -humulene, germacrene-B, and widdrol (Ahmed *et al.*, 1994). According to Amato *et al.* (2015), chia leaves are a rich source of several flavonoids (i.e. acetyl vitexin, acetyl orientin, apigenin and luteolin glycosides).

Although chia shows a high potential as an alternative crop in many countries throughout the world (Porras-Loaiza *et al.*, 2014), little information is available about its cultivation practices in Europe. Chia is sown directly in soil at a rate of 5-6 kg ha⁻¹ in rows 70-80 cm apart (Coates and Ayerza, 1996; Ayerza and Coates, 2009; Ayerza, 2010). So far, commercial cultivation takes place mainly in South America, where in Argentina, Coates and Ayerza (1996) reported that seed yield of chia varied between 175 kg ha⁻¹ and 918 kg ha⁻¹. However, in southern Europe, flowering of plants within mid-to-late autumn is a major drawback, especially when crop intended for seed production, since plants are susceptible to low temperatures. According to Jamboonsri *et al.* (2012), domesticated chia lines have photoperiod requirements for floral induction of about 12 h; therefore in the northern hemisphere, plants begin to flower during October.

Another limiting factor for commercial cultivation of chia is weed interference, since weeds can severely affect both yield and quality of the final product. Cultivation and hand hoeing are still the main weed control practices. However, chemical control of weeds could be a useful means for commercial cultivation of chia, since usually hand hoeing is not practicable due to high cost and limited availability of labour. On other crops belonging to Lamiaceae family, several herbicides have been registered as safe for weed control. According to Schmatz *et al.* (2009) the herbicides bentazon, fluazifop-p-butyl, pyridate and clopyralid can be safely used in peppermint (*Mentha × piperita*). In another study, oxyfluorfen applied at a rate of 0.20 kg ha⁻¹ prior to emergence increased Japanese mint (*Mentha arvensis* L.) herb yield (Kaur *et al.*, 2013), while Singh and Saini (2008) observed that diuron applied at a rate of 0.6 kg ha⁻¹ was safe for weed control in Japanese mint crop. To our knowledge, there are no registered herbicides for chemical control of broad-leaved or grass weeds in chia crop, in European Union. Therefore, the aim of this study was to evaluate the efficacy and selectivity of pre- and post-emergence herbicides in chia crop. The experiment were carried out at two locations with different soil types and climate conditions in order to assess different scenarios of growing conditions.

Materials and Methods

Experimental site

Chia crop was established at two locations in Central Greece: a) at the experimental field of the University of Thessaly in Velesino, Thessaly Region (22.756E, 39.396N; Site 1), where soil was sandy clay loam (38% sand, 36% silt, and 26% clay) with pH 7.4 (1:1 soil/H₂O), and b) at the province of Domokos in Central Greece (22.336E, 39.036N; Site 2), where soil was clay (50.7% clay, 23.3% silt, and 26% sand) with pH 7.6 (1:1 soil/H₂O). Meteorological data of both experimental sites are presented in Fig. 1. Seeds were sown on May 5th, 2014 and May 10th, 2014, at site 1 and 2, respectively. Seedbeds were prepared with moldboard

plough to a depth of 25-30 cm, followed twice by a rotary tiller to a depth of 15 cm. Seeds were sown by hand in rows 60 cm apart and a depth of 1 cm.

Experimental design

The experimental design was a randomized complete block design with three replicates per treatment for both sites. The experiments were set up over an area of 163 m² (21.75 × 7.5 m) at each location, with plots of 2 m wide and 3 m long, while the corridors between plots were 0.75 m wide. A range of herbicides available for use on Lamiaceae species was selected. The treatments were randomized within the blocks and were as follows: bentazon (1.2 kg a.i. ha⁻¹; Basagran 48 SL), fluazifop-p-butyl (0.25 kg a.i. ha⁻¹; Fusilade 12.5 EC), linuron (0.9 kg a.i. ha⁻¹; Afalon 47.5 WP), pendimethalin (1.137 kg a.i. ha⁻¹; Stomp Aqua 455 CS), oxyfluorfen (0.6 kg a.i. ha⁻¹; Goal 48 SC), and control treatment (no herbicides; weeds were controlled by hand hoeing at 60 days after sowing (DAS)).

Pendimethalin, oxyfluorfen, and linuron were applied pre-emergence. After the application of these herbicides and for incorporation purposes, experimental plots were irrigated using a sprinkler irrigation system. In addition, bentazon and fluazifop-p-butyl were applied when chia plants reached the 6-8 true leaf stage. Herbicides were applied with a field plot sprayer, with constant pressure at 250 kPa, using a total water volume of 300 L ha⁻¹. At both sites, after weed assessment (60 DAS), all the experimental plots were kept weed free by hand hoeing until final harvest. At 30 DAS, ammonium nitrate (34.5-0-0) was applied at a rate of 200 kg ha⁻¹, while irrigation was applied when needed.

Sampling, measurements and methods

To determine the effect of selected herbicides on plants growth, the density of the emerged plants was recorded at 21 days after sowing (DAS), while plant height and dry weight were determined throughout the growing period (at 36, 50, 64 and 82 DAS). Photosynthetic rate ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) and stomatal conductance ($\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$) measurements took place at 10:30 am to 2:30 pm under clear sky, at 36 and 50 DAS, on the 2nd pair of fully expanded leaves from the apex, using a LCi Leaf Chamber Analysis System (ADC, Bioscientific, Hoddesdon, UK), with three measurements per plot. For plant height and dry weight determination, 5 plants were randomly selected from each plot.

At both locations, chia plants flowered at the beginning of October; however, although plants produced seeds the crop was damaged by frost prior to seed maturity, therefore seed yield was not determined. Furthermore, at 60 DAS, the number and dry weight of the main weeds were assessed. For weed assessments, a wooden square quadrat (0.40 m × 0.40 m) was placed twice in each plot. The dry weight was determined after drying at 60 °C to a constant weight. Weed density and biomass were expressed as number m⁻² and g m⁻², respectively.

Statistical analysis

The data were subjected to analysis of variance and the means were compared using Fisher's Least Significant Difference (LSD) test ($P < 0.05$). These analyses are

Fig. 1. Meteorological data at experimental sites (Velesino, Domokos) in Greece, during the growing season (May to October 2014)

Table 1. Results of two-way analysis of variance (ANOVA) for effects of herbicide (H) and experimental site (S) on plant height (PH), dry weight (DW), photosynthetic rate (A) and stomatal conductance (gs) at 36, 50, 64 and 82 days after sowing

Source	df	PH 36	PH 50	PH 64	PH 82	DW 36	DW50
H	5	44.00***	51.30***	104.80***	34.14***	297.86***	197.59***
S	1	127.52***	15.46***	21.14***	14.20***	6.35*	2.75 ^{ns}
H × S	5	2.87*	1.29 ^{ns}	1.41 ^{ns}	0.22 ^{ns}	1.74 ^{ns}	1.76 ^{ns}
	df	DW64	DW82	A36	A50	gs36	gs50
H	5	1071.87***	602.26***	138.69***	71.06***	48.166***	60.88***
S	1	49.57***	1.43 ^{ns}	35.33***	2.11 ^{ns}	10.47**	11.79**
H × S	5	12.48***	1.70 ^{ns}	0.664 ^{ns}	9.52***	1.66 ^{ns}	2.63*

F-test values are shown. ns = not significant; *, ** and *** denotes significant differences at $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively.

presented in Table 1, while the data are presented in figures as means of three replicates. Pearson's correlation analysis was also performed to examine the relationships between chia traits. All statistical analyses were carried out with SigmaPlot software package, version 12 (Systat Software Inc., San Jose, CA).

Results

Weed flora

At site 1, the main weeds were *Convolvulus arvensis* L., *Portulaca oleracea* L., *Solanum nigrum* L. and *Sorghum halepense* (L.) Pers., while at site 2, the main weeds were *C. arvensis*, *P. oleracea*, *S. nigrum*, *Hibiscus trionum* L. and *Echinochloa crus-galli* (L.) P. Beauv. Regarding the total weed density and biomass yield, there were significant differences between herbicides treatments (Fig. 2). The lowest weed density and biomass were observed in oxyfluorfen plots, followed by pendimethalin application. Oxyfluorfen had the best performance for *P. oleracea* control, pendimethalin provided more than 85% control of *S. nigrum* and *E. crus-galli*, while fluzifop-p-butyl resulted in more than 65% and 90% control of *S. halepense* and *E. crus-galli*, respectively. Bentazon was also found to be the less effective herbicide against *S. nigrum* and *P. oleracea* (data not shown).

Chia growth and biomass yield

Regarding the chia seedlings emergence, there were significant differences between treatments ($F=4.284$,

$P < 0.05$). At both sites, chia seedlings emergence was suppressed by linuron, pendimethalin and oxyfluorfen. At site 1, pendimethalin, linuron and oxyfluorfen reduced the chia emergence by 56%, 41% and 81%, respectively, while at site 2, the application of pendimethalin, linuron and oxyfluorfen resulted in a reduction of chia emergence by 55%, 34% and 75%, respectively.

The pre-emergence herbicides had a negative effect on chia plants growth (Fig. 3), while oxyfluorfen caused substantial crop injury (i.e. leaf necrosis and limited root system). At the first measurement, the lowest height of plants (7 and 6 cm, at site 2 and site 1, respectively) was recorded for oxyfluorfen treatment, while the highest height was recorded for control plots (21 and 18 cm, at site 2 and site 1, respectively). At this growth stage, chia height was reduced by 67%, 42% and 48% in oxyfluorfen, linuron and pendimethalin plots, respectively.

The effect of pre-emergence herbicides on chia growth resulted in a significant reduction in biomass yield compared to control treatment, while significant differences were also observed between herbicide treatments. At 36 DAS, the lowest dry weight was recorded for oxyfluorfen treatment (16.63 kg ha^{-1} and 18.68 kg ha^{-1} at site 2 and site 1, respectively), while the highest chia biomass was recorded for control plots (Fig. 4). A positive and significant correlation was also observed between biomass and crop density ($r=0.924$, $P < 0.001$). The post-emergence herbicides (bentazon and fluzifop-p-butyl) did not affect chia growth. Slight necrosis of leaves was observed on some plants after spraying with bentazon. However, the symptoms were

transient and disappeared within 2-3 weeks. Moreover, no significant differences were observed between bentazon, fluazifop-p-butyl and control treatment concerning the height and dry weight of chia plants at 36 and 50 DAS (Figs. 3 and 4). At 64 DAS, bentazon reduced significantly dry weight in comparison to the fluazifop-p-butyl and control treatments at site 1. Moreover, at 82 DAS, there were no significant differences between bentazon, fluazifop-p-butyl and control concerning biomass yield of chia crop (Fig. 6).

Regarding the stomatal conductance and photosynthetic rate of chia plants, there were significant

differences between herbicide treatments. At 36 DAS, photosynthetic rate of chia plants was reduced by 59-62%, 42-48% and 43-45% in oxyfluorfen, linuron and pendimethalin plots, respectively. These results are in accordance with the above-mentioned dry weight reduction caused by oxyfluorfen, linuron and pendimethalin application. However, there were no statistically significant differences between bentazon, fluazifop-p-butyl and control treatments concerning the stomatal conductance and photosynthetic rate of chia plants (Fig. 5).

Fig. 2. Total weed density (no m⁻²) and biomass (g m⁻²) in chia crop as affected by the herbicide treatments at the experimental sites: Velestino (site 1) and Domokos (site 2). At each site, bars followed by different letters indicate significant differences according to Fisher's Least Significant Difference (LSD) test ($P < 0.05$)

Fig. 3. Effects of herbicide treatments on plant height (cm) at 36, 50, 64 and 82 days after sowing (DAS) at the experimental sites: Velestino (site 1) and Domokos (site 2). At each measurement, means followed by the same letter are not significantly different according to Fisher's Least Significant Difference (LSD) test ($P < 0.05$).

Fig. 4. Effects of herbicide treatments on dry weight (kg ha⁻¹) of chia crop at 36, 50 and 64 days after sowing (DAS) at the experimental sites: Velestino (site 1) and Domokos (site 2). At each measurement, means followed by the same letter are not significantly different according to Fisher's Least Significant Difference (LSD) test ($P < 0.05$)

Fig. 5. Effects of herbicide treatments on a) photosynthetic rate ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) and b) stomatal conductance ($\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$) of chia plants at 36 and 50 days after sowing (DAS) at the experimental sites: Velestino (site 1) and Domokos (site 2). At each measurement, means followed by the same letter are not significantly different according to Fisher's Least Significant Difference (LSD) test ($P < 0.05$)

Fig. 6. Effects of herbicide treatments on biomass yield (kg ha^{-1}) of chia crop, at 82 days after sowing (DAS) at the experimental sites: Velestino (site 1) and Domokos (site 2). Means followed by the same letter are not significantly different according to Fisher's Least Significant Difference (LSD) test ($P < 0.05$)

Discussion

The results of the present study indicated that maximum weed density reduction was observed from oxyfluorfen followed by pendimethalin treatment. Oxyfluorfen application resulted in efficient control of almost all broad-leaved weeds (i.e. *S. nigrum* and *P. oleracea*), while pendimethalin provided more than 85% control of *S. nigrum* and *E. crus-galli*. Similar levels of weed control efficacy for pendimethalin and oxyfluorfen have been previously reported by Karkanis *et al.* (2012). Bentazon was found to be the less effective herbicide against annual broad-leaved weeds. Villegas Rojas (2012) also found that the post-emergence application of bentazon provided poor weed control in chia crop.

The post-emergence herbicides bentazon and fluazifop-p-butyl did not affect significantly the growth of chia plants. Foliar injury was observed only in bentazon plots, which

was not reflected in biomass yield. In a recent study, Villegas Rojas (2012) have also reported that bentazon and quizalofop-p-ethyl caused foliar injury in chia crop (i.e. chlorosis and apical meristem necrosis). In addition, the pre-emergence herbicides had a negative effect on chia plants growth. At both sites, chia seedlings emergence was suppressed by linuron, pendimethalin and oxyfluorfen. Similarly, Villegas Rojas (2012) reported that the application of trifluralin, metolachlor, linuron and pendimethalin reduced chia seedlings emergence. However, the suppression of seedlings emergence by linuron and oxyfluorfen was greater at site 1 than that at site 2, indicating a significant effect of soil types on herbicide selectivity. However, the main reason for lower phytotoxicity of the above-mentioned herbicides at site 2 may be attributed to soil physical properties, since the soil at this site was heavier than that at site 1. According to Rahman *et al.* (2011) the level of phytotoxicity of soil-applied herbicides varies between different soils types. In particular, herbicide injury in field crops is more likely to occur on light textured soils with low clay content. In a recent study, Jones *et al.* (2013) reported that hybrid bermudagrass [*C. dactylon* (L.) pers. $\times$ *C. transvaalensis* Burtt-Davy] injury by indaziflam was greater in sandy soil than in silt loam soil. Therefore, the fact that the soil at site 2 contained more clay than site 1, may contributed to the reduction of linuron and oxyfluorfen phytotoxicity. In contrast, there was no significant difference on chia seedlings emergence in plots treated with pendimethalin at both fields, probably due to the capsule suspension formulation of pendimethalin that was being used. The controlled release of the active ingredient from microcapsules resulted in a similar effect on seedlings emergence at both sites.

Biomass yield was also decreased significantly by the pre-emergence application of herbicides. The lowest chia biomass was recorded for oxyfluorfen, while the greatest biomass was found in control plots. In a recent study, Serreau and Raimondo (2015) also observed that the pre-emergence application of flurochloridone, diflufenican and linuron caused slight crop injury which ranged from 10 to 15%, while sulfetrazone caused severe injury (up to 50%) to chia plants. According to James *et al.* (1991), linuron at 1.5

kg a.i ha⁻¹ and prometryn at 0.5 kg a.i ha⁻¹ applied pre-emergence resulted in a 7-43% damage to sage (*Salvia officinalis* L.), while linuron at 1 kg a.i ha⁻¹ and prometryn at 0.5 kg a.i ha⁻¹ resulted in a 7-17% damage of clary sage plants (*Salvia sclarea* L.). In contrast, Mitchell *et al.* (1995) reported that linuron at 0.75 kg a.i ha⁻¹ and pendimethalin at 1.5 kg a.i ha⁻¹ were well tolerated by transplanted plants of sage.

The lowest photosynthetic rate of chia plants was recorded for pre-emergence herbicides. At early growth stages, the limited root system of plants in oxyfluorfen and pendimethalin treated plots may contributed significantly to higher reduction of stomatal conductance and photosynthetic rate of these plants, comparing to the other treatments.

The main reason for the observed differences between the tested herbicides may be attributed to their different persistence in soil. The dissipation of linuron and pendimethalin in soil is faster than that of oxyfluorfen. According to Triantafyllidis *et al.* (2012), the half-life of pendimethalin in loam soil ranged between 26.7 and 27.5 days. Under different environmental and soil conditions, Tsiropoulos and Lolas (2004) observed that the half-life of the same herbicide ranged between 43 and 62 days. In addition, the dissipation rates also depend on soil properties. According to Swarczewicz *et al.* (2013), the half-life of linuron in sandy loam and clay loam soils was 37 days and 44 days, respectively. Furthermore, oxyfluorfen is more persistent in soil than pendimethalin, regardless of soil type. In particular, Alister *et al.* (2009) observed that the half-life of pendimethalin ranged between 10.5 and 31.5 days, while the half-life values of oxyfluorfen, in the same soil, ranged between 34.3 and 52.3 days.

Conclusions

Our results indicate that the application of pre-emergence herbicides pendimethalin, linuron and oxyfluorfen, at the tested rates, affects significantly plants' growth and seedlings emergence. Among the tested herbicides, oxyfluorfen caused the most severe damage in chia crop. In addition, oxyfluorfen was more efficient against almost all broad-leaved weeds, while pendimethalin provided more than 85% control of *Solanum nigrum* and *Echinochloa crus-galli*. However, further studies have to be conducted to evaluate the selectivity of herbicides in chia crop under different application rates, soil types and environmental conditions in order to make safe suggestions for chemical control of weeds.

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