

Research Article

Vegetable oil characteristics enhance the phytotoxicity of pinoxaden and haloxyfop-R-methyl on littleseed canarygrass *Phalaris minor* Retz.

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Abstract: It is possible to increase the efficacy of herbicides by adding vegetable oils to the spray tank. In order to evaluate and study this fact a greenhouse study was conducted. Three vegetable oils: coconut, sesame and almond oils at 0.3% (v/v) were applied to compare their influence on enhancing the efficacy of pinoxaden and haloxyfop-R-methyl on littleseed canarygrass *Phalaris minor* Retz. (Poaceae) control, in 2012. The experimental design was completely randomized design with six replications in a factorial arrangement for each herbicide. The factors for each herbicide included vegetable oils in three levels and herbicide dose at six levels (0, 6, 12.5, 25, 50 and 100 percent of recommended doses). The results of experiment revealed that by adding each vegetable oils haloxyfop-R-methyl ester weed suppressing influence was further increased compared to pinoxaden which was attributed to a positive relationship between oil receptivity and the high octanol–water partition coefficient of haloxyfop-R-methyl ester herbicide. Among the evaluated vegetable oils, coconut oil had the highest ability to enhance the efficacy of the two herbicides.

Keywords: adjuvant, gallant super, axial, weeds

Introduction

Herbicides are still used to supply adequate food for ever increasing population of the world, despite the potential damage of their applications on environment, human health and food safety (Kudsk, 2008). In developing countries like Iran, application of herbicides in agricultural lands are considered as an important factor for increasing yield and also one of the main components of weed management programs (Baghestani *et al.*, 2008). Reports of studies from Iran show that the average wheat yield of irrigated and rain fed fields increased from 3100 and 1000 kg ha⁻¹ to

3800 and 1200 kg ha⁻¹ during 1994–2004, respectively, which is mainly attributed to a raise in herbicide use from 2100 to 3700 t year⁻¹ (Baghestani *et al.*, 2008).

Public concerns about possible effects of herbicides on human health and the environment has led to increased pressure on farmers to optimize their usage and to look for approaches to use herbicides at recommended levels with less adverse side effects. Amongst various strategies to reduce pesticide dose below the recommended ones, application of adjuvants to the herbicide is contemplated as one of the most convenient and available methods that is straightforward for farmers to apply and reduces the risk of adverse side effects, appreciably (Zabkiewicz, 2000; Kudsk, 2008). Adjuvants are used worldwide because of their ability to increase the efficacy of foliage applied herbicides and are commonly

Handling Editor: Eshagh Keshtkar

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Received: 14 January 2020, Accepted: 19 August 2020

Published online: 01 September 2020

added to the sprayer tank (Holloway *et al.*, 2000). However, their precise mechanism of enhancing herbicide uptake is still unclear (Sharma and Singh, 2000). It seems that adjuvants improve spraying solution characteristics, modify amount of depositions in spraying solution and enhance the herbicide retention and penetration (Green, 2002). Furthermore, they decrease surface tension of spraying solution and prevent droplets from bouncing off after dealing with leaves which is regarded as a main feature of adjuvants. As a result, by adjuvants we can achieve better plant coverage (Green and Beestman, 2007). As an adjuvant vegetable oils can increase absorption of active ingredient by softening, solubilizing and disrupting nature of cuticular waxes. Apart from the regarded characteristics, It is also believed that vegetable oils can increase drying period of droplets via their flying time before they hit plant leaves and also improve wetting ability of target surface by spreading depositions on surface that is difficult-to-wet (mainly Poaceae) (Cabanne, 2000).

Not all Adjuvants are environmentally friendly as stated above. There are some synthetic ones that are very harmful to environment, plants, animals and underground water (Parr, 1982). For instance, alkyl phenol ethoxylates were replaced by other types of non-ionic adjuvants, due to their low biodegradability (White, 1993). The most widely applied example of alkyl amine ethoxylates in pesticide formulations are polyethoxylated tallow amines. Previous studies also indicated that polyethoxylated tallow amines are toxic to amphibians (Howe *et al.*, 2004; Relyea 2005; Relyea *et al.*, 2005).

In contrast to the above mention adjuvants, vegetable oils are ecotoxicologically compatible, environmentally friendly and easily degraded by microorganisms in the soil (Cornish *et al.*, 1993). It has also been reported that vegetable oils are often more effective than their paraffinic petroleum oils (Cabanne, 2000), and some vegetable oils increased effectiveness of herbicides such as imazamethabenz-methyl, sethoxydim, and sulfosulfuron on wild oat *Avena ludoviciana* Dur. (Izadi Darbandi *et al.*, 2013) or sulfosulfuron and sulfosulfuron plus

metsulfuronmethyl activity on wild barley *Hordeum spontaneum* Koch. (Izadi Darbandi and Aliverdi, 2015).

It is believed that various adjuvants interact differently with distinct herbicides (Stagnari *et al.*, 2006); therefore, it is difficult to select a suitable adjuvant for each herbicide without examination. In conformity with the above reasons, we carried out this research to achieve the following goals: (1) to determine the best vegetable oil that boosts activity of pinoxaden and haloxyfop-R-methyl ester to control littleseed canarygrass (2) to compare pinoxaden and haloxyfop-R-methyl ester herbicide efficacy with and without addition of vegetable oils to control littleseed canarygrass.

Materials and Methods

Plant material, site description and procedure

Experiments were conducted in 2012 at the Research Greenhouse of Agricultural Faculty of Ferdowsi University of Mashhad, Iran (longitude 36° 18' 24" N, latitude 59° 31' 38" E and elevation of 980 meters above sea level). Littleseed canarygrass seeds were collected from the field near Research Greenhouse. Seeds were treated for 6 min in 98% sulphuric acid (Derakhshan *et al.*, 2014) due to having hard shell and then were sown in potting trays filled with peat moss. After emergence, at one leaf stage, 10 seedlings were transplanted to 1 L-plastic pots (13cm top diameter, 10 cm diameter at the base and 11cm depth) in a mixture of clay-loam soil, sand and peat (1: 1: 1; v/v/v). Plants were grown in greenhouse conditions with temperatures at 24/18 °C day/night and 45 ± 7% relative humidity with a 12 h photoperiod. The pots were irrigated daily. After four weeks when plants were at the 3-4 true leaf stage, plants with uniform height were selected and thinned to 4 plants per pot.

Experimental design

Phytotoxicity of vegetable oils alone

A preliminary phytotoxicity experiment was designed to ensure that littleseed canarygrass are not harmed by tested vegetable oils and that vegetable oils act only as additives. In this study, coconut (Healthy Origins[®], 100%

Liquid Coconut Oil, Pittsburgh, PA15241, USA), sesame *Sesamum indicum* and bitter almond *Prunus amygdalus* var. *amara* oil (extracted from grains via the mechanical extraction method from Iran) at 0.3% (v/v) were applied in a completely randomized design without herbicide on littleseed canarygrass with 6 replications.

Phytotoxicity of herbicide with and without vegetable oils

For each herbicide, the experiment was arranged in a completely randomized design with a factorial arrangement of treatments and 6 replications. Pinoxaden at 0, 2.81, 5.62, 11.25, 22.5 and 45 g. a.i. ha⁻¹ (Axial[®], 10% EC, syngenta) and haloxyfop-R-methyl ester at 0, 6.75, 13.5, 27, 54 and 108 g. a.i. ha⁻¹ (Gallant super[®], 10.8% EC, Dow Agrosience) were used in dose-response experiments. Each of the above concentrations of the two herbicides was applied alone and with each one of coconut, sesame and almond oils at 0.3% (v/v).

Emulsifiable vegetable oils were prepared by dissolving alkyl aryl polyglycol ether (C₈H₁₆C₆H₄ (C₂H₄O)₁₀ H) (a nonionic emulsifier) (Merck, Germany) in each vegetable oil (95% vegetable oil + 5% emulsifier).

Weeds were sprayed at three to four-true leaf stages using greenhouse sprayer delivering 290 l ha⁻¹ at 400 k Pa utilizing 8002 flat-fan nozzle tip; boom height was 50 cm. All above ground living plant organs were cut 4 weeks after treatment from the soil surface and oven dried at 70 °C for 48 h and then weighed.

Statistical analysis

All experiments were repeated but because of similar results which were detected in the two trials, the results were combined into one analysis (Data not shown). The dose-response curves were fitted with Gompertz model (Eq. 1) for haloxyfop-R-methyl ester, and log- logistic four-parameter model (Eq. 2) for pinoxaden with the drc add-on package to the R software (Ritz and Streibig, 2005).

$$f(x(b, c, d, e)) = c + (d - c)e^{-e^{b(\log(x) - e)}} \quad \text{Eq. 1}$$

$$f(x(b, c, d, e)) = \frac{d - c}{1 + e^{b(\log(x) - \log(e))}} + c \quad \text{Eq. 2}$$

In these formulas, the parameter e in logistic model denotes ED_{50} and in Gompertz model denotes the logarithm of the ED_{50} point. The parameter b denotes the relative slope around e . The parameters c and d are the lower and upper limits; b is the relative slope around e . The Gompertz model is not symmetric around any point and the logistic function is symmetric around e (Ritz and Streibig, 2005). The ED_{50} -parameter can be replaced by any ED level, e. g. ED_{90} , which denotes the required dose of herbicide to give 90% weed control.

Relative potency (R) is measured according to Eq. 3 (Ritz and Streibig, 2005).

$$R = \frac{Z_b}{Z_a} \quad \text{Eq. 3}$$

Here, Z_b is the ED_{50} of commercial herbicides formulation alone and Z_a is the ED_{50} of commercial herbicides formulation in combination with sesame, almond or coconut oil. If R is equal to 1, the addition of vegetable oils has had no effect on herbicides performance. But if R were smaller or bigger than 1, it implies that herbicides performance in combination with oils would be less or more than when the herbicides alone were used (Ritz and Streibig, 2005). Mean comparison test and analysis of variance were calculated using Minitab 16.1 software according to LSD at 0.05 probability level.

Results

Phytotoxicity experiment of vegetable oils as a single treatment or alone

The following preliminary results were noticed when plants were treated with vegetable oils alone. Little seed canary grass growth was not

affected when oils alone were applied and with no phytotoxic effects. Hence, dry weight of littleseed canarygrass, did not decrease significantly in comparison with the control ($F = 1.23$; $df_e = 20$; $p = 0.124$) (Fig. 1).

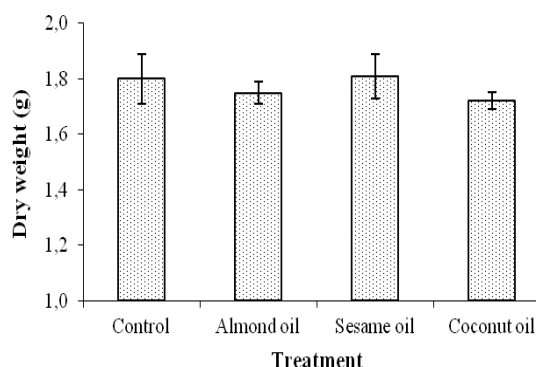


Figure 1 Effect of different vegetable oils at 0.3% (v/v) alone on dry weight of littleseed canarygrass 4 weeks after treatment. Treatments are not significantly different ($F = 1.23$; $df_e = 20$; $p = 0.124$). Error bars represent Standard Error (SE).

Phytotoxicity of herbicides with and without vegetable oils

The estimated ED_{50} and ED_{90} parameters of little seed canary grass on dry weight in the presence of emulsifier were slightly lower than herbicides alone (Fig. 2 and Table 1). Application of emulsifier alone (without adding vegetable oils) did not have any effect on the herbicides and littleseed canarygrass dry weight.

Results of these experiments showed that vegetable oils remarkably increased foliar activity of pinoxaden and haloxyfop-R-methyl ester herbicides in the presence of the vegetable oils. The determined ED_{50} for pinoxaden and haloxyfop-R-methyl ester alone were 22.13 and 44.42 g a.i. ha^{-1} , respectively; however, when they were mixed with vegetable oils, their ED_{50} decreased and the amounts varied from 7.24 to 16.41 g a.i. ha^{-1} for pinoxaden and from 9.02 to 14.84 g a.i. ha^{-1} for haloxyfop-R-methyl ester. Also the ED_{90} values showed a similar trend. So that, reduction in ED_{90} occurred from 42.89 g a.i. ha^{-1} for pinoxaden alone to a range from 29.07 to 40.93 g a.i. ha^{-1} and from 78.53 g a.i. ha^{-1} for

haloxyfop-R-methyl ester to a range from 15.52 to 33.63 g a.i. ha^{-1} when vegetable oils were added to spray solution. Therefore, Based on ED_{50} and ED_{90} values given in Table 1, all of the vegetable oils remarkably, enhanced the efficacy of two herbicides in comparison with herbicides alone except for sesame and almond oil with pinoxaden. The non-linear regression analysis of the dose-response also demonstrated the following results (Fig. 2).

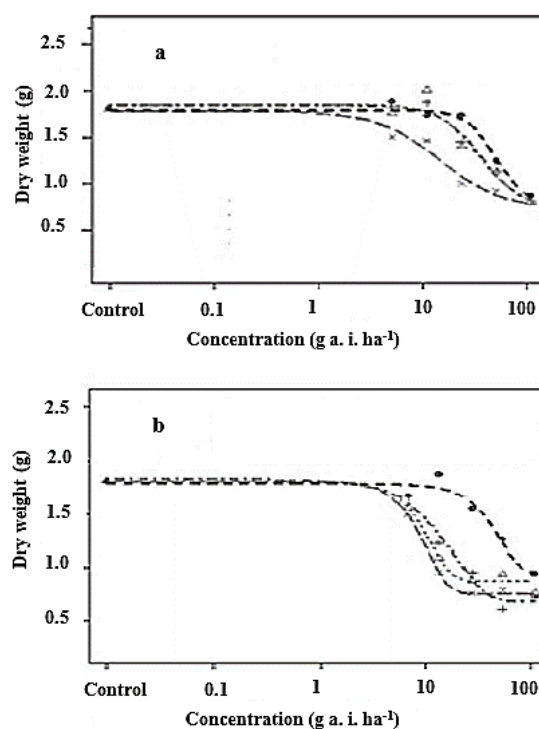


Figure 2 Dose-response curves of the effect of (a) pinoxaden: Pin (○), Pin + Se (Δ), Pin + Al (+), Pin + Co (×), and (b) haloxyfop-R-methyl ester: Hal (○), Hal + Se (Δ), Hal + Al (+), Hal + Co (×) herbicides on dry weight of littleseed canarygrass 4 weeks after treatment with and without different vegetable oils. The points are observed data and lines resulted from fitting Gompertz (Eq. 1) and log-logistic four parameter (Eq. 2) models. Pin: Pinoxaden, Co: Coconut oil, Se: Sesame oil, Al: Almond oil, and Hal: Haloxyfop-R-methyl ester.

In the first experiment that was conducted with pinoxaden, the acquired relative potency values were higher than 1 that implies the efficacy of herbicide for 50 percent reduction in littleseed canarygrass dry weight, the

potency value increased by addition of coconut, sesame and almond oils 3.05, 1.35 and 1.34 -fold, respectively in comparison with herbicide alone. Among these vegetable oils, only coconut oil significantly increased efficacy of pinoxaden. Also, results of second experiment indicated that the performance of haloxyfop-R-methyl ester with coconut,

sesame and almond oils increased to 4.92, 4.61 and 2.99 -fold respectively compared to haloxyfop-R-methyl ester alone (Fig. 3). Therefore, according to relative potency in both experiments, vegetable oils can be ranked in the following order based on the enhancement of herbicide efficacy: coconut > sesame > almond oil (Fig. 3).

Table 1 Estimated values of ED_{50} and ED_{90} of the effect of pinoxaden and haloxyfop-R-methyl ester herbicides, with and without vegetable oils on dry weight of littleseed canarygrass resulted from fitting Gompertz (Eq.1) and log-logistic four parameter (Eq.2) models.

Treatment	$ED_{50} \pm SE$ (g a.i. ha ⁻¹)	$ED_{90} \pm SE$ (g a.i. ha ⁻¹)
Pinoxaden alone	22.13 ± 3.02 ^{a2}	42.89 ± 4.74 ^a
Pinoxaden + coconut oil ¹	7.24 ± 1.98 ^b	29.07 ± 5.56 ^b
Pinoxaden + sesame oil	16.28 ± 3.52 ^a	40.93 ± 2.09 ^a
Pinoxaden + almond oil	16.41 ± 4.29 ^a	39.53 ± 3.82 ^a
Haloxyfop-R-methyl ester alone	44.42 ± 1.52 ^a	78.53 ± 4.80 ^a
Haloxyfop-R-methylester + coconut oil	9.02 ± 1.34 ^c	15.52 ± 3.11 ^c
Haloxyfop-R-methyl ester + sesame oil	9.62 ± 1.79 ^c	16.44 ± 5.07 ^c
Haloxyfop-R-methyl ester + almond oil	14.84 ± 2.70 ^b	33.63 ± 8.01 ^b

1. The vegetable oils were added at 0.5% (v/v).

2. Indices with the same letter are not significantly different according to confidence intervals at 95% probability ($X \pm t_{\alpha/2} \times SE$).

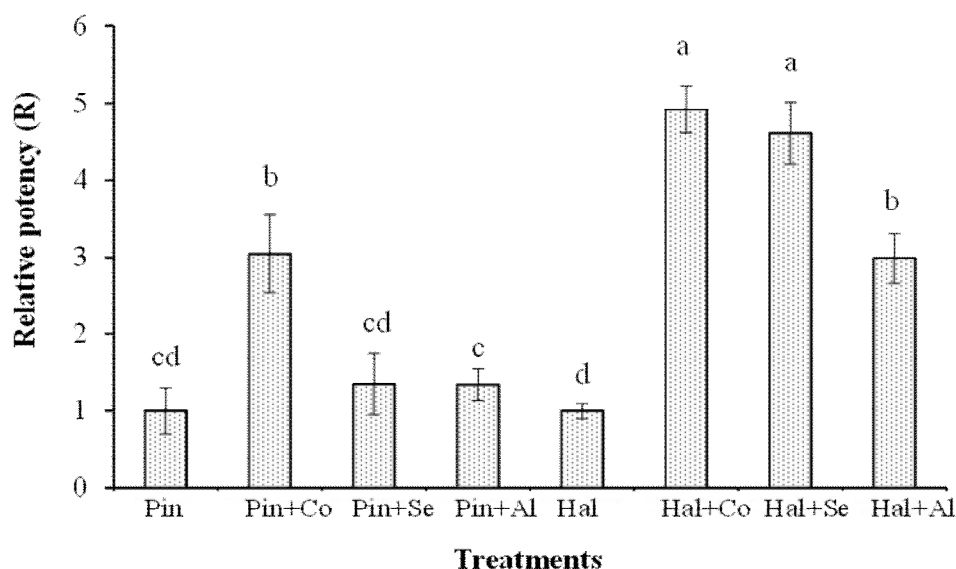


Figure 3 Effect of almond, sesame and coconut oils at 0.3% v/v on relative potency (R) of pinoxaden and haloxyfop-R-methyl ester on littleseed canarygrass. Error bars represent Standard Error (SE). Values with the same letter are not significantly different according to confidence intervals at 95% probability ($X \pm t_{\alpha/2} \times SE$). Pin: Pinoxaden, Co: Coconut oil, Se: Sesame oil, Al: Almond oil, and Hal: Haloxyfop-R-methyl ester.

By using of Eq. 3, the relative potency values of studied herbicides in the presence of each vegetable oil were measured and compared according to standard error of the means (Fig. 3). Coconut oil was the most efficient on herbicide performance in littleseed canarygrass, thereby causing greatest dry weight reduction in comparison with other vegetable oils, particularly with almond oil.

Discussion

The foliar activity of two herbicides increased due to addition of vegetable oils which is consistent with previous studies (Cabanne, 2000; Stagnari *et al.*, 2006; Aliverdi *et al.*, 2009; Rashed-Mohassel *et al.*, 2009; Izadi Darbandi *et al.*, 2013; Izadi Darbandi and Aliverdi, 2015). There are several factors related to these findings as Nalewaja *et al.* (1996) stated that performance of herbicides, physiochemical characteristics of herbicides and adjuvants are affected when they are applied together so that lipophilic adjuvants work best when they are mixed with water-insoluble herbicides ($\log K_{ow} > 1$) (K_{ow} represents octanol-water partition coefficient), while the water-soluble herbicides ($\log K_{ow} < 1$) work best when they are mixed with hydrophilic adjuvants. Therefore, vegetable oils with lipophilic properties, increased the performance of these herbicides as their $\log K_{ow}$ is higher than 1. According to previous studies, lipophilic adjuvants enhanced foliar activity of quazalofop-P ester ($\log K_{ow} = 4.7$) (Nalewaja and Matysiak, 1993; Nalewaja *et al.*, 1996). Also, Hazen (2000) stated vegetable oils increased absorption and movement of the active ingredients into more hydrophilic structures, because of softening and disrupting of cuticular waxes which are the main barrier against penetration of herbicides.

There are other probable reasons related to adjuvants that modify spraying process. There are impressive factors in the fate of the spraying droplets, such as droplet size, impact velocity, leaf surface micro-morphology and the dynamic surface tension of the spray solution (Sharma

and Singh, 2000). In high surface tension, the spray droplets will bounce off and improper leaf coverage might occur (Hazen, 2000). Effect of vegetable oils on reduction of surface tension of spray solution was demonstrated in previous studies (Sharma and Singh, 2000; Shu *et al.*, 2008; Rashed-Mohassel *et al.*, 2011). Moreover, the enhanced efficacy of tested herbicides may be attributed to surface tension reduction of the spray solution that leads to spread of the spray droplets and wetness of leaves (Bunting *et al.*, 2004) and ultimately increases herbicide absorption and penetration through the plant cuticle (Monaco *et al.*, 2002).

Other research indicated that vegetable oils enhanced the performance of clodinafop-propargyl and diclofop-methyl fenoxaprop-p-ethyl (Stagnari *et al.*, 2006), glyphosate (Gauvrit *et al.*, 2007), diclofop-methyl, cycloxydim, and clodinafop-propargyl herbicides (Rashed-Mohassel *et al.*, 2010), imazamethabenz-methyl, sethoxydim, and sulfosulfuron (Izadi Darbandi *et al.*, 2013) and sulfosulfuron and sulfosulfuron plus metsulfuronmethyl (Izadi Darbandi and Aliverdi, 2015).

In addition to the mentioned data above, the other reason for efficacy of coconut oil is the high values of short-chain fatty acids in coconut oil in comparison with almond and sesame oils (Soler *et al.*, 1988; Were *et al.*, 2006), for example, lack of oleate (C 18:1) and abundance of short fatty acid and palmitate (C 16:0) (Soler *et al.*, 1988; Were *et al.*, 2006; Chowdhury *et al.*, 2007) in coconut oil with respect to other vegetable oils. Pline *et al.* (1999) declared short-chain fatty acids such as pelargonic acid (C 9:0) created rapid damage to the plant. It also stated that there is a positive relationship between the surface tension with the length of fatty acid hydrocarbon chain (Allen *et al.*, 1999; Shu *et al.*, 2008). It means that the surface tension is reduced with decrease in hydrocarbon chain length of fatty acids (Freitas *et al.*, 2011). Therefore, according to mentioned reasons, because of having short fatty acids such as caprylic 8:0, capric 10:0 and lauric 12:0, coconut oil (Chowdhury *et al.*, 2007) has

probably reduced the surface tension of spray solution more than the other oils and thereby produced smaller droplets than other oils. This process has reduced the level of energy in smaller droplets, their contact angle and increased the retention of droplets on the leaf surface (Rashed-Mohassel *et al.*, 2009). Finally, improvement in retention of droplets increases the amount of absorption into plant leaves and translocation of contents in plant; that implies the more herbicide absorption and translocation, the greater control achievement (Jinxia, 1996).

In addition, vegetable oils that have more saturated / unsaturated fatty acids ratio (Soler *et al.*, 1988; Were *et al.*, 2006; Chowdhury *et al.*, 2007) were more effective in increasing the performance of herbicides. For instance, coconut oil with highest amount of this ratio (11.8) (Chowdhury *et al.*, 2007) had the most impact on performance of herbicides.

Adding vegetable oils with lower oleate and palmitate content, such as coconut and sesame oils (Were *et al.*, 2006; Chowdhury *et al.*, 2007) enhanced the performance of herbicides more than almond oil. For instance coconut oil with the least amount (6.2%) of oleate (Chowdhury *et al.*, 2007), led to best control with pinoxaden and haloxyfop-R-methyl ester against littleseed canarygrass.

The averaged results of three vegetable oils indicated that haloxyfop-R-methyl ester efficacy was increased more than pinoxaden by vegetable oils for littleseed canarygrass control (Fig. 2). These results are presumably related to the different physiochemical characteristics of two herbicides such as $\log K_{ow}$. It seems that haloxyfop-R-methyl ester with $\log K_{ow} = 4$ in comparison with pinoxaden with $\log K_{ow} = 3.2$, worked better when vegetable oils applied. This finding agrees with other researchers (Nalewaja *et al.*, 1996; Izadi-Darbandi *et al.*, 2013; Izadi Darbandi and Aliverdi, 2015).

Some synthetic adjuvants such as mineral oils cause adverse effects to the environment. In contrast, natural oils like vegetable ones are compatible with the environment. Based on above reason and our findings, it seems that vegetable oils are regarded as a more proper

option than synthetic adjuvants. It is also concluded that: 1) all tested vegetable oils at 0.3% v/v (coconut, sesame and almond) potentially increased efficiency of both herbicides. 2) The efficacy of both herbicides was enhanced when they were mixed with vegetable oils and which ranked from the highest to the lowest as: coconut, sesame and almond oil, respectively. 3) The mixture of haloxyfop-R-methyl ester with vegetable oils was more effective than pinoxaden in control of littleseed canarygrass.

Based on the results of this study, it seems that vegetable oils, especially coconut oil, can be used at 0.3% v/v to increase the efficacy of haloxyfop-R-methyl ester herbicide in controlling littleseed canarygrass. Accordingly, while significantly increasing the efficacy of herbicide, the risk of environmental damage caused by synthetic adjuvants, will also be eliminated.

Acknowledgments

This study was carried out as a research program (Project No. 2/23239) at Ferdowsi University of Mashhad. The authors wish to thank Seyyed Hamid Moghaddam for advice on language revision.

Disclosure statement

The authors declare no conflicts of interest.

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ویژگی‌های روغن‌های گیاهی سمیت گیاهی پینوکسادن و هالوکسی فوپ آر متیل بر علف قناری دانه ریز *Phalaris minor* Retz. را افزایش می‌دهد

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دریافت: ۲۴ دی ۱۳۹۸؛ پذیرش: ۲۶ مرداد ۱۳۹۹

چکیده: افزایش کارایی علف‌کش‌ها از طریق افزودن روغن‌های گیاهی به مخزن سمپاش امکان‌پذیر است. به‌منظور ارزیابی و مطالعه این موضوع، یک پژوهش گلخانه‌ای انجام شد. سه روغن گیاهی (نارگیل، کنجد و بادام تلخ) به‌میزان ۰/۳ درصد حجم به حجم مورد استفاده قرار گرفت تا تأثیر آنها بر افزایش کارایی پینوکسادن و هالوکسی فوپ آر متیل در سال ۱۳۹۳ روی کنترل علف قناری دانه ریز مورد ارزیابی قرار گیرد. طرح آزمایشی کاملاً تصادفی در شش تکرار و به‌صورت فاکتوریل برای هر علف‌کش بود. فاکتورها شامل روغن گیاهی در سه سطح و دز علف‌کش در شش سطح (۰، ۶، ۱۲/۵، ۲۵، ۵۰ و ۱۰۰ درصد دز توصیه شده) علف‌کش‌های هالوکسی فوپ آر متیل و پینوکسادن بود. نتایج آزمایش نشان داد که با افزودن هر یک از روغن‌های گیاهی، کارایی علف‌کش هالوکسی فوپ آر متیل استر بر روی علف هرز در مقایسه با پینوکسادن بیش‌تر تحت تأثیر قرار گرفت. که دلیل احتمالی آن رابطه بین قدرت جذب روغن و بالا بودن ضریب K_{OC} علف‌کش هالوکسی فوپ آر متیل بود. در بین روغن‌های گیاهی مورد مطالعه، روغن نارگیل بیش‌ترین توانایی را در افزایش کارایی دو علف‌کش داشت.

واژگان کلیدی: آکسیال، سوپر گالانت، علف هرز، ماده افزودنی