

ORIGINAL ARTICLE

Efficacy of glyphosate and fluazifop-P-butyl herbicides with adjuvants at different levels of cutting for the common reed (*Phragmites australis*)

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Vol. 58, No. 3: 282–288, 2018

DOI: 10.24425/jppr.2018.124636

Received: May 5, 2018

Accepted: September 7, 2018

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Abstract

Field experiments were conducted to evaluate the efficacy of glyphosate (H₁) and fluazifop-P-butyl (H₂) herbicides with adjuvants on the common reed without cutting and at two different cutting levels (10 and 30 cm). The adjuvants were urea, nitric acid and sulfonic acid. The relative importance value (RIV), leaf chlorophyll content and plant density were determined to assay the efficacy of herbicides. Glyphosate treatment only (H_{1a}) was more effective than fluazifop-P-butyl (H_{2a}) on reeds without cutting and at the 10 cm cutting level. However, no significant difference was observed between them at the 30 cm cutting level. A positive effect of plant cutting occurred on the efficacy of all herbicides applied alone or in a tank mix with adjuvants. Furthermore, the 10 cm cutting level was more effective in eradication of reeds than the 30 cm cutting level. The adjuvants significantly improved the efficacy of the recommended (Hb) and half recommended (Hc) herbicide rates in comparison to being used alone on uncut reeds. The reduction percentages were 94.5, 86.99, 76.61 and 69.94 for H_{1b}, H_{1c}, H_{2b} and H_{2c} treatments, respectively. However, the adjuvants did not improve the glyphosate effect at different levels of cutting. Conversely the reduction percentage of reeds was improved by the recommended rate of fluazifop-P-butyl with adjuvants (H_{2b}) to 92.77% and 84.62% at 10 and 30 cm cutting levels, respectively.

Keywords: adjuvants, fluazifop-P-butyl, glyphosate, herbicide, *Phragmites*

Introduction

The common reed (*Phragmites australis* (Cav.) Trin. ex Steud.) is a perennial rhizomatous grass. Nowadays, reed is recorded as an invasive species along brackish water marshes, canals, road sides, railways and waste lands in the Nile region, along the Mediterranean coast, near Sinai, and in oases especially in New Valley, Egypt (El-Sheikh 1996; Abd-El-ghani and Fawzy 2006; Derr 2008). Common reed is a flowering grass that spreads by vegetative reproduction and seeds (Marks *et al.* 1994; Chambers *et al.* 1999; Palmer *et al.* 2014). It can grow up to 4 cm a day and can reach heights of 2 to 5 m (John 2003; Derr 2008; Bonanno and Giudice 2010). Reeds can displace native vegetation rapidly

in one growing season (Marks *et al.* 1994; Chambers *et al.* 1999; Back and Holomuzki 2008). Monocultures consume the greatest amount of water, which can be as much as 2,000 l · m⁻¹ of standing reed, to supply its incredible rate of growth (Perdue 1958). Since it produces a significant amount of biomass it is quite flammable and can be a fire hazard (Perdue 1958; Sharma *et al.* 1998; Spencer *et al.* 2006). As a result, reed has been described as one of the world's '100 worst weed invaders' (ISSG 2011) and it is considered the most dangerous weed in Egypt (Ashour 1990).

Several control methods for common reed control have been suggested, including mowing, burning,

grazing, cutting and herbicide application (Moreira *et al.* 1999; Knezevic *et al.* 2013). A combination of cutting and herbicide application has been considered to be a successful control measure for common reed (Sale and Wetzel 1983; Buttler 1992; Kay 1995; Monteiro *et al.* 1999). Chemical control is the most common method and glyphosate herbicide is one of the most effective for killing reed (Spencer *et al.* 2008; Knezevic *et al.* 2013).

In addition to the expense many environmental problems, a risk of toxicity to non-target plants, and herbicide resistance by the weed can be caused by chemical control (Singh 1997; Adkins *et al.* 2006). Bhan *et al.* (1997) reported, that chemical control alone is not justifiable since the effect of herbicides will always be of a temporary nature and repeated operations will be required. Hence, the effectiveness of herbicide applications can be increased with the addition of adjuvants to spray tanks (Harker 1992; Bunting *et al.* 2004). This improvement primarily occurs when these preparations are used at reduced rates and under disadvantageous environmental conditions (Praczyk and Adamczewski 1996).

There have been previous studies which present specific details of the common reed's response to different herbicides, in particular, glyphosate and fluzifop-P-butyl. Additionally, there is insufficient data about the effect of fluzifop-P-butyl as a selective herbicide in the control of common reeds in Egypt. Therefore, new, suitable treatment and combined methods are required. Accordingly, the present study was aimed at comparing the efficacy of these herbicides with different modes of action and selectivity. Furthermore, this study may help reduce the amount of herbicides or the number of treatments required. The effect of adjuvants and cutting levels of plants on common reed control methods were investigated.

Materials and Methods

Study area

The experiments were conducted at the experimental farm of the Faculty of Agriculture, New Valley branch, Assiut University that is located 10 km off the New Valley government road to Assiut (25°31'26"N, 30°36'33"E, altitude 283 m). Soil at the site was virgin sand, characterized by a high salt content ranged from 3 to 40 dS · m⁻¹ that was suitable for widespread growth of common reed (*P. australis*) and was found in large areas.

Herbicides and adjuvants

Glyphosate (Rowand up 48% WSC, Monsanto[®]) and fluzifop-P-butyl (Fusilade Max 12.5% EC, Syngenta[®]) herbicides, nitric acid (62%), sulfonic acid (83%) and urea (46%) additives were purchased from reputed chemical suppliers in Egypt. Treatments, which consisted of glyphosate and fluzifop-P-butyl with adjuvants, are given in Table 1. Urea nitrate and urea sulfonyl were produced by mixing urea with nitric acid and sulfonic acid, respectively.

Field experiments

The trials were conducted from September 2016 to December 2017. The first approach consisted of foliar applications of glyphosate and fluzifop-P-butyl herbicides with adjuvants without cutting off stems during the seed filling stage. Plots, 3 × 2 m, were arranged end-to-end in a randomized complete block design (RCBD) with three replications. The second approach combined cutting of stems and foliar applications. Plots, 3 × 2 m were arranged end-to-end in a split-block design with

Table 1. Summary of herbicide and adjuvants treatments

Treatment	Spray volume [600 l · ha ⁻¹]				
	herbicide [kg a.i. · ha ⁻¹]		adjuvants [l or kg · ha ⁻¹]		
	glyphosate	fluzifop-P-butyl	nitric acid [l]	sulfonic acid [l]	urea [kg]
Control	–	–	–	–	–
H ₁ a	2.96*	–	–	–	–
H ₁ b	2.96*	–	1.5	0.772	3.86
H ₁ c	1.48**	–	1.5	0.772	3.86
H ₂ a	–	0.463*	–	–	–
H ₂ b	–	0.463*	1.5	0.772	3.86
H ₂ c	–	0.232**	1.5	0.772	–

(–) – not added

*recommended rate, **half of the recommended rate

three replications. Herbicide applications were the main factor and cutting levels were the subplot factor. *Phragmites* were cut in autumn during the flowering stage. The stems were cut with a machete at two levels, 10 and 30 cm, then the cut material was removed by hand. Foliar herbicide was applied 4–5 weeks after cutting when resprouted shoots were approximately 1 m high. Autumn treatments of herbicides were applied on 27 November 2016. The air temperature was 25°C ($\pm 2^\circ\text{C}$) in autumn and air humidity 40%. The herbicide was sprayed using a backpack sprayer at $600 \text{ l} \cdot \text{ha}^{-1}$. Six different treatments were applied individually under appropriate weather conditions (low wind and no precipitation). Control treatments were applied with water only.

Efficacy of herbicides

Responses of the reeds to each treatment were evaluated after one month (on 27th December 2016) and after one year (on 27th November 2017) of treatments. Responses were determined using many parameters: the relative importance value (RIV), percentage of density reduction and chlorophyll content.

RIV was determined to assess the efficacy of herbicide treatments by the following equations according to Mozdzer *et al.* (2008):

$$\text{RIV} = (d/D \times 100) + (h/H \times 100) + (c/C \times 100),$$

where: d – the stem density within an individual quadrat, D – maximum stem density of all quadrats, h – mean height within an individual quadrat, H – maximum height among all quadrats, c – percent cover reeds within an individual quadrat, and C – maximum percent cover reeds among all quadrats.

Density was measured using fixed $0.5 \times 0.5 \text{ m}^2$ squares in each plot. Shoots falling within the frames of the quadrat were counted. Density was determined by the number of living stems in 1 m^2 present in quadrats randomly placed within the common reed clumps (Ramalingam *et al.* 2013). Percentage cover was rated visually by two independent observers based on a scale of 0–100 (0 – no living weeds present and 100 – no reduction in weed biomass) (Monteiro *et al.* 1999).

Reduction of common reed density was determined using the following formula of Mulla *et al.* (1971):

$$\% \text{ Reduction} = 100 - (C_1/T_1 \times T_2/C_2) \times 100,$$

where: C_1 and C_2 – the means of stem numbers $\cdot \text{m}^{-2}$ in pre-treatment and post-treatment in the control area, respectively; T_1 and T_2 – the means of stem numbers $\cdot \text{m}^{-2}$ in pre-treatment and post-treatment in treatment areas, respectively.

Chlorophyll a , b content was determined according to Krishnan *et al.* (1996). Leaf samples (100 mg) were

placed in a graduated tube containing 25 ml of 80% acetone. The chlorophyll was extracted without grinding or centrifugation by incubating the leaf tissues into the solvent in a dark place at incubation temperatures of 4°C ($\pm 2^\circ\text{C}$). The contents of the tubes were shaken occasionally to accelerate pigment extraction. After 48 h of incubation the extract liquid was filtered through glass wool to remove leaf pieces and transferred to another graduated tube. The liquid extract was made up to a total volume of 25 ml with 80% acetone. The chlorophyll content was spectrophotometrically analyzed in a UV visible spectrophotometer (PG Instruments T80 UV/VIS Spectrometer – United Kingdom) using 3 ml sealed quartz-glass cuvettes with a path length of 1 cm. The chlorophyll content was calculated as $\text{mg} \cdot \text{g}^{-1}$ by the following equations cited in Dere *et al.* (1998):

$$\text{Chlorophyll } a = 11.75 A_{662} - 2.350 A_{645},$$

$$\text{Chlorophyll } b = 18.61 A_{645} - 3.960 A_{662}.$$

Data analysis

A randomized complete block design for cuttings and treatments was used with three replications. Analysis of variance (ANOVA) was carried out using Proc Mixed of SAS package version 9.2 (SAS 2008) and means were compared by Duncan comparison at 5% level of significance (Steel and Torrie 1981).

Results and Discussion

Common reed plants used in these experiments were typical of common reed (*P. australis*) throughout Egypt. Prior to these experiments, plants at these sites averaged 69.7 stems $\cdot \text{m}^{-2}$ (range: 32 to 148 stems $\cdot \text{m}^{-2}$). Plant widths averaged 6.2 mm (range: 3.0 to 9.4 mm) at the base of the clump and mean stem height 1.6 m (range: 0.91 to 2.38 m).

Experiment 1: Effect of the treatments on the common reed control without cut stems

The consequences of common reed control by glyphosate and fluazifop-P-butyl herbicide treatments after 1 month are presented in Table 2. The analysis of variance indicated that the recommended rate of glyphosate herbicide (H_1a) was significantly higher than fluazifop-P-butyl treatment (H_2a) in reducing the reeds' RIV. Conversely, fluazifop-P-butyl treatment (H_2a) reduced significantly leaf chlorophyll content. Furthermore, the

Table 2. Efficacy of herbicide, alone and combine with adjuvants* on common reed without cutting after one month of application

Treatments	Spray components	RIV ± SD	Chlorophyll (a, b) ± SD
Control	water	255 a ± 0.85	1.06 a ± 0.04
H ₁ a	glyphosate only	195 d ± 0.85	0.62 b ± 0.33
H ₁ b	glyphosate + adjuvants	225 c ± 5.67	0.31 dc ± 0.01
H ₁ c	glyphosate (half rate) + adjuvants	222 c ± 3.17	0.46 bc ± 0.03
H ₂ a	fluzifop-P-butyl only	238 b ± 2.97	0.26 dc ± 0.02
H ₂ b	fluzifop-P-butyl + adjuvants	245 ab ± 2.95	0.26 dc ± 0.02
H ₂ c	fluzifop-P-butyl (half rate) + adjuvants	133 e ± 5.58	0.19 d ± 0.00

*urea, nitric acid and sulfonic acid,
RIV – relative importance value, SD – standard deviation
Means with the same letter are not significantly different at p < 0.05

plants that were treated with fluzifop-P-butyl had the lowest values of leaf chlorophyll content. These results and those of Tu *et al.* (2001) indicated that the effect of fluzifop-P-butyl herbicide was faster than glyphosate herbicide on the shoot system at the beginning of the experiment. Nitric acid, sulfonic acid and urea were added according to Table 1 with recommended (Hb) and half recommended (Hc) rates of both glyphosate (H₁b and H₁c treatments) and fluzifop-P-butyl (H₂b and H₂c treatments). These adjuvants did not improve the effect of glyphosate treatments and there were no significant differences between them in reducing the RIV and chlorophyll content. The half recommended rate of fluzifop-P-butyl with adjuvants (H₂c treatment) showed a significantly higher efficacy in reducing the RIV and chlorophyll content that were 133 and 0.19 mg · g⁻¹, respectively. It is difficult to assess death in giant reeds due to the rhizomes underground that may still be alive (Silva *et al.* 2011). Therefore, the percentage of reduction in the reed density (no. of stems · m⁻²) after 1 year of the application was studied. It was found that all herbicides alone and herbicides with adjuvants provided significantly greater reed management than the control. Also, the plants with all glyphosate treatments had the highest percent of reed control (Fig. 1). The results of this study suggest that treatments provided excellent common reed control during the seed filling stage. Kenzevic *et al.* (2013) had similar findings. They observed that at the flowering and seed filling stages, the best *Phragmites* control (≥95%) was achieved with glyphosate treatments. Similar results were attained by Silva *et al.* (2011) who reported that *Arundo donax* control would be the most effective by using 5% glyphosate, without cutting stems in late September and October (flowering stage). Also, Monteiro *et al.* (1999) demonstrated that the cover of common reed after 1 year of glyphosate application was only about 20%. Finally, the evaluation of the herbicide effect based on the death speed of aerial parts over the soil surface may not be useful because the herbicides killed the total green parts quickly before being

translocated to the rhizomes. Therefore, it may be less effective in the eradication of perennial rhizomatous weeds. These results agree with Al-Juboory and Ali (1996) who found that glyphosate herbicide had the slowest impact on the total green parts of common reed compared to other herbicides but in the long run it was the best. The effect of fluzifop-P-butyl herbicide was the opposite (Tu *et al.* 2001). In comparison to being used alone the adjuvants significantly improved the efficacy of glyphosate and fluzifop-P-butyl herbicides. The H₁b treatment showed a higher effect (94.5%) in reducing reed density followed by the H₁c treatment (86.99%) with significant differences than glyphosate only (H₁a) (Fig. 1). The results are similar to those of Bekeko (2013), who found that lower concentrations

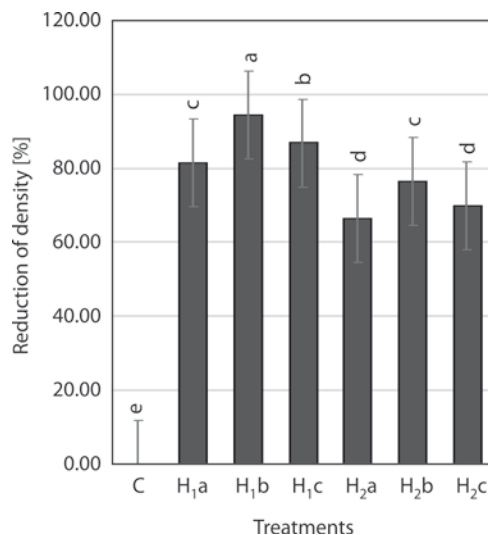


Fig. 1. Reduction percent of common reed density without cutting after 12 months of herbicide application. Adjuvants: urea + nitric acid + sulfonic acid. Treatments: C (control), H₁a (glyphosate only), H₁b (glyphosate + adjuvants), H₁c (half rate of glyphosate + adjuvants), H₂a (fluzifop-P-butyl only), H₂b (fluzifop-P-butyl + adjuvants) and H₂c (half rate of fluzifop-P-butyl + adjuvants). Means with the same superscript letter are not significantly different at p < 0.05

Table 3. Efficacy of herbicide, alone and combine with adjuvants* on relative importance value (RIV) and chlorophyll content in common reed at two levels of stem cutting after one month of applications

Treatments	RIV \pm SD		Means	Chlorophyll (<i>a, b</i>) \pm SD		Means
	10 cm	30 cm		10 cm	30 cm	
C	253 b \pm 4.3	264 a \pm 9.5	259 A \pm 09	1.27 a \pm 0.10	1.08 b \pm 0.06	1.18 A \pm 0.13
H ₁ a	114 f \pm 3.2	109 f \pm 8.3	112 F \pm 06	1.11 b \pm 0.05	0.99 c \pm 0.06	1.04 B \pm 0.08
H ₁ b	117 f \pm 2.9	162 d \pm 2.6	140 D \pm 25	0.36 e \pm 0.02	0.29 ef \pm 0.02	0.33 D \pm 0.04
H ₁ c	115 f \pm 1.8	146 e \pm 8.0	130 E \pm 18	0.27 c \pm 0.03	0.25 fg \pm 0.03	0.27 E \pm 0.03
H ₂ a	147 e \pm 2.2	115 f \pm 2.3	131 E \pm 18	0.18 g \pm 0.03	0.26 fg \pm 0.02	0.22 F \pm 0.05
H ₂ b	163 d \pm 2.3	190 c \pm 9.6	176 B \pm 16	0.33 ef \pm 0.02	0.54 d \pm 0.09	0.43 C \pm 0.13
H ₂ c	159 d \pm 9.5	159 d \pm 5.8	159 C \pm 07	0.52d \pm 0.02	0.29 ef \pm 0.03	0.40 C \pm 0.13

*urea, nitric acid and sulfonic acid

C – control, H₁a – glyphosate only, H₁b – glyphosate + adjuvants, H₁c – half rate of glyphosate + adjuvants, H₂a – fluazifop-P-butyl only, H₂b – fluazifop-P-butyl + adjuvants, H₂c – half rate of fluazifop-P-butyl + adjuvants, SD – standard deviation

Means with the same letter are not significantly different at $p < 0.05$ (the small letters – interaction effect, big letters – main effect)

of urea and common salt enhanced the phytotoxicity level of glyphosate on parthenium weed. Similarly, urea phosphate (UPP) as an adjuvant could increase the efficacy of glyphosate and make it possible to achieve effective weed control with glyphosate at lower doses (Pingliang *et al.* 2012).

Experiment 2: Effect of the treatments on common reed control after cutting stems at levels of 10 and 30 cm

After 1 month (Table 3) all herbicide treatments significantly RIV and leaf chlorophyll content at two levels of cutting. Also, interaction effects between herbicide treatments and cutting levels were significant for RIV

($df = 6, f = 27.7, p < 0.001$) and chlorophyll content ($df = 6, f = 13.2, p = 0.001$). Glyphosate treatment only (H₁a) was significantly higher than fluazifop-P-butyl treatment only (H₂a) in reducing reeds' RIV at the cutting level of 10 cm. A significant difference was not observed between them at the cutting level of 30 cm. The lowest value of chlorophyll content (0.18 and 0.26 $\text{mg} \cdot \text{g}^{-1}$) was observed with H₂a treatment without any significant difference between the two levels of cutting, 10 and 30 cm, respectively. Conversely, H₁a treatment had the lowest reduction in leaf chlorophyll content (Fig. 2). The addition of nitric acid, sulfonic acid and urea to the half recommended glyphosate rate (H₁c treatment) improved the efficacy in comparison to glyphosate only (H₁a) without any significant difference

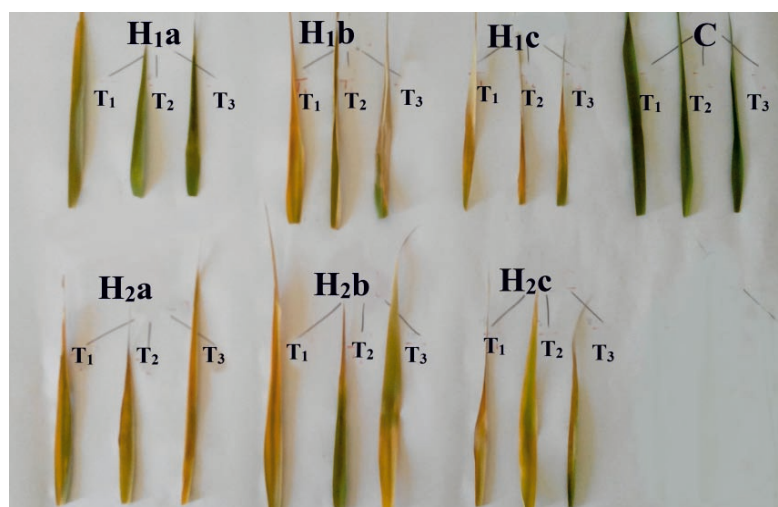


Fig. 2. Influence of herbicide, alone and combined with adjuvants, applications on leaf chlorophyll for common reed (*Phragmites australis*). Adjuvants: urea + nitric acid + sulfonic acid. Treatments: H₁a (glyphosate only), H₁b (glyphosate + adjuvants), H₁c (half rate of glyphosate + adjuvants), H₂a (fluazifop-P-butyl only), H₂b (fluazifop-P-butyl + adjuvants) and H₂c (half rate of fluazifop-P-butyl + adjuvants). Levels of cutting: T₁ (without cutting), T₂ (cutting 30 cm), T₃ (cutting 10 cm)

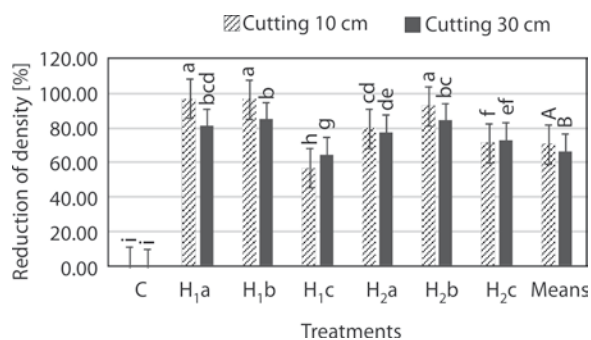


Fig. 3. Reduction percent of common reed density at two cutting levels after 12 months of herbicide application. Adjuvants: urea + nitric acid + sulfonic acid. Treatments: C (control), H_{1a} (glyphosate only), H_{1b} (glyphosate + adjuvants), H_{1c} (half rate of glyphosate + adjuvants), H_{2a} (fluzifop-P-butyl only), H_{2b} (fluzifop-P-butyl + adjuvants) and H_{2c} (half rate of fluzifop-P-butyl + adjuvants). Means with the same superscript letter are not significantly different at $p < 0.05$

between them at the 10 cm cutting level. However, these adjuvants did not improve the efficacy at the 30 cm cutting level. This observation agrees with the general assumption that glyphosate efficacy increases with the addition of urea ammonium nitrate (UAN) or ammonium sulfate to the spray solution by further increasing herbicide absorption (Miller *et al.* 1999; Bunting *et al.* 2004). A positive effect of plant cutting during the flowering stage was observed on the efficacy of all herbicide treatments compared to the control (Monteiro *et al.* 1999). This greater effect probably results from the elimination of dry aerial stems, the stimulation of many young leaves and depletion of rhizome reserves due to the cutting 30 days before application (Buttler 1992; Moreira *et al.* 1999; Silva *et al.* 2011). Our results after 1 month indicated that the cutting level of stems at 10 cm is significantly better in reducing reeds' RIV than cutting at 30 cm ($df = 1, f = 35.5, p \leq 0.001$). However, no significant difference was observed between the two levels of cutting in reducing chlorophyll content ($df = 1, f = 2.75, p = 0.239$). Thereafter, the percent of common reed density was decreased sharply at 12 months after application with H_{1a}, H_{1b} and H_{2b} treatments to 96.92, 96.66 and 92.77%, respectively at the 10 cm cutting level. Moreover, it indicated a high reduction of reeds cut at 30 cm. No significant differences were observed between these percentages at each level of cutting (Fig. 3). Eventually, the reeds with the 10 cm cutting level were significantly more affected (decreased density) than the cutting level at 30 cm ($df = 1, f = 19.7, p = 0.001$). Plant density was increased by short cutting of plants before treatment according to Silva *et al.* 2011. Probably it affects the translocation of herbicide doses to rhizomes depending on the cutting levels. Whereas the impact of common reed cutting on the density and liveliness of plants seems to be affected by ecological conditions and the time of cutting

(Monteiro *et al.* 1999). The present control of common reed after 12 months was significantly improved by the application of fluzifop-P-butyl with adjuvants (H_{2b}) over fluzifop-P-butyl herbicide only (H_{2a}) at two levels of cutting. The use of additives helps to increase the phytotoxicity of herbicides by enhancing absorption and translocation of herbicides leading to long term management of weeds by changing the weed spectrum into soft weed species (Brady 1970; Rao 1956). Conversely, glyphosate treatments with adjuvants did not give significantly improved results in comparison to glyphosate herbicide only (H_{1a}).

Conclusions

Chemical control of common reeds (during flowering and seed filling) without cutting of stems was more effective with glyphosate than fluzifop-P-butyl. However, fluzifop-P-butyl herbicide achieved high reduction rates of chlorophyll content. In this study the efficiency of these herbicides can be enhanced by mixing with urea, nitric acid and sulfonic acid as adjuvants. A point to note, the recommended rates of herbicides were reduced to half by adding these adjuvants. Hence, fluzifop-P-butyl can be used safely as a selective herbicide for controlling reeds that grow with broad-leaf crops by using a half rate of this herbicide with adjuvants. Also glyphosate can be used for common reeds that grow along canals, roadsides and wastelands. Furthermore, positive effects of herbicides were observed after plant cutting during the flowering stage. Results also showed that reed cutting at 10 cm was more effective in reducing density than cutting at 30 cm. The efficacy of fluzifop-P-butyl was improved by the adjuvants at each level of cutting. In contrast, glyphosate can be recommended to be used alone in this case of reed cutting.

Acknowledgements

The author wishes to thank all anonymous reviewers for their valuable comments on an earlier version, and Dr. Khaled A.M. Ibrahim, Department of Plant Production, Faculty of Agriculture, New Valley, Egypt for his help during field work.

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