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Effect of an auxin-like herbicide 2,4-dichloro phenoxy acetic acid on proline content of mesquite (*Prosopis juliflora* Swarz) DC

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Abstract
2,4-dichloro phenoxy acetic acid (2,4-D), known as auxin-like herbicide or synthetic auxin, mimic the effect of the natural auxin indole-3-acetic acid (IAA). Afield and laboratory experiments were arranged to study the effect of (2,4-D) on the proline content of leaves of natural stand mesquite trees (*Prosopis juliflora*) treated with 2,4-D at different rates (0, 6×10^3, 12×10^3, 18×10^3 and 24×10^3 mg a. i. / L). Herbicide rates dissolved in two solvents (diesel or water), which 2,4-D mixtures sprayed around the lower part of the tree stem. The experiment designed in the factorial experiment in Randomized Complete Block Design (RCBD) with three replicates. The results showed that: High rate of 2,4-D (24×10^3 mg) gave a high concentration of proline, which gave 115,59 and 108,10 mg/g.: 142.25 and 145.60 mg/g in the two winter, and two rainy seasons, respectively. In both winter seasons leaves proline increased as the 2,4-D concentration increased dissolved in diesel with the three sizes, while in both rainy seasons, all 2,4-D concentrations dissolved in water or diesel increased proline content compared to the control. The results in this research confirmed that: 2,4-D herbicide led to a significant increase in leaves proline content of mesquite trees to a degree that lead to toxicity of the tree, which is one of the mechanisms of action of auxin overdose.

Keywords: 2,4-dichloro phenoxy Acetic Acid (2,4-D) herbicide, mesquite trees, proline

1. Introduction
Mesquite (*Prosopis juliflora*) is a major plant in the dry lands of Africa, America, and Asia (Burkart, 1976) [3]. There are about 44 species in the genus *Prosopis*, four spread globally, *P. juliflora, P. pallida, P. glandulosa, and P. relutina* (Pasiecznik *et al.* 2004) [18]. Mesquite first introduced into Sudan from Egypt and South Africa in 1917 with the purpose of solving the problem of desertification in some regions of Sudan (Brown and Massey, 1929) [14]. It is an important species because of its high nitrogen fixing potential in very dry areas and in drought seasons and also because it provides shelter and food to many species of animals Chemical analyses of *Prosopis* species have shown that the hole pods contain 9-17 % protein, 13-31% sucrose and 17-31 % crude fiber. These considered very useful sources of protein for animals (Almaraz *et al*. 2007) [1].
2,4-D used to control broad-leaf weeds in cereal crops. (Gervais *et al.* 2008) [7]. The uptake and translocation of 2,4-D are greater under conditions of higher temperatures and humidity (Peterson *et al.* 2016). High concentration of 2,4-D stimulated the production of reactive oxygen species (ROS) e.g., OH·, O_2·, H_2O_2, which promotes oxidative stress which damages proteins, lipids, and nucleic acids (Sandalio *et al.* 2012) [19]. Plants keep the ROS level in check by and accumulating different types of organic, which are solute, highly soluble and non-toxic. These organic compounds protected the plants from stress by contributing to cellular osmotic adjustment, Ludlow *et al.* (1985) [13]. The adjustment achieved by the accumulation of osmolytes such as amino acid proline, sucrose, glycine, and alanine (Serraj and Sinclair 2002) [15].
Proline accumulation increased in many organisms in higher plants during oxidative stress as a response to adverse conditions or as a stress signal, proline play important role in primary metabolism and physiologic functions, (Yang and Murphy, 2009) [23]. In higher plants, proline biosynthesis occurs via either the glutamate or the ornithine pathway. (Verslues and Sharam, 2010) [21]. Proline accumulates in the plant cells speedily and degrades quickly when needed (Trovato *et al.* 2008) [20]. Despite the beneficial effects of proline, it imparts toxic effects if over - accumulated at excessive concentrations (Heuer, 2003) [9].

The objective of this work was to study the effect of 2,4-D as an auxin-like herbicide on leaves of mesquite proline content.

2. Materials and Methods

2.1 Field Experiments

Field experiments conducted during two consecutive winter seasons 2016/ 2017 and 2017/2018 and two consecutive rainy seasons 2017 and 2018 in Khartoum state-Sudan.

Naturally grown mesquite trees were selected randomly at two locations and districted to three spots, 30 mesquite trees in each spot were carefully selected and classified for three sizes, (small, medium, and large) ten in each, based on a number of stems per tree and diameter of the canopy (in meters). Small trees have 1 to 2 stems and less than 2 meters for the canopy; medium trees have 3 to 5 stems and 2-3 meters for the canopy and large trees have more than 5 stems and more than 3 meters for the canopy. 2,4 -D was applied at five rates 0, 6×10^3, 12×10^3, 18×10^3 and 24×10^3 mg a.i. / L in tank mixture with water or diesel. In addition, water and diesel used individually as control. The application method basal bark treatment was used in which 2,4-D mixtures (water or diesel) were sprayed around the lower part of the tree stem at about 30 cm above the soil level (Geesing *et al*.2004) [6].

2.2 Laboratory Experiment: Leaves collected from treated trees and proline content were determined using the method recommended by Bates, (1973) [2]. The leaves collected from treated trees were homogenized in extraction buffer (3% w/v aqueous sulphosalicylic acid) (0.01g/0.5 ml) for each treatment separately in mortar. The homogenate centerfugated at 6000 rpm for 15 minutes. 2.0 ml of filterer reacted with 2.0 ml of acid ninhydrin and 2.0 ml of glacial acetic acid in a test tube: and heated at 100ºC for one hour in a water bath and the reaction terminated in cold water. The reaction mixture extracted with 4 ml toluene, mixed in a vortex for 10-15 seconds, and warmed to room temperature. The aqueous phase layers containing proline separated from the mixture and absorption read at 520 nm in a spectrophotometer used toluene as blank. The proline concentration calculated as mg ⁄ g of fresh weight using the standard curve.

2.3 Experimental design: The experiments arranged in factorial in Randomized Complete Block Design (RCBD) with three replicates. The experimental unit consisted of three mesquite trees of different sizes (small, medium, and large) for any treatment in the block. Data subjected to analysis of variance test (ANOVA) and means statistically separated by least significant difference (LSD) test using a computer statistical software, Statistix 8, and differences between means at (0.05) level of significance.

3. Results

The three tree sizes treated with different rates of 2,4-D dissolved in diesel significantly increased leaves proline content compared to the corresponding 2,4-D rates dissolved in water. High rate of 2,4-D (24×10^3 mg) gave high leaves proline content with small, medium and large, which gave (209.3 and 175.7 mg/g), (163.8 and 157.1 mg/g) and (169.6 and 205.1 mg/g) in winter and rainy seasons, respectively (Tables 1and 2).

The results in both winter and rainy seasons showed that: all rates of 2,4-D irrespective of solvent type and size of trees: significantly increased leaves proline content compared to the control. The high rate of 2,4-D (24×10^3 mg) gave high proline content, which gave 115.89, 108.1 mg/g and 142.25, 145.6 mg/g, respectively (Table 3). The diesel solvent irrespective of 2,4-D rates and size of trees significantly increased leaves proline content by 199.73%, 225.21%, and by 55.12% and 98.13% than water solvent in the first and second winter and rainy seasons, respectively (Table 4). The different sizes between trees showed non-significant differences (Table 5). Proline content, significantly increased with the interaction of the three tree sizes and diesel solvent than with interaction with water solvent by (261.99%, 174.15%, and 199.73%) and (230.03%, 222.34%, and 222.73%) in both winter seasons and by (102.01%, 32,.43% and 46.3%) and (95.12%, 7,5.06%, and 131.81%) in both rainy seasons for small, medium and large trees, respectively (Table 6).

The interaction of 2,4-D rates and the diesel solvent significantly increased leaves proline content compared to the control. (Table 7). The interaction between 2,4-D rates and the three sizes of the tree showed significant differences compared to the control (Table 8).

Table 1: Proline content of leaves from three sizes of mesquite trees (Small, medium, and large) treated with different concentrations of 2,4-D dissolved in water or diesel (Data are means of three replicates).

Tree size	2,4-D concentrations in (10^3) mg	Proline mg/g			
		First winter season		Second winter season	
		Diesel	Water	Diesel	Water
Small	0	26.7 ij	24.4 j	37.6 f	38.9 f
	6	57.9 efgh	30.3 hij	56.9 f	24.7 f
	12	174.5 b	37.1 ghij	164.0 abc	40.2 f
	18	112.3 c	41.1 ghij	131.7 cd	33.5 f
	24	209.3 a	27.5 ij	175.7 abc	34.1 f
Medium	0	62.1 efg	32.0 hij	44.6 f	33.3 f
	6	47.9 ghij	30.5 hij	67.8 f	40.2 f
	12	94.9 cd	41.7 ghij	134.5 cd	25.7 f
	18	156.1 b	43.5 ghij	187.5 ab	49.6 f
	24	163.8 b	43.3 ghij	157.1 bcd	34.7 f
Large	0	54.5 fghi	31.2 hij	41.5 f	37.5 f
	6	84.2 cde	21.9 j	70.0 ef	39.3 f
	12	79.3 def	35.3 ghij	114.1 de	36.0 f
	18	158.7 b	32.7 hij	175.7 abc	32.2 f
	24	169.6 b	79.9 def	205.1 a	41.9 f
SE±		14.3		22.8	
CV		23.81		26.81	

Means with the same letters are not significantly different according to the Least Significant to LSD

Table 2: Proline content of leaves from three sizes of mesquite trees (Small, medium and large) treated with different concentrations of 2,4-D dissolved in water or diesel (Data are means of three replicates).

Tree size	2,4-D concentrations in (10^3) mg	Proline mg/g			
		First winter season		Second winter season	
		Diesel	Water	Diesel	Water
Small	0	69.5 j	23.1 k	73.6 efghi	22.7 j
	6	108.0 defghi	64.4 j	121.8 c	97.1 cdefg
	12	114.8 cdefgh	79.8 hij	99.6 cdef	88.1 cdefgh
	18	145.9 abc	65.4 j	200.7 ab	69.4 fghi
	24	166.0 a	66.4 j	203.5 ab	77.0 defghi
Medium	0	81.9 ghij	22.7 k	72.9 efghi	24.0 j
	6	118.3 cdef	83.5 fghij	87.2 cdefgh	106.0 cde
	12	117.1 cdef	78.9 ij	109.1 cd	77.0 defghi
	18	135.3 abcd	119.1 bcde	179.6 b	70.8 efghi
	24	159.4 a	153.7 ab	231.9 a	111.1 cd
Large	0	83.9 fghij	20.3 k	78.9 defghi	23.9 j
	6	117.5 cdef	22.7 k	92.9 cdefg	104.3 cdef
	12	116.3 cdefg	88.2 efghij	120.9 c	53.4 hij
	18	162.1 a	85.7 efghij	208.9 ab	51.5 ij
	24	153.9 ab	159.4 a	183.4 b	62.5 ghi
SE±		17.6		17.7	
CV		21.2		20.82	

Means with the same letters are not significantly different according to the Least Significant Differences (LSD) test.

Table 3: Influence of different concentrations of 2,4-D on leaf's proline content from treated mesquite trees (Data are means of three replicates).

2,4-D con. in (10^3) mg	Proline mg/g			
	First Season		Second Season	
	Winter	Rainy	Winter	Rainy
Control	38.48 d	50.24 d	38.9 c	49.3 d
6	45.44 d	95.19c	49.8 c	101.5 c
12	77.14 c	99.16 c	85.8 b	91.4 c
18	90.78 b	118.94 b	101.2 ab	130.1 b
24	115.89 a	142.25 a	108.1 a	145.6 a
SE±	5.83	7.15	9.31	7.19

Means with the same letters are not significantly different according to the LSD test

Table 4: Effect of different solvent types on leaf's proline content from treated mesquite trees (Data are means of three replicates).

Solvent types	Proline mg/g			
	First season		Second season	
	Winter	Rainy	Winter	Rainy
Diesel	110.3 a	123.0 a	117.4 a	137.7 a
Water	36.8 b	79.3 b	36.1 b	69.5 b
SE±	3.69	4.52	5.89	4.54

Means with the same letters are not significantly different according to LSD test

Table 5: Effect of different tree sizes on leaf's proline content from treated mesquite trees (Data are means of three replicates).

Tree sizes	Proline mg/g			
	First season		Second season	
	Winter	Rainy	Winter	Rainy
Small	74.1 a	90.3 b	73.72 a	105.8 a
Medium	71.6 a	106.5 a	77.51 a	107.0 a
Large	74.7 a	106.7 a	79 a	98.1 a
SE±	4.52	5.54	7.21	5.57

This means with the same letters are not significantly different according to the LSD test

Table 6: Effect of the interaction of three tree sizes and solvent types on leaf's proline content from treated mesquite trees (Data are means of three replicates).

Tree sizes	Proline mg/g							
	First season				Second season			
	Winter		Rainy		Winter		Rainy	
	Water	Diesel	Water	Diesel	Water	Diesel	Water	Diesel
Small	32.1b	116.2 a	59.8c	120.8a	34.3b	113.2 a	71.7 bc	139.9 a
Medium	38,3b	105.0 a	91.6b	121.3a	36.7b	118.3a	77.8b	136.2a
Large	40.2b	109.0 a	86.6b	126.7a	37.4b	120.7a	59.1c	137.0 a
SD±	6.39		7.83		10.19		7.88	

This means with the same letters are not significantly different according to the LSD test

Table 7: Influence of the interaction of different concentrations of 2,4-D and two solvents (Diesel and water) on leaf's proline content from treated mesquite trees (Data are means of three replicates)

2,4-D co.in (10^3) mg	Proline mg/g							
	First season				Second season			
	Winter		Rainy		Winter		Rainy	
	Diesel	Water	Diesel	Water	Diesel	Water	Diesel	Water
0	47.75e	29.21f	78.44c	22.03d	41.2 ed	36.6d	75.1 de	23.5 f
6	63.33d	27.56f	114.6b	75.78c	64.9c	34.8d	100.6bc	102.4 bc
12	116.22 c	30.06ef	116.03b	82.29c	137.5b	34.0d	109.9b	72.8de
18	142.44b	39.11ef	147.78a	90.1c	164a	38.4d	196.4a	63.9e
24	180.92a	50.25de	158.0a	126.51b	179.3a	36.9d	206.3a	85.0 cd
SE±	5.83		10.11		13.16		10.17	

Means with the same letters are not significantly different according to the LSD test

Table 8: Influence of the interaction of different concentrations of 2,4-D and the three tree sizes on leaf's proline content from treated mesquite trees (Data are means of three replicates).

2,4-D co. in (10^3) mg	Proline mg/g											
	First season						Second season					
	Winter			Rainy			Winter			Rainy		
	Large	Medium	Small	Large	Medium	Small	Large	Medium	Small	Large	Medium	Small
0	42.9 gh	47.1 g	25.5 h	52.1 f	52.3 f	46.3f	39.5 d	39.0 d	38.2 d	51.4 f	48.4 f	48.1 f
6	53.1 fg	39.2 gh	44.1 gh	98.5de	100.9cde	86.2 e	54.7 cd	54.1 cd	40.8 d	98.6de	96.6 e	109.4 cde
12	57.3 ef	68.3 ef	105.8abc	102.2cde	98.0 de	97.3 de	75.1 bc	80.1 bc	102.1ab	87.1 e	93.1 e	93.9 e
18	957 cd	99.8 bc	76.9 de	123.9bc	127.2 b	105.7bcde	102.4 b	118.6 a	82.6 bc	130.2bc	125.2 bc	135.1 b
24	124.8a	103.6 bc	118.4 ab	156.7 a	153.9 a	116.2 bcd	123.5 a	95.9 ab	104.9 b	123.0bcd	171.5 a	142.4 b
SE±	10.10			12.38			16.12			12.46		

Means with the same letters are not significantly different according to the LSD

4. Discussion

In both seasons, 2,4-D increased leaves proline content irrespective of the variations of the properties of the solvent (diesel or water) compared to the control. This result is in line with that obtained by many authors, foliage spray *Eupatorium adenophorum* with picloram (auxin-like herbicide) at different concentrations increased the proline content as the herbicide concentration increased (Xiaowen *et al.* 2014) [22]. Proline content increased as the 2,4-D concentration increased in *Azolla pinnata* treated with different concentrations of 2,4-D (Kumar *et al.* 2016) [11]. In addition, Yashodhara and Gupta (2010) found that *Pisum sativum* treated with 2,4-D at different concentrations: increased proline with a 2,4-D concentration increase. 2,4-D mimics the natural plant hormone indole - 3- acetic acid (natural auxin) and activates auxin response genes (Korasick *et al.* 2015) [10]. This is thought to be 2,4-D at high concentration, which acts as herbicide and persists for a long time in the plant and acts as an auxin overdose, which leads to an imbalance in auxin and interaction with other hormones (Song, 2014) [16]. High rates of auxinic herbicides lead to oxidative stress through the unregulated generation of reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2) (Sandalio *et al.* 2012) [19]. 2,4-D (as auxinic herbicide) synthesis of 1-carboxylic acid, which is the key enzyme in ethylene biosynthesis that stimulated H_2O_2 (Lin *et al.*2009) [12]. H_2O_2 conceder second messenger for the synthesis of abscisic acid (ABA), which leads to stoma closure and limiting CO_2 assimilation followed by a reduction in photosynthesis (Grossmann, 2010) [8]. H_2O_2 at low concentration act as signal molecules and regulate the expression of a large number of genes involved in cell response to different stress conditions and development (Mittler *et al.* 2011) [14]. However, a high accumulation of H_2O_2 promotes oxidative damage to proteins, lipids, and nucleic acids (Sandalio *et al.* 2012) [19]. In response to oxidative stress, plants accumulate large quantities of proline to keep the ROS level in check (Serraj and Sinclair, 2002) [15].

5. Conclusion

The results of the present study indicated that: in the two seasons 2,4-D increased leaves proline content irrespective of the variations of the properties of the solvent (diesel or water) and the sizes of the tree, to a degree that led to the toxicity of mesquite trees.

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