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Article

The Response of Different Weed Species to Glyphosate Using Ammonium Sulfate and Hard Water

Resposta de Diferentes Espécies de Plantas Daninhas ao Glyphosate com o Uso de Sulfato de Amônio e Água Dura

MIRZAEI, M.[1]
RASTGOO, M.[1*]
HAJMOHAMMADNIA GHALIBAF, K.[1]
ZAND, E.[2]

ABSTRACT - Water hardness antagonism and the effect of ammonium sulphate (AMS) on efficacy of glyphosate have been well documented. However conflicting results between weed species were noted by the authors. Greenhouse experiments were conducted twice at the Ferdowsi University of Mashhad in a randomized complete block design with a factorial arrangement and three replications during 2014-2015. Four experiments were arranged separately on cypress (*Kochia scoparia*), redroot pigweed (*Amaranthus retroflexus*), little seed canary grass (*Phalaris minor*) and winter wild oat (*Avena ludoviciana*) using ammonium sulphate and deionizad water and in the presence of different salts, (i.e. $NaHCO_3$, $CaCO_3$, $MgCl_2$ and $CaCl_2$ at 500 ppm) against three doses of glyphosate (256.25, 512.5 and 1,025 g a.i. ha^{-1}), with and without ammonium sulphate (AMS) as adjuvant (2% w/v). The results showed the application of AMS overcomes the inhibitory effects of salts in the spray solution in tested species. The degree of effectiveness in *A. retroflexus* was more than *A. ludoviciana* and *P. minor.* Glyphosate with AMS caused reduction in dry matter in grasses from 0.34 to 0.28 g, while glyphosate toxicity in *A. retroflexus* with AMS was 100 percent and all of the plants were destroyed (0.82 to 0 g). The application of AMS in overcoming the inhibitory effects of water hardness had no effect on *K. scoparia* control. However, Increasing AMS could overcome the inhibitory effects of hard water in the spray solution on glyphosate efficacy in *A. retroflexus* and *K. scoparia*, but it had no effect on tested grassy weeds. We may conclude that glyphosate work differently on weed species using AMS and hard water.

Keywords: adjuvant, redroot pigweed, Round up, summer cypress, water hardness, winter wild oat.

*RESUMO - Há estudos prévios sobre o antagonismo da dureza da água e o efeito do sulfato de amônio na eficácia do glyphosate. No entanto, os autores desses estudos observaram resultados conflitantes entre espécies de plantas daninhas. Experimentos em estufa foram conduzidos em duplicata na Universidade Ferdowsi de Mashhad, em um delineamento de blocos completos casualizados com arranjo fatorial e três repetições, no período 2014-2015. Quatro experimentos foram arranjados separadamente em cipreste de verão (**Kochia scoparia**), caruru (**Amaranthus retroflexus**), erva-cabecinha (**Phalaris minor**) e aveia selvagem de inverno (**Avena ludoviciana**), usando sulfato de amônio e água deionizada e na presença de diferentes sais ($NaHCO_3$, $CaCO_3$, $MgCl_2$ e $CaCl_2$ a 500 ppm) contra três doses de glyphosate (256,25, 512,5 e 1.025 g i.a. ha^{-1}), com e sem sulfato de amônio como adjuvante (2% p/v). Os resultados mostraram que a aplicação de sulfato de amônio supera os efeitos inibitórios de sais na solução de pulverização*

*** Corresponding author:**
<m.rastgoo@um.ac.ir>

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[1] Faculty of Agriculture, Ferdowsi University of Mashhad, Mashhad, Iran. [2] Iranian Research Institute of Plant Protection, Tehran, Iran.

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nas espécies testadas. O grau de eficácia em ***A. retroflexus*** *foi maior que em* ***A. ludoviciana*** *e* ***P. minor****. O glyphosate com o sulfato de amônio causou redução na matéria seca de 0,34 para 0,28 g nas gramíneas, enquanto a toxicidade do glyphosate em* ***A. retroflexus*** *com sulfato de amônio foi de 100% e todas as plantas foram destruídas (0,82 a 0 g). A aplicação do sulfato de amônio para superação dos efeitos inibitórios da dureza da água não teve efeito sobre o controle de* ***K. scoparia****. No entanto, o aumento de sulfato de amônio poderia superar os efeitos inibitórios da água dura na solução de pulverização em relação à eficácia do glyphosate em* ***A. retroflexus*** *e* ***K. scoparia****, mas não teve efeito sobre as plantas daninhas gramíneas testadas. Pode-se concluir que o glyphosate funciona de forma diferente em espécies de plantas daninhas usando sulfato de amônio e água dura.*

Palavras-chave: adjuvante, caruru, Roundup, cipreste de verão, dureza da água, aveia selvagem de inverno.

INTRODUCTION

Water is a universal solvent and it is used as a primary carrier for crop protection product applications. The properties of water used as a carrier in spray solutions can greatly influence the performance of herbicides, including glyphosate, dicamba, 2,4-D, imazethapyr, sethoxydim, nicosulfuron and many other herbicides (Altland, 2001; Cahal et al., 2012). Glyphosate has undergone extensive ecological risk assessments (Fairchild et al., 2002); it is a non-selective and systemic herbicide (Aladesanwa and Oladimeji, 2005) with no soil activity and is applied in a wide range of agricultural and non-agricultural ecosystems (Adu-Yeboah et al., 2014).

Glyphosate is the most widely studied herbicide in terms of water hardness, and most studies show a reduction in glyphosate performance (Buhler and Burnside, 1983a; Nalewaja and Matysiak, 1991; Mueller et al., 2006). It has been well documented that glyphosate efficacy is reduced when applied in water containing high level cations (Nalewaja and Matysiak 1991; Chahal et al., 2012; Devkota, 2016). Antagonistic effects of glyphosate by salts depend on both cations and anions. Nalewaja and Matysiak (1991) reported that sodium and magnesium salts were less antagonistic than calcium, zinc and iron salts. Calcium and magnesium sulphates were less antagonistic than chlorides, but sulphates and chlorides of sodium were similarly antagonistic to glyphosate toxicity on wheat (Nalewaja and Matysiak, 1991). Moreover, numerous studies have shown that cations or foliar fertilizers can influence glyphosate efficacy, depending on the weed species (Baily et al., 2002; Mueller et al., 2006).

The addition of ammonium (AMS) to glyphosate solution reduces the antagonistic effect of cations and enhances the control of certain weed species (Thelen et al.,1995; Pratt et al., 2003; Nurse et al., 2008). Zollinger et al. (2010) reported that the activity of the four weak acid herbicides, such as, dicamba plus diflufenzopyr, and glufosinate increased with addition of ammonium sulphate to the spray solution; they were all antagonized by calcium and magnesium, and ammonium sulfate overcame this antagonism. Pratt et al. (2003) showed that hard water, defined as 500 mg L^{-1} $CaCO_3$, reduced velvetleaf (*Abutilon theophrasti* L.) control by glyphosate while addition of AMS increased control. Although most of the research literature indicates that AMS can improve glyphosate efficacy (Wills and McWhorter 1985; Salisbury et al., 1991; Young et al., 2003), some authors reported no benefit of added AMS on glyphosate (Nurse et al., 2008; Soltani et al., 2011). Differential responses between weed species were noted by the authors. AMS adjuvant enhancement of glyphosate phytotoxicity varied with different reports on different or similar species (Nalewaja and Matysiak, 1992; Nurse et al., 2008; Soltani et al., 2011). AMS at 0.5% (w/v) in a spray carrier containing calcium choloride (500 ppm calcium) overcame calcium antagonism of glyphosate toxicity to wheat (*Triticum aestivum* L.), overcame antagonism and enhanced toxicity to sunflower (*Helianthus annuus*), but only partly overcame calcium antagonism of toxicity to *K. scoparia* and soybean (*Glycine max* L.) (Nalewaja and Matysiak, 1992).With glyphosate at 100 g a.i. ha^{-1}, ammonium sulphate at 2% (w/v) in a distilled water spray carrier enhanced toxicity to *H. annuus* from 11 to 55% fresh weight reduction but reduced glyphosate toxicity in *K. scoparia* and *G. max*. Soltani et al. (2011) reported no effect of water hardness on *A. theophrasti*, *A. retroflexus*, *Chenopodium album*, *Setaria viridis* and *Echinochloa crus-galli* control, when glyphosate was applied with or without AMS. With the widespread occurrence of glyphosate resistant (GR) weeds, and because the effect of hard water and AMS on glyphosate toxicity is species dependent, optimum weed control in different species should be studied in weed species in more detail.

By considering contrasting results and variation of glyphosate efficacy in hard water among different species through AMS, the objective of this research was to evaluate the influence of different salts and ammonium sulfate on *K. scoparia* L., *A. retroflexus* L., *P. minor* Retz., and *A. ludoviciana* and comparison grassy and broadleaved species response under greenhouse conditions.

MATERIALS AND METHODS

Four experiments were conducted at the research greenhouse of the Agricultural Faculty, Ferdowsi University of Mashhad, Iran (Lat 36°15' N, Long 59°28' E; 985 m Altitude) in 2014-2015. The aim of these experiments was to study the effect of different salts on glyphosate efficacy to control four weed species in the presence of ammonium sulfate. Each experiment was conducted twice. There was no significant difference between two time replications of each experiment, therefore data were combined over experimental runs and were averaged for the purpose of analysis.

Plant material and growth

Seeds of *K. scoparia*, *A. retroflexus*, *P. minor*, and *A. ludoviciana* were collected from the research field of Ferdowsi University of Mashhad, Iran. Ten Seeds of each species were planted in plastic pots (13 cm diameter and 14 cm height) and filled with a mixture of sand and clay loam soil (1:1; v/v). The pots were irrigated daily or as needed throughout the experiments to maintain adequate soil moisture for plant growth. After development of the second true leaf, the plants were thinned to five plants with uniform height per pot. The plants were grown in a greenhouse at the temperature of 28±2 °C and 16 h day length.

Inhibitory effect of different salts on glyphosate efficacy

This experiment evaluated the inhibitory effect of different salts on glyphosate efficacy for control of different weeds. For each weed species, the experiment was carried out in a randomized complete block design with a factorial arrangement and three replications per treatment. Experiments were conducted twice and data were pooled for analysis of variance. Factors included application of 500 mg L^{-1} of four types of salts ($NaHCO_3$, $CaCO_3$, $MgCl_2$, and $CaCl_2$ from Merck Company, Germany) and deionized water against three doses of glyphosate (256.25, 512.5, and 1,025 g a.i. ha^{-1}), and along with the presence and absence of ammonium sulfate. Glyphosate rates were selected upon preliminary experiments under greenhouse conditions and selected for the species. At four to six true leaf stage, the plants were treated with glyphosate (Roundup®, 41% SL) using a greenhouse bench sprayer (Matabi 121030 Super Agro 20 L sprayer; Agrotech Services-Crop Spraying Equipment, Rossendale, UK) fitted with a 8002 even flat-fan nozzle tip. The output volume of the sprayer was 290 L ha^{-1} at a pressure of 200 kPa.

The percentage of survival (Eq. 1) and dry weight of the plants were determined at 21 days after applying the treatments (DAT). The plants were considered alive when at least half of the leaves were green and dead when all the leaves were necrotic. At the end of the experiment, all the above-ground plants were cut at the soil surface, oven-dried at 68 °C for 48 h and then, weighed. The data underwent analysis of variance, and differences among mean values of treatments were compared by Fischer's Protected LSD test ($p \leq 0.05$) in the software SAS 9.1.

$$\text{Survival (\%)} = (\text{Number of alive plants in pot} / \text{Total plants per pot}) \times 100 \qquad \text{(eq. 1)}$$

RESULTS AND DISCUSSION

ANOVA showed that both degree of survival and dry weight of the weed species were significantly ($p \leq 0.01$) affected by the main effect of glyphosate and ammonium sulfate

(Tables 1 and 2). Inorganic salts that were added to the spray solution reduced the phytoxtoxicity of glyphosate in all weeds. The results indicated that interactions of glyphosate level/hardness and glyphosate level/ammonium sulfate were statistically significant on the survival and dry weight of *K. scoparia* and *A. retroflexus*, but they were not significant in *A. ludoviciana* and *P. minor* (Tables 1 and 2). Moreover, the interaction of hardness and ammonium sulfate was effective on the degree of survival and dry weight of *A. retroflexus*, *A. ludoviciana*, and *P. minor*, but not in *K. scoparia* (Tables 1 and 2). The results also showed that the effect of the triple interaction of glyphosate level, hardness, and ammonium sulfate was statistically significant ($p \leq 0.01$) on the survival and dry weight of *K. scoparia* and *A. retroflexus*, whereas this effect was not significant in *A. ludoviciana* and *P. minor* (Tables 1 and 2).

Table 1 **-** ANOVA of survival percentage and dry weight of *K. scoparia* and *A. retroflexus* affected by glyphosate level, salt type, and ammonium sulfate (AMS) application

Source of variation	df	Mean squares			
		K. scoparia		*A. retroflexus*	
		Survival	Dry weight	Survival	Dry weight
Block	2	11.90^{ns}	0.003**	131.90^{ns}	0.05^{ns}
Glyphosate Dose (GD)	2	30527.30**	0.51**	4694.40**	1.72**
Salt	4	1586.50**	0.04**	4677.10**	1.02**
AMS	1	31459.40**	0.46**	41173.60**	12.44**
GD×Salt	8	487.80**	0.007*	666.70**	0.14 *
AMS×GD	2	3734.00**	0.04**	4111.10**	1.50*
AMS×Salt	4	222.30^{ns}	0.008^{ns}	4003.50 **	0.82 **
GD×Salt×AMS	8	355.70**	0.02**	795.10**	0.16**
Error	58	113.80	0.005	160.70	0.05
CV		31.18	28.67	26.99	21.59

Note: ns, non-significant, *, ** significant at $P \leq 0.05$ and $P \leq 0.01$, respectively; df, degree of freedom.

Table 2 **-** ANOVA of survival percentage and dry weight of *A. ludoviciana* and *P. minor* affected by glyphosate dose, salt type, and ammonium sulfate (AMS) application

Source of variation	df	Mean squares			
		A. ludoviciana		*P. minor*	
		Survival	Dry weight	Survival	Dry weight
Block	2	1020.83^{ns}	0.05*	671.11^{ns}	0.01^{ns}
Glyphosate Dose (GD)	2	11645.83**	0.10**	12457.78**	0.15**
Hardness	4	989.58**	0.01^{ns}	855.55**	0.005^{ns}
AMS	1	8027.78**	0.12**	9000 **	0.09*
GD×Hardness	8	812.50 ^{ns}	0.01^{ns}	635.55^{ns}	0.02^{ns}
AMS×GD	2	1340.28^{ns}	0.03^{ns}	413.33^{ns}	0.02^{ns}
AMS×Hardness	4	3392.36**	0.03*	2122.22*	0.03*
GD×Hardness×AMS	8	611.11^{ns}	0.01^{ns}	302.22^{ns}	0.02^{ns}
Error	58	905.89	0.01	643.52	0.01
CV		25.59	20.40	26.99	21.59

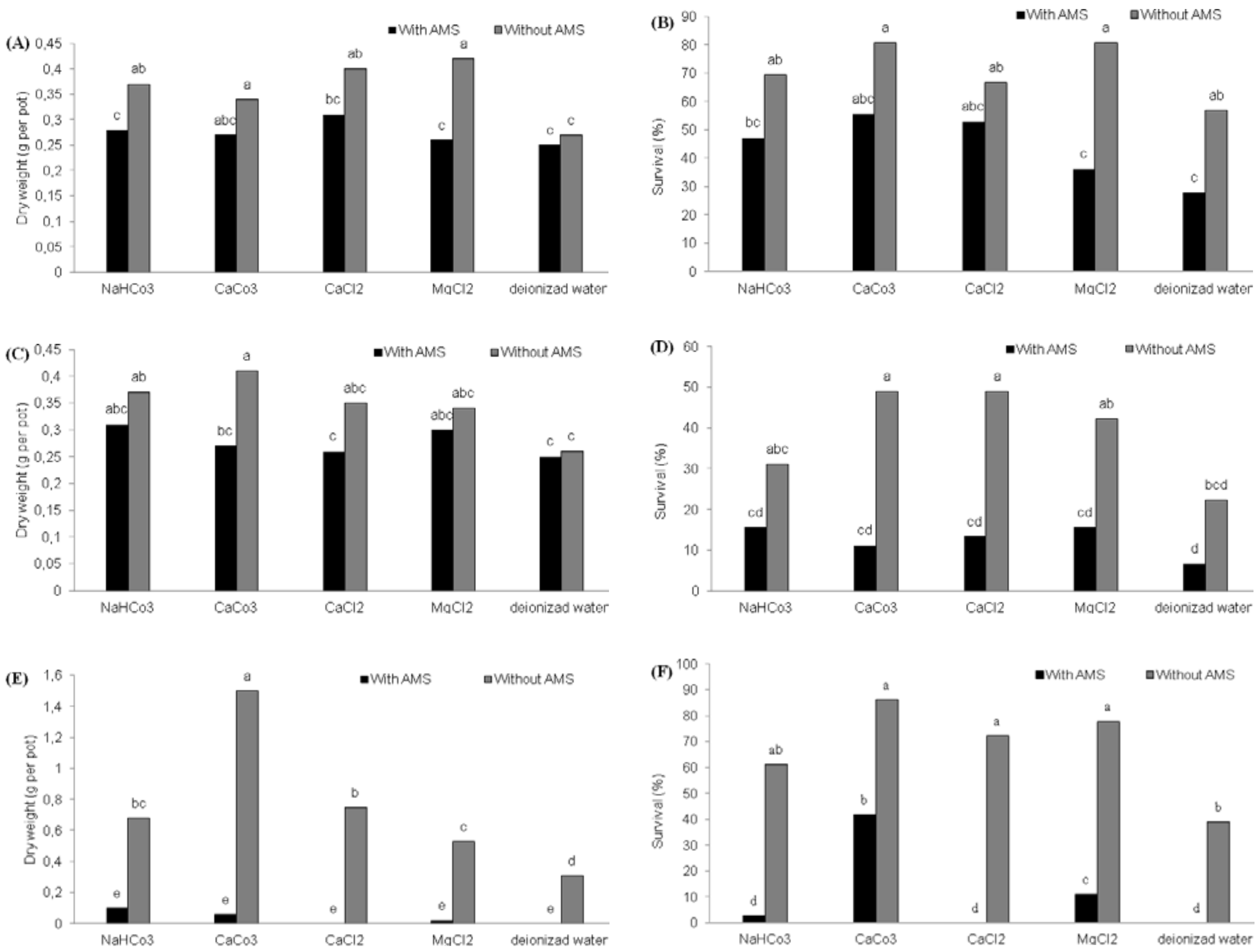
Note: ns, non- significant, *, ** significant at $P \leq 0.05$ and $P \leq 0.01$, respectively; df, degree of freedom.

Similar results were reported on the efficacy of other weak acid herbicides as affected by water hardness. Reduction of the efficacy of 2,4-D, glyphosate, and mesotrione was attributed to the presence of Ca^{2+}, Mg^{2+}, Mn^{2+}, and Zn^{2+} cations in the spray solution (Nalewaja and Matysiak, 1991; Bernards et al., 2005; Mueller et al., 2006; Roskamp et al., 2013; Devkota et al., 2016a). Wills and McWhorter (1985) described that the reduction of phytotoxicity of glyphosate in purple nutsedge (*Cyperus rotundus*) was attributed to the addition of various inorganic salts to the spray solutions.

Zollinger et al. (2010) showed that the activity of the five weak acid herbicides including glyphosate, aminopyralid, tembotrione, dicamba plus diflufenzopyr, and glufosinate were reduced by $CaCl_2$ and $MgCl_2$. Mueller et al. (2006) reported that calcium and magnesium concentrations greater than 250 ppm antagonized glyphosate activity on weeds such as broadleaf signalgrass

(*Brachiaria platyphylla*), pitted morningglory (*Ipomoea lacunosa*), palmer amaranth (*Amaranthus palmeri*), and yellow nutsedge (*Cyperus esculentus*). Pratt et al. (2003) found that 500 mg L^{-1} calcium carbonate reduced glyphosate control of velvetleaf (*A. theophrasti*). Nalewaja and Matysiak (1991) showed that glyphosate phytotoxicity to wheat was antagonized by sodium, calcium, and magnesium. Thus, cations are primarily responsible for reducing glyphosate activity.

Previous studies also reported that the use of AMS enhanced glyphosate efficacy in the presence of minerals (Pratt et al., 2003; Zollinger et al., 2010). However, information is limited to differences among species in response to glyphosate applied with AMS. In this study, the influence of ammonium sulfate on glyphosate toxicity in the presence of hard water cations is species dependent (Figure 1). Toxicity to *H. annuus* from glyphosate applied alone at rates which gave less than 90% fresh weight reduction were enhanced by AMS but not in *K. scoparia* and *G. max* (Nalewaja and Matysiak, 1992). The results of the current study for *K. scoparia* control with glyphosate as affected by hard water and AMS coincides with the result found by Nalewaja and Matysiak (1992) (Table 1).



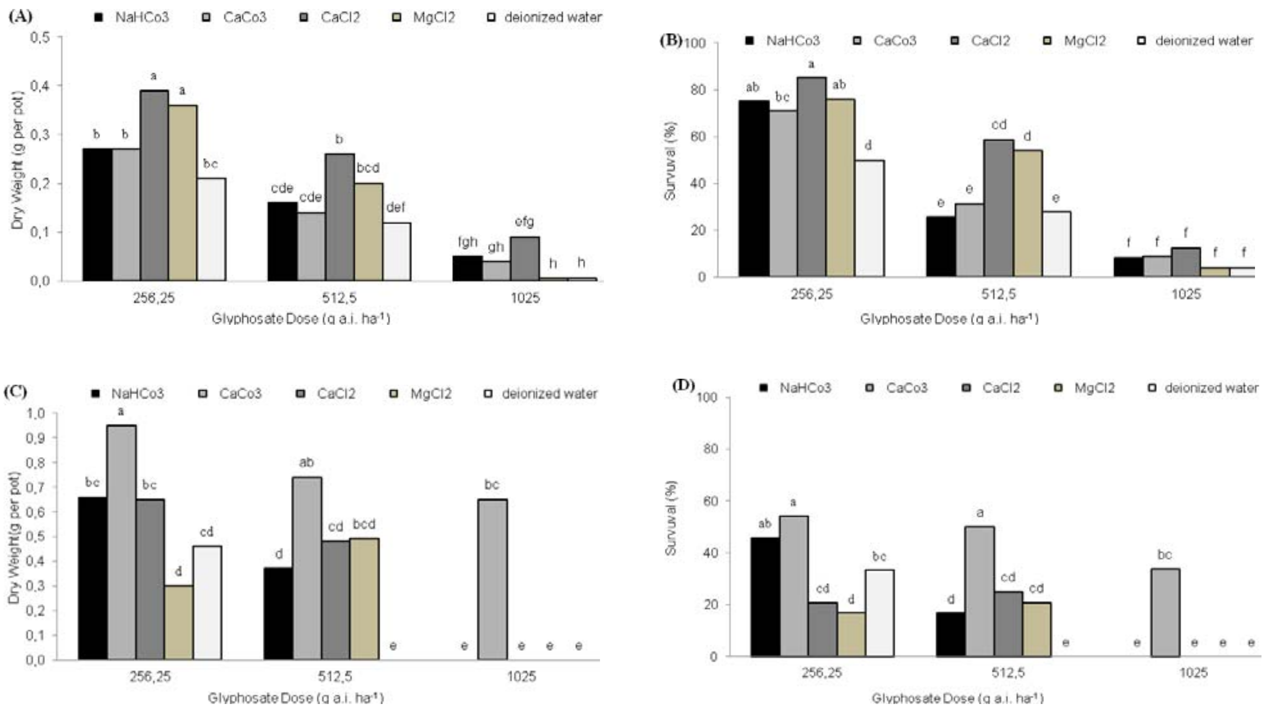
Means with the same letters are not statistically significant at 5% probability level.

Figure 1 **-** The effect of water hardness effect on dry weight (A) and survival (B) of *A. ludoviciana*, dry weight (C) and survival (D) of *P. minor* and dry weight (E) and survival (F) of *A. retroflexus* with and without ammonium sulfate (AMS) application, at 21 days after treatment.

Similarly, Hajmohammadnia et al. (2016) reported that sodium bicarbonate and calcium carbonate reduced glyphosate efficacy in spray tanks in velvetleaf and barnyardgrass. Using AMS enhanced glyphosate efficacy and reduced the antagonistic effect of cations in both species, but it was more effective in *A. theophrasti*. In contrast to this finding, Soltani et al. reported that AMS had no effect in overcoming hard water in glyphosate efficacy for *E. crus-galli*, *C. album*, *S. viridis*, *A. retroflexus* and *A. theophrasti* control on full label dose.

The present study confirmed that AMS could overcome the antagonistic effects of water hardness in control *A. ludoviciana*, *P. minor* and *A. retroflexus*. In contrast and in absence of water hardness, in the control of *A. retroflexus*, application of AMS in deionized water was remarkable while the application of AMS in deionized water did not have a significant effect on glyphosate efficacy in controlling *A. ludoviciana*, and *P. minor* (Figure 1).

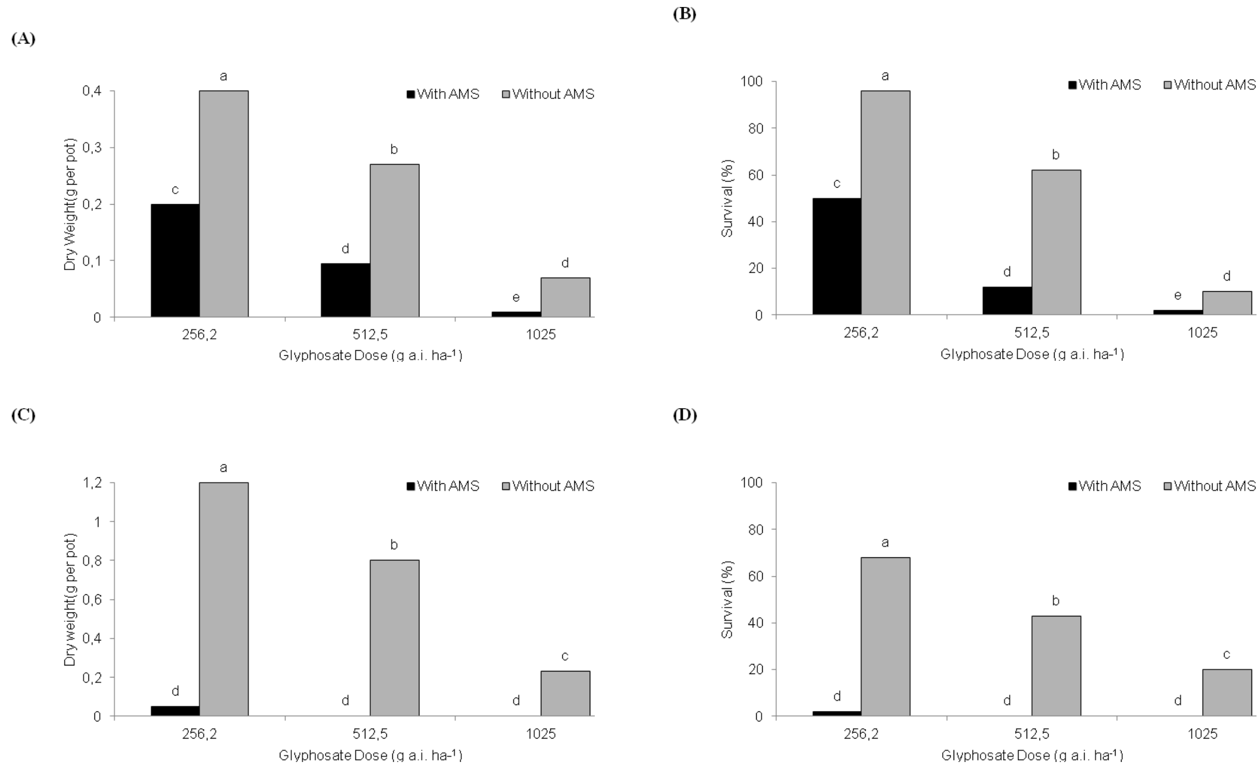
Based on the result of glyphosate, antagonism by salts can be overcome by high use rates of AMS. The influence of antagonistic salts in glyphosate spray carriers would be most pronounced with conditions that would cause marginal or inadequate control (Nalewaja and Matysiak, 1991). By increasing glyphosate dose, some of the antagonistic effects on glyphosate efficacy were neutralized. Stahlman and Phillips (1979) reported that increasing glyphosate application can overcome some of the reduction of activity. Several studies have documented an increase in overcoming some of the antagonistic effects by increasing glyphosate dose (Stahlman and Phillips, 1979; Buhler and Burnside, 1983b). This finding can be attributed to fewer cations such as Ca^{2+} and Mg^{2+} in the spray solution to associate with deactivates the glyphosate molecule (Thelen et al., 1995). However, in this experiment, overcoming the negative effects of salts in the spray solution by increasing the concentration of glyphosate was affected by weed species. So that increasing glyphosate dose could not compensate for the effects of hard water on grassy weeds but in *K. scoparia* and *A. retroflexus* could overcome (Figure 2).



Means with the same letter are not statistically significant at 5% probability level.

Figure 2 **-** The effect of different glyphosate doses on dry weight (A) and survival (B) of *K. scoparia* and dry weight (C) and survival (D) of *A. retroflexus* in the presence and the absence of water hardness, at 21 days after treatment.

In *A. retroflexus*, using AMS in the lowest glyphosate dose (256.25 g a.i. ha^{-1}) was the same effect as the highest dose (1,025 g a.i. ha^{-1}) without AMS (Figure 3). This increased herbicide activity with AMS was also attributed to improved herbicide movement through the leaf cuticle as a result of the presence of the ammonium ion of AMS. It has been reported that the ammonium ion enhances the transcuticular movement of other herbicides such as bentazon in *Elytrigia repens* (Wanamarta et al., 1993). Numerous studies have indicated that AMS increases foliar absorption of herbicides and consequently increases herbicide efficacy (Wilson and Nishimoto, 1975; Costa and Appleby, 1986; Fielding and Stoller, 1990; Gronwald et al., 1993; Kent et al., 1991). Other studies by Hall et al., (2000) have shown that the addition of AMS to glyphosate solutions has reduced binding of cations that are present on the leaf surfaces and within plants of some weed species such as *A. theophrasti*, *Conyza canadensis* or *A. retroflexus*.



Means with the same letter are not statistically significant at 5% probability level.

Figure 3 **-** The effect of different glyphosate doses on dry weight (A) and survival (B) of *K. scoparia* and dry weight (C) and survival (D) of *A. retroflexus* with and without ammonium sulfate (AMS) application, at 21 days after treatment.

The current research demonstrates that water hardness acts differently, depending on weed species. Meanwhile, this effect in broad-leaved weeds (*K. scoparia* and *A. retroflexus*) was more pronounced than in grassy weeds (*A. ludoviciana* and *P. minor*). The results indicated antagonism of glyphosate effectiveness by salts may be overcome with higher glyphosate doses. Overcoming the negative effects of salts in the spray solution by increasing the concentration of glyphosate depends on weed species. Increasing glyphosate dose did not alter the effect of hard water in the tested grassyweeds, but it did in *K. scoparia* and *A. retroflexus*. The addition of AMS in the spray solution at 2% (w/v) has potential to improve glyphosate efficacy for *A. retroflexus* control and, to a lesser extent, for control of *P. minor* and *A. ludoviciana*. However, at this rate, AMS could not overcome hard water antagonism for *K. scoparia*. Our studies provide information on how calcium chloride, calcium carbonate, sodium bicarbonate, and magnesium choloride can influence the performance of glyphosate on different weeds with or without AMS. Because of weed species responded differently to different doses of herbicide, cations type in spray solution, and AMS application, advice the same recommendation for herbicide application in all conditions of water hardness of spray solution, is not logic.

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