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Effect of Synthetic Auxin on Water Content of Seedlings of *Prosopis juliflora Schwarz* DC

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ABSTRACT

This study evaluated the effect of 2,4-D on the water content of the shoot and root of mesquite seedlings, which were raised from seeds collected from trees treated during the flowering stage with various weights of 2,4-D (6, 12, 18, and 24 grams a.i.), dissolved in either diesel or water. The results showed that seedlings grown from seeds collected from different tree sizes treated with 12, 18, and 24 grams a.i dissolved in diesel, and those treated with 24 grams a.i dissolved in water, exhibited a significant decrease in shoot water content compared to the control group. Additionally, the root water content of seedlings from seeds collected from trees treated with 18 and 24 grams a.i. of 2,4-D dissolved in either diesel or water also showed a significant decrease compared to the control. Overall, the study demonstrated a reduction in water content of seedlings raised from seeds of mesquite trees treated with 2,4-D. This reduction in water content weakened the establishment of the seedlings and ultimately led to their death in the early stages

Keywords: *Mesquite shoot, root, 2,4-D, diesel, and water*

1. Introduction

The synthetic auxin 2,4-dichlorophenoxy acetic acid (2,4-D) mimics the effects of the natural auxin indole-3-acetic acid (IAA) in plants [1]. Unlike IAA quickly inactivated, 2,4-D persists for extended periods. This prolonged presence can create an imbalance in auxin levels and disrupt interactions with other hormones at the tissue level[2]. 2,4-D accumulates in chloroplasts and is metabolized within these organelles. At low doses, 2,4-D can promote plant growth. However, at high doses, it leads to overgrowth characterized by cupping and stunting leaves, brittleness, stunting and twisting of stems, and overall abnormal growth. High concentrations of 2,4-D induced the enzyme aminocyclopropane-1-carboxylic acid (ACCS), which increased ethylene biosynthesis. This is followed by an accumulation of abscisic acid (ABA) [3]. The effects of high 2,4-D concentrations include abnormal growth patterns, reductions in stomata aperture, and overproduction of reactive oxygen species (ROS). These changes can lead to chloroplast damage, progressive chlorosis, and the destruction or blockage of the membrane and vascular system in the stem [3].

2,4-D is actively transported into plant cells via a common carrier protein. Metabolism of 2,4-D in plants occurs through direct conjugation and ring hydroxylation. In sensitive dicots, 2,4-D can bind to the TIR1, which leads to the expression of auxin-responsive genes. However, the ring structure of 2,4-D is not hydroxylase by metabolic enzymes. The direct conjugation of 2,4-D with amino acids or glucose results in phytotoxic metabolites that can be hydrolyzed back to 2,4-D[4]. In intolerant monocots, the ring hydroxylation of 2,4-D leads to a non- or partially phytotoxic metabolite[5]. This research aims to study the effect of 2,4-D on the water content of mesquite seedlings raised from seeds collected from 2,4-D-treated trees.

2. Materials and Methods

2-1. Plant Materials, chemical solutions, and application methods

An experiment was conducted in about four hectares, divided into three replicates. In each replicate, thirty mesquite trees were carefully selected and classified into three sizes: small, medium, and large. Each size group consisted of ten trees categorized based on tree height and the number of branches per tree. The experiment took place during the flowering stage of the mesquite trees. Before herbicide application, ten inflorescences were chosen and marked on each tree. The herbicide 2,4-D was applied at four weights: 6, 12, 18, and 24 grams (a.i.). Each weight was dissolved in either one liter of water or diesel. Additionally, water and diesel were used as individual controls for comparison. The mixture was applied as a directed spray to the base of the stem, approximately 20-30 cm above ground level, on each of the selected trees [6].

2-2. Pods collection, seeds prepared, planting, and data collection

The ripe pods from the marked inflorescences were collected from each treatment within sixty days of application. The seeds were extracted from pods using a sharp knife and scissors. Seed dormancy was broken by soaking it in 98% sulfuric acid for a few minutes and in distilled water for 24 hours. Twenty seeds from each treatment were planted in plastic bags, each measuring 40 cm in diameter, and equipped with holes in the bottom. The bags were filled with a 5 kg mixture of loam and sand in a 1:1 volume ratio. These plastic bags were kept under temperature and day-length conditions, and seeds were irrigated every two days.

One month after planting, the seedlings from each treatment were removed from the bag and separated into stems and roots. The Mean fresh weight of both was calculated. The shoot and root were dried in the air for three days, and in an oven at 70 °C, until the constant weights were achieved. and the water content for both parts was calculated [7].

Fresh weight – dry weight × 100
fresh weight

2-3. Experimental design and data analysis

The experiments were arranged as a Factorial Randomized Complete Block Design (FRCBD) with three replications for each treatment. Data were analyzed using analysis of variance (ANOVA). Means were compared for significance with the least significant difference (LSD) test at a significance level of 0,05.

3. Results and Dissuasion

All weights of 2,4-D tested achieved significant differences in shoots and roots water content compared to the control group. The interactions of the weights of 12, 18, and 24 grams a.i. of 2,4-D with diesel solvent resulted in notable differences in shoot water content compared to the control. All tested weights of 2,4-D when combined with diesel or water significantly reduced root water content relative to the control (Table 1).

Diesel solvent significantly decreased shoot water content compared to water solvent. However, no significant difference in root water content between the two solvents. Additionally, there were no significant differences in shoot and root water content among the seedlings from seeds collected from trees of different sizes, small, medium, and large. The interaction between tree sizes and diesel solvent resulted in a significant decrease in shoot water content compared to the water solvent, but root water content showed no significant differences across the interaction of tree sizes and the two solvents (Table 2).

The interaction between the application of 24 grams a.i. of 2,4-D and the three tree sizes resulted in significant differences in shoot water content compared to the control. The seedlings from the three tree sizes were treated with either 18 grams **o**r 24 grams a.i. of 2,4-D dissolved in diesel or 24 grams a.i. Dissolved in water exhibited a significant decrease in shoot water content compared to the control. There were no significant differences in shoot water content of seedlings raised from the seeds collected from medium and large trees treated with 6, 12, and 18 grams a.i. of 2,4-D dissolved either in diesel or water compared to the control (Table 3).

The interaction between various amounts of 2,4-D and the three tree sizes resulted in significant differences in root water content compared to the control. The root water content of seedlings grown from seeds collected from the three tree sizes and treated with 18 and 24 grams a.i. of 2,4-D dissolved in either diesel or water was significantly lower than that of the control group (Table 4).

The results in the present study indicated that the use of the herbicide 2,4-D during **t**he flowering stage of mesquite trees led to a decrease in the shoot and root water content of seedlings as the concentration of 2,4-D increased. These findings are consistent with those of [8] who reported that; the use of 2,4-D as a soil application at 1000 g a.i. ha^{-} applied at various times before sowing soybeans decreased both the fresh and dry weight of shoots and roots. Similarly, [9] found that applying 2,4-D at five different rates (1-5 ppm) resulted in a reduction in the fresh and dry weight of *Abelmoschusesculentus*after five days of treatment Additionally, *Cassia tora*also treated with different rates of 2,4-D, resulted in a decrease in the dry and fresh weight of both shoots and roots, correlating with increased concentration of the herbicide [10].

In the mesquite stem (dicotyledoneae), the vascular tissues are arranged in rings and contain a cambium. In this case, [11] reported that synthetic auxins are not metabolized as quickly as the endogenous auxin indole-3-acetic acid IAA. In contrast, monocotyledoneae stems have vascular tissues that are scattered in bundles and lack a vascular cambium, in these stems, synthetic auxins can rapidly inactivate through conjugation. Mesquite pods ripen 60 days after flowering[12]. This extended period allows for the absorption of 2,4-D into the seed coat, increasing its concentration within the seeds, and affecting them as a pre-emergence herbicide. At high concentrations, 2,4-D acts as an herbicide and remains persistent in the plant for an extended time [2]. This led to an overproduction of ethylene, which stimulates the production of hydrogen peroxide (H2O2) [13]. Excess H2O2 can cause oxidative damage, resulting in reduced plant biomass production [14] and the destruction of xylem tissues [3]. Additionally, alkanes and polycyclic aromatic hydrocarbons from diesel increased the toxicity, further reducing the growth of both stems and roots [15].

Table 1: Effect of 2,4-D and solvent types on shoot and root water contents

2,4-D (grams a.i.)	Shoot water content		Effect of 2,4-D weights	Root water content		Effect of 2,4-D weights
	Diesel	Water		Diesel	Water	
Control	67.11 ab	67.61 ab	67.36 a	62.72 a	63.8 a	63.26 a
6	61.08 bc	60.84 bc	60. 96 b	52.33 b	48.00 b	50.17 b
12	56.69 cd	69.68 a	63.19 ab	54.42 b	50.82 b	52.62 b
18	51.27 d	65.53 ab	58.4 b	48.73 b	52.49 b	50.61 b
24	51.2 d	68.4 ab	59.8 b	51.84 b	51.13 b	51.49 b
	2,4-D xSolvents		2,4-D	2,4-D xSolvents		2,4-D
SE±	4.26		3.01	3.83		2.71

Table 2: Effect of Tree sizes, solvent types, and the interaction between tree sizes and solvent types on shoot and root water contents

Tree sizes	Shoot water content		Tree sizes	Root water content		Tree sizes
	Diesel	Water		Diesel	Water	
Small	55.99 c	65.92 ab	60.96 a	53.24 ab	54.88 ab	54.06 a
Medium	59.66 bc	66.16 ab	62.91 a	54.82 ab	49.36 b	52.09 a
Large	56.77 c	67.16 a	61.97 a	53.96 ab	55.49 a	54.73 a
Effect of Solvents	57.47 b	66.41 a		54.00 a	53.24 a	
	Tree sizes xsolvent types	Tree sizes	Solvent types	Tree sizes xsolvent types	Tree sizes	Solvent types
SE±	3.30	2.33	1.90	2.96	2.10	1.71

Table 3: Effect of the interaction of 2,4-D weights, tree sizes, and solvent types, and the interaction of 2,4-D weights and tree sizes on shoot water content

2,4-D weights (grams a.i.)	Tree sizes	Solvent types		2,4-D weights x Tree sizes
		Diesel	Water	
0	Small	66.25 abcde	75.33 a	70.81a
6	Small	62.21 abcdefg	66.80 abcde	64.51ba
12	Small	53.35 efgh	69.89 abc	61.12 ab
18	Small	55.12 fgh	67.27 abcde	61.20ab
24	Small	48.12 gh	50.36 fgh	49.24 b
0	Medium	67.03 abcde	71.79 ab	69.41 a
6	Medium	62.72 abcdefg	66.54 abcde	64.63 ab
12	Medium	56.90 cdefgh	64.33 abcdef	60.62 ab
18	Medium	56.48 cdefgh	69.61 abcd	63.05 ab
24	Medium	50.39 fgh	63.27 abcdef	56.83b
0	Large	63.30 abcde	75.13 a	69.22 a
6	Large	58.31 bcdefg	65.61 abcde	61.96 ab
12	Large	59.81 bcdefg	69.49 abcd	64.65 ab
18	Large	54.32 fgh	65.86 abcde	60.09ab
24	Large	48.12 gh	59.7 bcdefg	53.31 b
	2,4-D weights x Tree sizes x Solvent types			2,4-D weights x Tree sizes
SE±	7.37			5.21

Table 4: Effect of the interaction of 2,4-D weights, tree sizes, and solvent types, and the interaction of 2,4-D weights and tree sizes on root water content

2,4-D weights (grams a.i.)	Tree sizes	Solvent types		2,4-D weights x Tree sizes
		Diesel	Water	
0	Small	62.85 abc	64.11 ab	63.48 a
6	Small	56.80abcdefg	57.07 abcdefg	56.94 c
12	Small	54.73abcdefg	56.21abcdefg	55.47 c
18	Small	44.44 gh	52.37cdefgh	48.41 c
24	Small	47.37 efgh	44.67 gh	46.02c
0	Medium	62.85 abc	61.92 abcd	62.39a
6	Medium	54.06abcdefgh	51.52 cdefgh	52.79 c
12	Medium	53.33defgh	47.54efgh	50.44c
18	Medium	51.98efgh	44.89fgh	48.44c
24	Medium	50.52efgh	41.95 h	46.24 c
0	Large	60.21 abcde	65.36 a	62.79 ab
6	Large	54.47abcdefgh	58.14abcdef	56.31 c
12	Large	56.15 fg	56.38abcdefg	56.27 c
18	Large	51.57fgh	48.83defgh	50.20c
24	Large	48.42 fgh	48.77defgh	48.60 c
	2,4-D weights x Tree sizes x Solvent types			2,4-D weights x Tree sizes
SE±	6.63			4.69

4. Conclusion

This research demonstrated how an auxin-like herbicide (2,4-D) reduced the water content in mesquite seedlings, which hindered their establishment and ultimately resulted in their death during the early stages.

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