

## **Influence of 2,4-dichloroPhenoxyAcetic Acid on Seeds Germination and Early growth of Mesquite (*Prosopis juliflora* Swarz) DC Seedlings.**

**Abstract:** This study was conducted to evaluate the effect of 2,4-dichlorophenoxy acetic acid (2,4-D) on seed germination and the early growth of mesquite (*Prosopis juliflora*) seedlings. The three sizes of mesquite at the flowering stage (small, medium, and large) were treated with 2,4-D at different rates (0,  $6 \times 10^3$ ,  $12 \times 10^3$ ,  $18 \times 10^3$  and  $24 \times 10^3$  mg a.i. / L) dissolved in two solvents (diesel or water), the application method basal bark treatment was used in the two different seasons. The results showed that: the three factors (2,4-D rates, tree sizes, and solvent types) significantly decreased germination percentage with the increase of 2,4-D rate dissolved in diesel with all sizes of trees, while the emergence percentage, radical length, and shoot height were significantly decreased as 2,4-D rate increase dissolved in diesel or water. In the winter season, the root length of seedlings raised from seeds collected from the three tree sizes treated with 2,4-D dissolved in water was significantly decreased as a 2,4-D rate increased, while in the rainy season, the root length was affected with 2,4-D dissolved in diesel. In addition, the effect of 2,4-D rates and solvent types was evaluated for all parameters, the results found that: 2,4-D dissolved in diesel significantly decreased germination percent than the corresponding rates dissolved in water. Emergence percentage and shoot height and radical length were significantly decreased with 2,4-D dissolved in diesel or water compared to their control. In the winter season, the root length is affected by 2,4-D dissolved in water, while in the rainy season affected by 2,4-D dissolved in diesel.

**Keywords:** Mesquite, seedlings, emergence, 2,4-D, shoot height and roots length germination, radical length.

### **1. Introduction**

The fruits of mesquite (*P. juliflora*) known as pods are green, becoming commonly white-yellow when fully mature after 60 days of flowering (Harden and Reza, 1988). The pods of mesquite do not open at maturity, both domestic and wild animals play a main role in breaking the dormancy

of mesquite seeds by grazing upon the pods, the un-chewed seeds passing through the guts of animals that, enhances seeds germination(Almaraz *et al.* 2007).Mesquite trees commonly propagated by producing a large number of small-sized seeds: about 25 per pod,germinated, seeds expended most of their energy in developing a root system and locating a water source as soon as possible (Pasiiecznik *et al.* 2001).The primary product of photosynthesis in higher plants is starch, which is stored as a carbohydrate that supports metabolism and growth during the dark when photosynthesis is not possible (Zeeman *et al.* 2004).The mobilization of starch by the  $\alpha$ -amylase enzyme initiated seed germination (Fincher, 1989). In addition, nitrogen compounds convert stored starch to soluble sugar, whichneeded for germination and adjusting the potassium and sodium ratio; and increasing the production of ATP and seed respiration (Zheng *et al.* 2009). 2,4-dichloro phenoxy acetic acid (2,4-D) mimics the effects of the natural auxin (Indole- 3-acetic acid) (IAA) in plants (Pazmiño *et al.*2012). There are two types of formulations of 2,4-D: amine salts and esters (Peterson *et al.* 2016). Natural auxins IAA usually inactivated very quickly through conjugation and degradation in the plant, while synthetic auxins such as 2,4-D persist for long periods of time within the plant, this phenomenon is described as an auxin overdose which leads to an imbalance in auxin and interactions with other hormones at the tissue level (Song, 2014).

Chloroplasts have been considered the initial target of 2,4-D- since the chemical is mainly accumulated and subsequently metabolized in these organelles (Grossmann, 2009). Plants

usually metabolize herbicides via processes that convert the parent molecule to more polar products and insoluble residues (Hatzios *et al.* 2005). The selectivity of auxinic herbicides may be due to the vascular tissue structure of dicot (Scarpella *et al.* 2006), which possesses a cambium and the synthetic auxin not metabolized as quickly as the endogenous auxin (Taiz and Zeiger, 2013). Application of 2,4-D herbicide inhibited the seed germination percentage by penetrating the seed coat and is effective as a pre-emergence treatment. Auxins like 2,4 -D are metabolically broken down by the isozyme oxidase with the release of phenols, which accumulated in the tissues and inhibit the germination of seeds (Sathiyamoorthy 1990).

This work aims to evaluate the effect of 2,4-D on the germination percentage and the radical length, emergence percentage, and shoot height, root length of seeds collected from 2,4-D-treated mesquite trees at the flowering stage.

## **2. Materials and Methods**

### **2-1. Field Experiments**

#### **2-1-1. Sites of the field experiments**

A Field experiment was conducted during the winter season of 2017/ 2018 and the rainy season of 2018 in the demonstration farm of the Faculty of Agriculture University of Khartoum- Sudan.

The site lies at lat.15, 40 N, long 32, 32E, in a semi-arid zone, characterized by a great variation

in temperature, which ranges from 18° C to 40° C, and relative humidity of 34% to 75% (Gabbani, 2007).

### **2-1-2. Planting Materials**

Naturally stand mesquite trees were carefully selected in the two locations, selected trees were districted to three spots, and for each spot, thirty mesquite trees were carefully selected and classified for three sizes (small, medium, and large), ten in each based on the number of stems per tree and diameter of the canopy (in meters). Small trees, 1 to 2 stems and less than 2 meters for canopy, medium trees, 3 to 5 stems and 2-3 meters for canopy and large trees, more than 5 stems and more than 3 meters for canopy. At the flowering stage, ten inflorescences in any tree (small, medium, and large) were randomly selected, and marked before treatment application.

### **2-1-3. Chemical solutions and application method**

2,4 -D was applied at four rates  $6 \times 10^3$ ,  $12 \times 10^3$ ,  $18 \times 10^3$  and  $24 \times 10^3$  mg a.i. / L in tank mixture with water or diesel. In addition, water and diesel are used individually as control. The application was conducted in two seasons (Winter and rainy season), method of basal bark treatment was used in which 2,4-D mixtures were sprayed around the lower part of the tree stem at about 30 cm above the soil level (Geesing *et. al.*2004).

### **2-2. Collection of Pods and seed preparation**

Ripen pods produced from marked inflorescences were collected in paper bags from any group of treated trees (small, medium, and large) from any block, seeds were removed from pods using

a Sharpe knife and scissors and used immediately after extraction. Dormancy broken by soaking the seeds in sulfuric acid (abt. 98%) for a few minutes and washing them thoroughly with distilled water until the acid removed; the seeds are soaked for 24 hours in distilled water.

### **2-3. Germination Experiment and data collection**

Ten seeds from any treatment placed in a Petri – dish (9-cm. id) lined with two Whatman filter papers moistened with 2.5 ml of distilled water. Petri - dishes wrapped in black polyethylene under laboratory conditions and subsequently examined for germination when the radical protruded through the seed coat, the germination percent was calculated, and the radical length measured after one week.

### **2-4. Emergence Experiment and data collection**

Ten seeds from any treatment were transferred to the greenhouse and planted in polyethylene bags, (ten seeds in each), filled with 5 kg of soil mixed (1:1 v/v) loam and sand (40 cm. in diameter) with drainage holes in the bottom. The experiments conducted during the period from May to November 2017 and from January to March 2018 for seeds collected from trees treated in the winter and rainy seasons, respectively. The polyethylene were bags kept in the greenhouse under natural conditions of temperature and day length and irrigated every two days. The seedling emergence percent was watched over a period of 15 days and the emergence percent was calculated. After one month, seedlings taken out of the bags for any treatments and cleaning, and seedling height: and root length measured in cm.

### **2-5. Experimental design and data analysis**

Field 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. Treated with five rates of 2,4-D, dissolved in two solvents (Table 1). The laboratory experiment arranged in factorial in a Completely Randomized Design (CRD) with three replicates. Data subjected to analysis of variance test (ANOVA) and means statistically separated by least significant difference test (LSD) using a computer statistical software, Statistix 8, and differences between means at (0.05) level of significance.

Table 1: The different treatments and 2,4-D rates

Treatment	2,4-D rate.in (10 <sup>3</sup> ) mg
S/D/(2,4-D)/R0	2,4-D at rate zero dissolved in diesel
S/D/(2,4-D)/R1	2,4-D at rate 6 dissolved in diesel
S/D/(2,4-D)/R2	2,4-D at rate 12 dissolved in diesel
S/D/(2,4-D)/R3	2,4-D at rate 18 dissolved in diesel
S/D/(2,4-D)/R4	2,4-D at rate 24 dissolved in diesel
S/W/(2,4-D)/R0	2,4-D at rate zero dissolved in water
S/W(2,4-D)/R1	2,4-D at rate 6 dissolved in water
S/W(2,4-D)/R2	2,4-D at rate 12 dissolved in water
S/W/(2,4-D)/R3	2,4-D at rate 18 dissolved in water
S/W/(2,4-D)/R4	2,4-D at rate 24 dissolved in water
M/D/(2,4-D)/R0	2,4-D at rate zero dissolved in diesel
M/D/(2,4-D)/R1	2,4-D at rate 6 dissolved in diesel
M/D/(2,4-D)/R2	2,4-D at rate 12 dissolved in diesel
M/D/(2,4-D)/R3	2,4-D at rate 18 dissolved in diesel
M/D/(2,4-D)/R4	2,4-D at rate 24 dissolved in diesel
M/W/(2,4-D)/R0	2,4-D at rate zero dissolved in water
M/W/(2,4-D)/R1	2,4-D at rate 6 dissolved in water
M/W/(2,4-D)/R2	2,4-D at rate 12 dissolved in water
M/W/(2,4-D)/R3	2,4-D at rate 18 dissolved in water
M/W/(2,4-D)/R4	2,4-D at rate 24 dissolved in water
L/D/(2,4-D)/R0	2,4-D at rate zero dissolved in diesel
L/D/(2,4-D)/R1	2,4-D at rate 6 dissolved in diesel
L/D/(2,4-D)/R2	2,4-D at rate 12 dissolved in diesel
L/D/(2,4-D)/R3	2,4-D at rate 18 dissolved in diesel
L/D/(2,4-D)/R4	2,4-D at rate 24 dissolved in diesel
L/W/(2,4-D)/R0	2,4-D at rate zero dissolved in water
L/W/(2,4-D)/R1	2,4-D at rate 6 dissolved in water
L/W/(2,4-D)/R2	2,4-D at rate 12 dissolved in water
L/W/(2,4-D)/R3	2,4-D at rate 18 dissolved in water
L/W/(2,4-D)/R4	2,4-D at rate 24 dissolved in water

S=Small, M=Medium and L=Large

R=Rate, D=Diesel, W=Water

### 3. Results

#### 3-1. Effect of 2,4-D, solvent types, and tree sizes on seeds germination percentage of seeds from the three tree sizes

Table 2 shows the effect of 2,4-D on the germination percentage of seeds collected from the three mesquite tree sizes treated with different rates of 2,4-D dissolved in diesel, showing a significant decrease in germination percent with an increase of 2,4-D rate. The results showed that the two high rates of 2,4-D ( $18 \times 10^3$  and  $24 \times 10^3$  mg a.i.) dissolved in diesel, decreased the germination percentage of seeds collected from three tree sizes compared to the control. The overall mean of the effect of 2,4-D dissolved in diesel on germination percentage was decreased by 26.52%, 26.4%, 32.57%, and 47.37%, 25.51%, 47.44% than, the water solvent for small, medium, and large trees in the winter and rainy seasons, respectively.

Table 2: Effect of 2,4-D, solvent types, and tree sizes on germination percentage of seeds collected from different tree sizes

Treatment	Germination Percentage					
	Winter season			Rainy season		
	S	M	L	S	M	L
D2,4-DR0	93.3 ab	93.3 ab	93.3 ab	70.0 defg	86.7 abcd	76.7 cdef
D2,4-DR1	83.3 bcd	90.0 abc	83.3 bcd	83.3 abcde	76.7 cdef	76.7 cdef
D2,4-DR2	70.0 def	60.0 fg	63.3 efg	80.0 bcdef	63.3 fg	56.7gh
D2,4-DR3	40.0 h	63.3 efg	63.3 efg	40.0 h	70.0 efg	63.3 fg
D2,4-DR4	53.3 gh	60.0 fg	53.3 gh	43.3 h	63.3 fg	56.7 gh
Mean	67.98	73.32	71.3	63.32	72.00	66.02
W2,4-DR0	86.7 abc	96.7 ab	100.0 a	100.0 a	100.0 a	100.0 a
W2,4-DR1	93.3 ab	96.7 ab	96. ab	96.7 ab	96.7 ab	100.0 a
W2,4-DR2	76.7 cde	90.0 abc	93.3 ab	100.0 a	93.3 abc	90.0 bc
W2,4-DR3	83.3 bcd	96.7 ab	90.0 abc	66.7 efg	93.3 abc	96.7 ab
W2,4-DR4	90.0 abc	83.3 bcd	93.3 ab	100.0 a	100.0 a	100.0 a
Mean	86.00	92.68	94.52	92.68	96.66	97.34
SE±	8.07			9.24		

**3-2. Effect of 2,4-D, solvent types, and tree sizes on seed emergence percentage of seedlings from the three tree sizes**

The statistical analysis of variance indicated significant differences ( $P= 0.05$ ) in response to the effect of the two high rates of 2,4-D dissolved in diesel or water in seed emergence percent of mesquite seeds in the two seasons. The decrease of emergence percentage in response to 2,4-D was increased in the rainy season than in winter season for seeds collected from the three tree sizes. The overall mean of the emergence percentage were decreased with 2,4-D dissolved in diesel than dissolved in water.

Table 3: Effect of 2,4-D, solvent types, and tree sizes on the emergence percentage of seeds collected from different tree sizes

Treatment	Emergence Percentage					
	Winter season			Rainy season		
	S	M	L	S	M	L
D2,4-DR0	53.33 fghijk	70 abcdefg	70.0 abcdefg	63.33 abc	56.67 cde	46.67 cdef
D2,4-DR1	56.67 efghij	56.67 efghij	66.7 bcdefgh	46.67 cdef	16.67 ghi	20.00 ghi
D2,4-DR2	43.33 im	63.33 cdefghi	50.0 ghijk	30.00 ghi	13.33 hi	20.00 ghi
D2,4-DR3	26.67 nop	23.33 mnop	46.67 ki	10.00 i	10.00 i	16.67 ghi
D2,4-DR4	13.33 op	10.00 p	23.33 mnop	10.00 i	10.00 i	10.00 i
Mean	38.67	44.67	50.14	32.00	21.33	22.67
W2,4-DR0	83.33 ab	86.67 ab	90.00 a	80.00 ab	73.33 ab	86.67 a
W2,4-DR1	73.33 abcdef	60.00 defghi	76.67 abcde	73.33 ab	63.33 abc	76.67 ab
W2,4-DR2	80.00 cd	60.00 defghi	63.33 cdefghi	46.67 cdef	60.00 bcd	40.00 cdefg
W2,4-DR3	63.33 cdefghi	46.67 hijkl	70.00 bcdefg	26.67 fghi	46.67 cdef	36.67 defgh
W2,4-DR4	33.33 klmno	36.67 jklmn	66.7 bcdefgh	33.33 efghi	56.67 cde	30.00 fghi
Mean	66.66	58.00	73.34	52.00	60.00	54.00
SE±	10.60			13.67		

### **3-3. Effect of 2,4-D, solvent types, and tree sizes on radical length, shoot height, and root length of seedlings from the three tree sizes**

A greater mean value of shoot height and root length were observed in seedlings raised from seeds collected from mesquite trees treated in the winter season compared to that raised from rainy season seeds (Table 4). It found that all treatments in the winter season gave higher values of shoot height and root length compared to their respective treatments in the rainy seasons. In the rainy season, there were non-significant differences in shoot height between all 2,4-D rates dissolved in diesel, while all 2,4-D rates dissolved in water significantly decreased shoot height compared to the control. In the winter season, the three high rates of 2,4-D dissolved in diesel or water: significantly decreased the shoot height of seedlings. Seedlings raised from seeds collected from treated trees with 2,4-D dissolved in diesel in the winter season showed non-significant differences in root length, while the three high rates of 2,4-D dissolved in water significantly decreased root length compared to the control. In the rainy season, all 2,4-D rates dissolved in diesel significantly decreased root length, while only the high three rates dissolved in water significantly decreased root length compared to the control. In addition, the two high rates of 2,4-D dissolved in diesel or water significantly decreased the radical length in both seasons (Table 4).

Table 4: Effect of 2,4-D, solvent types and tree sizes on radical length, shoot height and root length

Treatment t	Radical length						Shoot height						Root length					
	Winter			Rainy			Winter			Rainy			Winter			Rainy		
	S	M	L	S	M	L	S	M	L	S	M	L	S	M	L	S	M	L
D2,4-DR0	9.03 ab	6.87 abc d	9.33 a	7.77 abcd e	6.00 efg h	5.67 efgh i	32.73 abc	32.83 abc	30.83 abcde	11.9 0 b	8.60 bcde f	8.93 bcdef g	19.60 cdef	22.17 abcde f	26.27 abc	16.0a	14.77ab	12.23 cdefgh i
D2,4-DR1	8.03 abcd	7.00 abcd d	9.03 ab	7.87 abcd	4.80 ijkl	5.33 efgh i	28.97 abcdef g	28.67 abcdef g	31.30 abcd	10.6 7 bcd	9.60 bcde f	9.66 bcdef f	22.97 abcdef f	21.77 bcdef	26.43 abc	10.67ghijklm n	10.27ijklm n	9.93 klmn
D2,4-DR2	6.50 cd	7.83 abcd d	5.70 efg h	6.47 efgh	5.17 ijk	5.20 fghi	24.20 efg	24.43 efg	23.83 fg	10.1 0 bcde f	9.60 bcde f	6.47 fg	20.00 bcdef	18.07 def	20.03 bcdef	11.10 Fghijklm	9.00n	9.00 n
D2,4-DR3	4.47 efg	5.50 efg h	4.90 efg h	3.50 n	4.67 lm	3.67 m	25.33 defg	23.83 fg	23.57 g	8.50 bcde f	6.83 efg	4.00 g	19.27 cdef	20.57 bcdef	19.63 cdef	10.50 Hijklmn	9.67lmn	9.63 mn
D2,4-DR4	4.60 efg h	3.27 i	4.27 gh	3.83 n	3.80 n	3.50 m	24.30 efg	23.77 fg	23.33 g	8.50 bcde f	6.67 efg	4.00 g	17.73 ef	17.97 def	18.33 cdef	6.63 o	10.1jklmn	9.67 lmn
Mean	6.54	6.09	6.66	7.09	6.09	4.67	27.11	26.71	26.57	9.93	8.26	6.61	19.91	20.11	22.14	10.98	10.76	10.09
W2,4-DR0	9.13 a	9.33 a	8.00 abcd d	8.03 abc	8.57 a	8.33 abc	30.67 abcde	33.27 ab	34.87 a	30.1 0 a	29.6 7 a	32.20 a	27.93 ab	30.10 a	26.00 abcd	14.0abc	12.43 cdefgh	12.83 bcdef
W2,4-DR1	6.57 bcd	8.77 abc	8.57 abc	8.43 ab	8.10 abc	8.00 abc	30.23 abcdef	29.50 abcdef g	27.27 bcdef g	11.2 0 bc	11.3 3 bc	10.77 bcd	24.77 abcde f	21.70 bcdef	20.86 bcdef	13.87bc	12.07 cdefghij	12.30 cdefgh i
W2,4-DR2	8.23 abcd	7.40 abcd d	5.80 efg	7.57 abcd	7.47 abcd d	7.20 bcd	24.90 defg	25.63 defg	24.70 defg	11.0 0 bc	11.0 0 bc	10.43 bcde	17.30 ef	19.10 cdef	16.73 f	13.4bcde	12.67 cdefg	12.40 cdefgh
W2,4-DR3	5.10 efg h	7.10 bcd	6.00 efg	6.60 defg	7.33 bcd	6.50 ij	29.90 abcdef g	25.17 defg	26.57 bcdef g	11.8 3 b	8.60 bcde f	9.43 bcdef	23.43 abcde f	19.73 cdef	16.56 f	12.77bcdef	11.33 fghijklm	12.23 cdefgh i
W2,4-DR4	5.40 efg h	7.07 bcd	5.80 efg	6.33 fg	7.10 cd	6.23 ijk	26.40 cdefg	24.07 efg	25.80 defg	9.43 bcde f	10.0 0 bcde f	8.00 cdef	19.73 cdef	20.33 bcdef	16.67 f	11.87defghij k	11.73 efghijkl	8.67 no
Mean	6.89	7.93	6.83	7.39	7.71	7.25	28.42	27.53	27.82	14.7 1	14.1 2	14.17	22.63	22.19	20.07	11.20	12.07	11.69
SE±	1.26			0.66			3.40			1.88			4.02			1.05		

### **3-4. Effect of 2,4-D, and solvents types on germination %, emergence %, radical length Shoot height, and root length**

All rates of 2,4-D dissolved in diesel solvent had significantly less germination percent and radical length compared to the corresponding rates dissolved in water. The two high rates of 2,4-D ( $18 \times 10^3$  and  $24 \times 10^3$  mg a.i.) gave less germination percent, which gave (55.6%, 55.6%) and (57.8%, 54.4%) and less radical length (4.96 cm, 4.04 cm) and (4.61 cm, 3.71 cm) in winter and rainy seasons, respectively. In the rainy season, all 2,4-D rates dissolved in diesel significantly decreased emergence percentage, while in the winter season, only the two high rates of 2,4-D dissolved in diesel solvent showed significant differences in emergence percentage compared to the control. All 2,4-D rates dissolved in diesel had significantly less shoot height compared to the corresponding rates in water. In the winter season, all 2,4-D rates dissolved in water significantly decreased root length, while in the rainy season only the two high rates of 2,4-D ( $18 \times 10^3$  and  $24 \times 10^3$  ppm a.i.) dissolved in diesel showed significant difference in root length compared to the control (Table 5).

Table 5: Effect of 2,4-D and solvent types on germination %, emergence %, radical length, Shoot height, and root length

Treatment	Germination % (Petri dishes)		Emergence % (Greenhouse)		Radical length		Root length		Shoot height	
	W	R	W	R	W	R	W	R	W	R
D2,4-DR0	93.3ab	77.8c	57.78bcd	50.0b	8.41ab	6.48cd	22.68bcd	13.57a	29.65ab	9.81bc
D2,4-DR1	85.6b	78.9c	60.0bcd	33.33cd	8.02abc	6.00de	23.72ab	11.06cd	24.15cd	9.98bc
D2,4-DR2	64.4c	66.7d	58.89bcd	21.11de	6.68cd	5.61e	19.37bcd	9.93de	24.15cd	8.72c
D2,4-DR3	55.6c	57.8de	23.33e	13.33e	4.96ef	4.61f	19.82bcd	9.7e	24.24de	6.44d
D2,4-DR4	55.6c	54.4e	23.33e	10.0e	4.04f	3.71g	18.01de	8.8e	23.8e	6.39d
Mean	70.9	67.12	44.67	25.55	6.42	5.28	20.73	10.61	25.20	8.27
W2,4-DR0	94.4ab	100.0a	86.67a	74.44a	8.82a	8.31a	28.01a	12.38ab	32.93a	30.66a
W2,4-DR1	95.6a	97.8a	70.0b	48.89b	7.97bc	8.18a	22.44bcd	12.38ab	29.02bc	11.1b
W2,4-DR2	86.7ab	94.4ab	52.22d	35.0b	7.14bcd	7.41b	17.71e	12.82a	25.08e	10.81bc
W2,4-DR3	88.9ab	85.6bc	65.56bc	36.67bc	6.07de	6.81bc	19.37bcd	12.45ab	27.21bc	9.95bc
W2,4-DR4	88.9ab	100.0a	55.56cd	40.0bc	6.09de	6.56cd	27.74bc	11.42bc	25.42de	9.14bc
Mean	90.0	95.56	66.00	47.00	7.22	7.44	23.05	12.29	27.93	14.33
SE±	4.69	5.29	6.12	7.66	0.73	0.38	2.35	0.60	1.96	1.09

#### 4. Discussion

In this study, 2,4-D (auxin-like herbicide) dissolved in diesel was applied at the flowering stage of a mesquite tree, and the germination percent of seeds collected from the treated tree significantly decreased as the rate of 2,4-D increased. The present study is in line with many researchers, (Miller and Norsworth, 2017) who found that; Dicamba (auxinic herbicide) applied at the reproductive stage of soya beans, reduced seeds germination percentage. Application of 2,4-D at the beginning of seed formation of soybean caused a reduction by 8% in seed germination compared to the application at the beginning of flowering (Neves, 1998). 2,4-D was also, found to be efficient in inhibiting seed germination and radical length of *Hibiscus cannabinus* at a rate of 5000 ppm compared to the control which gave 98% and 15.7 cm for germination and radical length, respectively (Sanjay, 2006). Liu *et al.* (2012) found that The application of 2,4-D in wheat at the soft dough stage of seeds, resulted in a higher level of residue of the active ingredient of 2,4-D in seeds, compared to the application of herbicide at ripening stages. This may possibly result from the increased translocation of photoassimilates from the various plant structures to the seeds (Cessna and Holm, 1994). This period concedes shorter time for the metabolism of the 2,4-D herbicide which penetrates the seed coat and is effective as a pre-emergence herbicide. Synthetic auxins, like 2,4-D, are metabolically broken down by the isozyme oxidase with the release of phenols, the accumulated of these phenols in the tissues might inhibit the germination of seeds (Sathiyamoorthy 1990).

Henner *et al.* (1999) reported that small molecules of petroleum hydrocarbons are phytotoxic for germination. The toxic hydrocarbon molecules in diesel solvents inhibit the activities of amylase and starch phosphorylase (Achuba, 2006).

The emergence and seedling establishment constitute the most critical periods in the life cycle of the plants. The emergence percent of seeds collected from treated trees in both rainy and winter seasons showed significant decrease. 2,4-D at low concentration acts as auxin and stimulates growth of plant cells and interacting with endogenous hormones like ethylene, gibberellic acid, abscisic acid and auxins. (Grossmann, 2009). 2,4-D at high concentration which acts as herbicide and persists for a long time within the plant and described as an auxin overdose. (Song, 2014).

The application of 2,4-D in wheat at the soft dough stage of seeds, resulted in a higher level of residue of active ingredient of 2,4-D in seeds, compared to the application of herbicide at ripening stages (Liu *et al.*, 2012). Largest level of residue of herbicide 2,4-D found at the soft dough stage, compared to the ripening stages, may possibly result from increased translocation of photo assimilates from the various plant structures to the seeds (Cessna and Holm, 1994). In monocot stems, the vascular tissues (the phloem and xylem) are scattered in bundles and lack a vascular cambium and synthetic auxin can quickly inactivate by conjugation, while in dicot stems, the vascular tissues are formed in rings and possess a cambium and the synthetic auxin is not metabolized as quickly as the endogenous auxin (Taiz and Zeiger, 2013). Application 2,4-D herbicide at the flowering stage of mesquite trees (dicot stem), increased the herbicide residue in

seeds, this is due to that, mesquite pods ripen 60 days after flowering (Harden and Reza, 1988). This period considered is a shorter time for the metabolism of the 2,4-D herbicide and penetrating the seed coat and is effective as a pre-emergence herbicide and decreased emergence percent. In addition, the toxic hydrocarbon molecules in diesel solvents inhibit the activities of amylase and starch phosphorylase, (Achuba, 2006). The primary product of photosynthesis in higher plants is starch, which is stored as a carbohydrate that supports metabolism and growth during the dark when photosynthesis is not possible (Zeeman *et al.* 2004). The mobilization of starch by the  $\alpha$ -amylase enzyme initiated seed germination (Fincher, 1989). The importance of amylase activities in germination, which convert stored starch to soluble sugar, which adjust the  $K^+/Na^+$  ratio and increases ATP production and seed respiration (Zheng *et al.* 2009).

The decrease in root length and shoot height observed in the present study is in line with that observed by (Mahender *et al.* 2014), who found that the root length and shoot height of *Coriandrum sativum* treated with 2,4-D decreased as 2,4-D concentration increase. Dicamba (auxinic herbicide) applied at the reproductive stage of soybean reduced plant height (Miller and Norsworthy, 2017). Khan *et al.* (2006) reported that the shoot and root length of *Triticum aestivum* and *Phalaris minor* raised from seeds treated with different concentrations of 2,4-D, decreased with the increase of 2,4-D concentration. Also, the application of 2,4-D near soybean sowing caused a decrease in shoots and root length (Elisa *et al.* 2015). *Abelmoschus esculentus* treated with 2,4-D at five rates (1-5 ppm) showed a reduction in shoot height and root length decreased after five days of treatment (Periyannayagi and Senthikumar, 2015). 2,4-D lasts for a long time resulting in the overproduction of ethylene (Lin *et al.* 2009). Ethylene production in

response to 2,4-D is the stimulation of  $H_2O_2$ , which is considered the second messenger in abscisic acid synthesis (ABA) (Yan *et al.* 2007). Overproduction of  $H_2O_2$ , which mediates stomata closure to reduce water loss and promotes oxidative damage, which reduces the production of plant biomass (Vanderauwera *et al.* 2011). In addition, alkanes and polycyclic aromatic hydrocarbons (PAHs) in the diesel solvent increased the toxicity, which reduced the growth of the stem and the root (Alkio *et al.* 2005). The performance of 2,4-D in the rainy season decreased the shoot height more than in the winter season, this may have been due to high humidity in the rainy season which increased the uptake and translocation of 2,4-D (Peterson, *et al.* 2016).

## **5. Conclusion**

Mesquite tree commonly propagated by producing a large number of viable seeds, The result of this study showed that 2,4-D decreased the viability of seeds which reflected in decreased germination and emergence percentage and also decreased the shoot height and root length, which affected seedlings' establishment. The results found that the sizes of the trees have no effect in all parameters. The results of this study contribute to reducing the spread of this tree. Therefore, we recommend more studies to find out whether this effect was continuing or for a single production season.

## **6. References**

Achuba, F. I. (2006). The effect of sublethal concentrations of crude oil on the growth and metabolism of Cowpea (*Vigna unguiculata*) seedlings. *Environmentalist*, 26(1), 17-20.

Alkio, M.; Tabuchi, T. M.; Wang, X. and Colón-Carmona, A. (2005), Stress responses to polycyclic aromatic hydrocarbons in Arabidopsis include growth inhibition and hypersensitive response-like symptoms. *Journal of Experimental Botany*. 56(421), 2983-2994.

Almaraz, A. N.; Graça, C. M.; Avila R. J. A.; Naranjo, J. N.; Corral, J. H. and González, V. L. S. (2007). Antioxidant activity of polyphenolic extract of monofloral honeybee collected pollen from mesquite (*Prosopis juliflora*). *J Food Compost Anal*:20(2), 119-124.

Cessna, J.A. and Holm, F. A. (1994). Residues of 2,4-D in wheat following application after heating. *Canadian Journal of Plant Science*, 74: 199-203.

Elisa, P.; Daniel, M. K.; Arthur, P.; Evandro, M. V. N.; Ricardo, F.; Matheus, G.; Paulo, E.T. and Carlos, A. (2015). Residual activity of 2,4-D amine on soybean plant development. *Journal of Agronomy*. 14(4), 247-250.

Fincher, G. B. (1989). Molecular and cell biology associated with endosperm mobilization in germinating cereal grains. *Ann. Rev. Plant Physiol. Plant Mol. Biol.*, 40(1), 305-346.

Gabbani, M. F. *Amelioration of Biotic Stress Induced by Onion Yellow Dwarf Virus on Onion Seed Crop Using Nutrition*. M.Sc.(Agric) thesis. University of Khartoum, Khartoum, Sudan. 2007, p. 25

Geesing, D.; Al-Khawlani, M. and Abba, M. L. (2004). Management of introduced *Prosopis juliflora* species: can economic exploitation control an invasive species *Unasylva*, 55(217), 36-44.

Grossmann K. (2009). Auxin herbicides: Current status of mechanism and mode of action. *Pest management science* 66(2) 113 -120.

Harden, M. L. and Reza Z. (1988). Nutritive composition of green and ripe pods of honey mesquite *Prosopis glandulosa*, Fabaceae. *Springer* Vol. 42, No.4 pp 522-532

Hatzios, K., Hock, B., Elstner, E. F. (2005). *Metabolism and elimination of toxicants*. Plant Toxicology, CRC Press. 4<sup>th</sup> ed: pp. 469-518.

Henner, P.; Schiavon, M.; Druelle, V. and Lichtfouse, E. (1999), Phytotoxicity of ancient gas work soils. Effect of polycyclic aromatic hydrocarbons (PAHs) on plant germination. *Organic Geochemistry*, 30(8), 963-969.

- Khan, M. R. and Aslam, K. M. (2006). Effect of 2,4 -D on Seedling Physiology and cytogenetical studies in *Triticum aestivum* and *Phalaris minor* (Gramineae). *Acta Bot. Yun.* 28 (4): 394-398.
- Lin, Z.; Zhong, S. and Grierson, D. (2009). Recent advances in ethylene research. *J Exp Bot* 60(12), 3311–3336.
- Liu, c., (2012). Dissipation and residue of 2,4-D isooctyl ester in wheat and soil *Environ. Monit Assess.* 184(7) 4247-4251.
- Mahender, K.; Agnihotri, R. K. and Vamil, R., (2014). Effect of phytohormones on seed germination and seedling growth of *Coriandrum sativum* L. *Pakistan Journal of Biological Sciences* 17(4), 594-596.
- Miller, M. R., Norsworthy, J. K. (2017). Soybean sensitivity to florasulfuron-benzyl during reproductive growth and the impact on subsequent progeny. *Weed Technology.* 32 (2), 135–140.
- Neves, R., (1998). The action of systemic non-selective herbicides on soybean progeny when applied during the reproductive period of mother plants. *Cienc. Rural* 28(3), 367-371.
- Pasiecznik, N. M.; Felker, P.; Harris, P. J.; Harsh, L.; Cruz, G.; Tewari, J. C.; Cadoret, K. and Maldonado, L. J. (2001). The *Prosopis juliflora-Prosopis pallida* complex: A monograph. 172. HDRA, Coventry, UK.
- Pazmiño, D. M.; Rodríguez-Serrano M.; Romero-Puertas, M. C.; Archilla-Ruiz, A.; Del Río, L. A. and Sandalio, L. M. (2011). Differential response of young and adult leaves to herbicide 2,4-dichlorophenoxyacetic acid in pea plants: role of reactive oxygen species. *Plant Cell Environment* 34(11), 1874-1889.
- Periyannayagi, G. and Senthilkumar, N. (2015). Effect of 2,4-D on the growth and biochemical characteristics of (*Abelmoschus esculentus* L.). *European Journal Applied Sci.* 7(3), 105-107.
- Peterson, M. A.; McMaster, S.A.; Riechers, D. A.; Skelton, J. and Stahlman, P. W. (2016). 2,4-D past, present, and future: A review. *Weed Technology* 30(2), 303-345.
- Sanjay, I. Kamble (2006). Effect of herbicide 2,4-D on seeds germination and early seedling growth of *Hibiscus cannabinus* L. *Biosciences Biotechnology Research Asia.* Vol. 3(1), 227-232.
- Sathiyamoorthy, P. (1990). Identification of vanillic and p-Coumaric acid as endogenous inhibitors of soybean seeds and their inhibitory effects on germination.. *J Plant Physiology,* 136(1), 120—121.
- Scarpella, E.; Marcos, D.; Friml, J. and Berleth, T. (2006). Control of leaf vascular patterning by polar auxin transport. *Genes Dev* 20(8), 1015–1027.

Song, Y. L. (2014). Insight into the mode of action of 2,4-dichlorophenoxyacetic acid (2,4-D) as an herbicide. *Journal of Integrative plant biology* 56(2), 106-113.

Taiz, L. and Zeiger, E. (2013). *Plant physiology*. 5<sup>th</sup> Ed. Sinauer Associates Inc., Publishers Sunderland, U.S.A.

Vanderauwera, S.; Suzuki, N.; Miller, G.; van de Cotte, B.; Morsa, S. and Ravanat, J. L. (2011). Extranuclear protection of chromosomal DNA from oxidative stress. *Natl. Acad. Sci. U.S.A.* (108), 1711 – 1716.

Yan, I.; Tsuchihara, N.; Etah, T. and Iwan, S. (2007). Reactive oxygen species and nitric oxide are involved in ABA inhibition of stomatal opening. *Plant cell environment*. 30 (10):1320-1325.

Zeeman, S.C.; Thorneycroft, D.; Schupp, N.; Chapple, A.; Weck, M.; Dunstan, H.; Haldimann, P.; Bechold, N.; Smith, A. M. and Smith, S. M. (2004). Plastidial  $\alpha$ -glucan phosphorylase is not required for starch degradation in Arabidopsis leaves but has a role in the tolerance of abiotic stress. *Plant Physiol.*, 135, pp. 849-858.

Zheng, C.; Jiang, D.; Liub, F.; Dai, T.; Liu, J. Q. and Cao, W. (2009). Exogenous nitric oxide improves seed germination in wheat against mitochondrial oxidative damage induced by high salinity. *Env. Exp. Bot.* 67(1), 222-227.