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Evaluation of Autotoxic Potential of Johnsongrass and Its Integrated Application with Herbicides

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Abstract: Johnsongrass (*Sorghum halepense* (L.) Pers.) is one among the worst weeds of the world which have evolved resistance against herbicides. The autotoxic potential of Johnsongrass can be used for its control. In this study, the different concentrations (2% and 4%) of Johnsongrass water extract (JGWE) were applied, alone or in combination with lower doses of foramsulfuron and clethodim, as post-emergence spray. A non-treated control was maintained for comparison. Data on plant height, fresh weight and dry weight of Johnsongrass were recorded on 30 days after spray. Fresh weight, dry weight and plant height of Johnsongrass were decreased significantly (37-46%, 46-60% and 30-39%, respectively) as compared to non-treated control after the application of JGWE (2 and 4%). A statistically similar control was noted for half doses of herbicides (foramsulfuron and clethodim) or a combination of herbicides + JGWE. These results conclude that allelopathic (autotoxic) potential of JGWE to suppress the Johnsongrass plants, however, their combination with herbicides could not significantly improve the control of Johnsongrass compared with individual use of these JGWEs.

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1. Introduction

Johnsongrass (*Sorghum halepense* (L.) Pers.) is a perennial grass species, considered native to the Mediterranean region (Holm et al., 1977). Johnsongrass is ranked as the sixth worst weed and has been reported to infest 30 different crops in 53 countries of the world (Holm et al., 1977). Owing to its high competition ability with plants in the vicinity, Johnsongrass can significantly depress the growth and productivity of neighboring crops or other plants (Vasilakoglou et al., 2005). This is a C_4 weed and possesses a high degree of adaptability to a wide variety of environments (Paterson et al., 1995). In addition to high competition ability, allelopathy is the other mechanism through which Johnsongrass interferes the growth and development activities of crops (Vasilakoglou et al., 2005).

Most importantly, Johnsongrass was found to have evolved resistance against the most widely used broad spectrum, non-selective herbicide glyphosate. For example, glyphosate-resistant Johnsongrass plants were found in herbicide-resistant soybean fields from Argentina (Vila-Aiub et al., 2007). Quizalofop-P, sethoxydim, clethodim,propaquizafop and nicosulfuron are a few other herbicides against which Johnsongrass biotypes have evolved a resistance (Bradley et al., 2001; Burke et al., 2006; Kaloumenos and Eleftherohorinos, 2009; Hernández et al., 2015). According to International Survey of Herbicide Resistant Weeds (ISHRW, 2016), Johnsongrass plants were found to be resistant to herbicides with mechanisms of action such as 5-enolpyruvylshikimate-3-phosphate (EPSP) synthase (glyphosate), acetolactate synthase (ALS) inhibitors (imazethapyr, nicosulfuron), acetyl-CoA synthase carboxylase (ACCase) inhibitors (clethodim, fenoxaprop-P-ethyl, fluazifop-p-butyl, quizalofop-p-ethyl, sethoxydim), dinitroanilines and others (pendimethalin).

Johnsongrass has been frequently noted in Marmara region of Turkey. A survey (2633 samples) was conducted to document the presence of this weed in Marmara region with high frequency (13-30 m^{-2}) and density (34-100%) (Yazlık, 2014). The results clearly revealed the insufficient control of Johnsongrass in Marmara region in response to herbicides. Evolution of herbicide resistance due to repeated use of same herbicides was the most probable reason for the poor control of this weed (Yazlık and Üremiş, 2015). Inappropriate (late) timing of herbicide application may be the other

reason of poor control of this weed in this region (Yazlik and Uremis, 2015).

In wake of such situations, alternative methods in addition to chemical control are required in order to control Johnsongrass. Allelopathic weed control is potential alternate in this regard (Weston et al., 2013). Allelopathic weed control makes use of the allelochemicals from plants to control other plants (weeds) (Farooq et al., 2011; Jabran et al., 2015). Autotoxicity is a form of allelopathy where the chemicals exuded from a plant inhibit the growth activities of members of its own species (Singh et al., 1999). The phenomenon of autotoxicity has been also reported in Johnsongrass (Abdul-Wahab and Rice, 1967). The use of allelopathic water extracts for weed control has been previously reported by several researchers (Jabran et al., 2010; Farooq et al., 2011).

This study was conducted to evaluate autotoxic properties of Johnsongrass to control this weed. Hence, it was tested whether allelopathic water extracts from Johnsongrass will provide an effective control of this weed and provide some new directions for controlling this noxious weed. Similarly, another objective was to combine the allelopathic water extracts with lower doses of two herbicides (foramsulfuron and clethodim) in order to obtain the synergistic effects of these two control methods.

2. Materials and Methods

2.1. Study site and treatments

The studies were conducted in the open field conditions of West Mediterranean Research Institute, Antalya, Turkey during the year 2012. Johnsongrass water extracts (JGWE) were prepared according to method of Cheema et al., (2000). The plant materials of Johnsongrass were collected at flowering time (02.08.2012) and air dried under the shade for one week. The dried plant materials were soaked in distilled water for 24 hours. The filtrate was collected after sieving the mixture using a cloth sieve and used to prepare two different concentrations (2 and 4%, w/v) of JGWE.

Moreover, half doses of two herbicides i.e. foramsulfuron (Bayer - Ekipp Super WG 61 = 75 g ha^{-1}) and clethodim (Agrobest – Sondelect EC = 625 ml ha^{-1}) were used either alone or in combination with different concentrations of JGWE (2 and 4%). The rates of herbicides used in this study were decided according to their local recommended doses for application in corn (foramsulfuron = 150 g ha^{-1}) and cotton (clethodim = 1255 ml ha^{-1}). Foramsulfuron is a systematic herbicide with a mode of action acetolactate synthase ALS (acetohydroxyacid synthase AHAS) inhibitor. Clethodim is also a systematic herbicide which reaches the roots very quickly after it is applied to green foliage of plants. This herbicide inhibits the synthesis of fatty acids by inhibiting the activity of acetyl CoA carboxylase (ACCase) enzyme (Rao, 2000).These two post-emergence herbicides were selected owing to their different mechanism of action.

Two sets of the same experiment were established in an open field on 07 August 2012. The pots used in the experiments had a diameter of 14 cm. Each pot was filled with a mixture of 'soil from Johnsongrass rhizosphere' + turf to a depth of 10 cm. Each pot was sown with 3 rhizomes of Johnsongrass. There were nine treatments in the experiment including 'control'. The treatments were applied when the Johnsongrass plants reached a height of almost 15 cm (post-emergence application) on 17.08.2012. Allelopathic water extracts, herbicides or their combinations were applied using 200 L ha^{-1} as volume of spray. The JGWE/herbicides/ JGWE+herbicides were applied using a hand sprayer on the foliage of Johnsongrass. Control was treated with distilled water. A summary of treatments has been presented in Table 1.

Table 1. A summary of treatments used in this study

Treatments	Doses
Control	Distilled water
JGWE	2%
JGWE	4%
Foramsulfuron (½ dose)	75 gha^{-1}
Clethodim (½ dose)	625 $mlha^{-1}$
JGWE + Foramsulfuron (½ dose)	2% JGWE + 75 gha^{-1} Foramsulfuron
JGWE + Clethodim (½ dose)	2% JGWE + 625 $mlha^{-1}$ Clethodim
JGWE + Foramsulfuron (½ dose)	4% JGWE + 75 gha^{-1}Foramsulfuron
JGWE + Clethodim (½ dose)	4% JGWE + 625 $mlha^{-1}$ Clethodim

JGWE = Johnsongrass water extract

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Table 2. Effect of different Johnsongrass water extract concentrations alone or in combination with herbicides on fresh weight, dry weight and plant height of *Sorghum halepense*

Treatments	Fresh weight (g)	Reduction (%)	Dry weight (g)	Reduction (%)	Plant height (cm)	Reduction (%)
Control	22.67 ± 2.52 a	-	10.33 ± 2.08 a	-	91.00 ± 8.72 a	-
JGWE 2%	14.47 ± 1.50 bc	37.0	5.60 ± 2.09 b	46.0	64.00 ± 9.54 bc	30.0
JGWE 4%	12.43 ± 3.16 bc	46.0	4.07 ± 2.10 b	60.3	55.33 ± 12.58 bc	39.2
Foramsulfuron ½ dose	10.20 ± 5.70 c	42.0	6.40 ± 0.90 b	38.0	66.00 ± 6.56 bc	28.0
Clethodim ½ dose	16.67 ± 1.42 b	26.0	7.20 ± 1.11 b	30.3	64.33 ± 7.51 bc	29.3
JGWE 2% + Foramsulfuron ½ dose	13.53 ± 1.47 bc	41.2	4.87 ± 1.95b	52.0	72.33 ± 6.81 b	21.0
JGWE 2% + Clethodim ½ dose	16.23 ± 1.20 bc	29.4	7.33 ± 1.53 b	29.0	58.33 ± 15.28 bc	36.0
JGWE 4% + Foramsulfuron ½ dose	11.17 ± 3.68 bc	51.3	4.70 ± 1.13 b	55.0	57.00 ± 3.00 bc	37.3
JGWE 4% + Clethodim ½ dose	11.77 ± 5.01 bc	48.0	5.33 ± 2.08 b	48.4	51.67 ± 10.41 c	43.0

Within the column, mean values followed by the same letter are not significantly different according to Duncan's Multiple Range Test ($p \leq 0.05$); JGWE =Johnsongrass water extract.

2.2 Data recording

The Johnsongrass plants were harvested in each treatment four weeks after application of allelopathic water extracts, herbicides or their combinations i.e. on 18.09.2012.The data of plant height (cm), fresh weight (g) and dry weight (g) were recorded. The plants harvested from each treatment were kept in oven at 60 ^{o}C for 48 h in order to record the dry weight. Moreover, the effect of treatments on Johnsongrass mortality was calculated using formula of Abbott (1925) as given in Eq. [1].

$$M = \left[\frac{C-T}{C}\right] \times 100 \qquad [1]$$

Where M is Mortality (%), C is fresh weight, dry weight or plant height of Johnsongrass in control treatment and T is fresh weight, dry weight or plant height of Johnsongrass in a certain treatment.

2.3. Statistical analysis

The collected data were analyzed using statistical software SPSS (Statistical Package for Social Sciences 10.0) to calculate the means and standard errors for each treatment. Moreover, Duncan's Multiple Range Test was used to determine the difference among the treatments at $p \leq 0.05$. The data from two sets of same experiment were statistically similar, hence, the means from two sets of experiments have been presented.

3. Results

Significant reduction in Johnsongrass fresh weight (37 and 46%, respectively), dry weight (46 and 60%, respectively), and plant height (30 and 39%, respectively) of Johnsongrass was recorded with the application of JGWE when compared with control treatment (Table 2;Fig. 1). Efficacy of JGWE 2% was little lower than JGWE 4% in suppressing different parameters of Johnsongrass (Table 2; Fig. 1). Half doses of foramsulfuron and clethodim suppressed fresh weight (42 and 26%, respectively), dry weight (38 and 30%, respectively) and plant height (28 and 29%, respectively) of the Johnsongrass plants (Table 2). Foramsulfuron was more effective than clethodim in decreasing the fresh and dry weights of Johnsongrass (Table 2; Fig. 1). Nevertheless, half doses of herbicides had resulted in a lower decrease in fresh weight, dry weight and plant height of Johnsongrass compared with JGWEs (2 and 4%) (Table 2).

Combining JGWE 2% with half dose of foramulfuron improved only the decrease in dry weight of Johnsongrass compared with individual use of JGWE or herbicide (Table 2). Similarly, combining JGWE 4% with half dose of foramsulfuron improved only the decrease in fresh weight of Johnsongrass compared with sole application of herbicide or JGWE 4%. Combining half dose of clethodim with 4% JGWE was more effective than combining it with JGWE 2% (Table 2). Moreover, JGWE 4% + clethodim half dose combination was more effective in suppressing the Johnsongrass over alone clethodim half dose, however, this combination could not significantly improve the Johnsongrass control over sole application of JGWE 4% (Table 2).

4. Discussion

The results of this research highlight that the Johnsongrass water extracts (JGWE) possess the potential to suppress the noxious weeds such as Johnsongrass. However, integration of allelopathic materials with herbicides did not provided some synergistic effects.

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Fig. 1. Effect of applications of Johnsongrass water extracts, herbicides or their combinations on growth of *Sorghum halepense*(first set of experiment). 1.Control, 2.Johnsongrass water extract (JGWE) 2% + clethodim ½, 3. Clethodim ½, 4. Foramsulfuron ½, 5.JGWE 4% + clethodim ½, 6. JGWE 2% + foramsulfuron ½, 7. JGWE 4% + foramsulfuron ½, 8. JGWE2%, 9. JGWE4%.

Johnsongrass has been documented as potent allelopathic plant in several of previous studies (Vasilakoglou et al., 2005; Yan et al., 2010; Haung et al., 2008; Huang et al., 2015) containing various allelochemicals in its body parts which can express an allelopathic activity to suppress other plants or weeds (Haung et al., 2008; Liu et al., 2011). The most important allelochemicals present in *S. halepense* are dhurrin, *p*-hydroxybenzaldehyde, ethyl *p*-hydroxybenzoate, diosmetin, apigenin, luteolin, diosmetin, tricin and *p*-hydroxybenzoic acid (Liu et al., 2011; Haung et al.,2010; Huang et al.,2015).The allelopathic activity of these allelochemicals helped to suppress the Johnsongrass plants as it was noted in our studies. Phenolic compounds are important in imparting the allelopathic potential to plants (Weston and Duke, 2003; Li et al., 2010). The results of previous studies indicate that various allelopathic materials could be a potential source to control Johnsongrass. For example, allelochemicals including isothiocyanate benzyl and isothiocyanate allyl from black radish (*Raphanus sativus* L. var. *niger*) were found to be effective in suppressing Johnsongrass plants (Uremis et al., 2009). Similarly, the JGWE had a higher efficacy in controlling the weeds and increasing soybean yield over herbicide (trifluralin) application (Movahedpouret al.,2010).

Nevertheless, the integration of allelopathic water extracts and herbicides could not provide expected results in our studies. Also, the half doses used alone offered a very low effectiveness against Johnsongrass. Herbicide resistance in the Johnsongrass plants used in the experiment may be a reason for this poor control after herbicide application (Yazlık, 2014). Combining allelopathic JGWE with other herbicides (than the ones included in this experiment) or other weed control methods may, however, serve as good integrated weed management option for controlling Johnsongrass.

5. Conclusion

Fresh weight and dry weight of Johnsongrass were decreased significantly due to its autotoxic effect. The results of this study conclude that the allelopathic JGWE possesses the potential to suppress the Johnsongrass plants. However, the integration of JGWE with herbicides could not produce expected results. Future research direction may include the identification and quantification of allelochemicals involved in the suppression of Johnsongrass. Similarly, higher concentrations of JGWE (other than 2 and 4%) are desired to be studied alone and in combination with other weed control methods for effective control of Johnsongrass. The options such as combination of JGWE with mechanical weed control may provide effective and sustainable control of Johnsongrass.

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