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Research Article

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The Impact of Synthetic Auxin on Correlation Between Leaf Carotenoids and Mortality Percentage of Mesquite (*Prosopis juliflora* Swarz) DC.

Eltayeb A. H. Suliman*[1], Sayda O. Elhewairis[1] and Salah E. Elamin[2]
[1]*Department of Botany and Agricultural Biotechnology*
[2]*Department of Crop Protection*
Faculty of Agriculture, University of Khartoum, Sudan
**Corresponding author e-mail: eltayeb71_sha@yahoo.com*
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ABSTRACT
In the Faculty of Agriculture - University of Khartoum – Sudan, experiments were conducted in both winter and rainy seasons to assess the impact of the synthetic auxin 2,4-dichloro phenoxy acetic acid (2,4-D) on the relationship between leaves carotenoid content and the mortality percentage of mesquite trees (*Prosopis juliflora*). Mesquite trees of small, medium, and large sizes were treated with the 2,4-D at four weights 6-, 12-, 18-, and 24-grams' active ingredient, each dissolved in either diesel oil or water. The results indicated that the three sizes treated with different weights of herbicide dissolved in diesel showed a high mortality percentage and fewer leaf carotenoid levels, compared to the same weights of 2,4-D dissolved in water. During the rainy season, the high weight of 2,4-D (24 grams a.i.) dissolved in water resulted in a high mortality percentage and lower carotenoid levels, compared to the other 2,4-D weights dissolved in water. The study concluded that; 2,4-D increased the negative relationship between mesquite mortality percentage and the leaf's carotenoid content.
Keywords: 2,4-D, Herbicide, Diesel, Natural, and Negative relationship.

INTRODUCTION

Carotenoids are pigments found in plants, they are lipid-soluble compounds that include beta-carotene, lutein, and zeaxanthin. Carotenoids are one of the most important members of the tetraterpene family of pigments (Arvayo-Enriquez *et. al*, .2013), Carotenoids are important to plant physiology and regulating plant development (Águila and Rodríguez, 2012). 2,4-D is the first synthetic auxin herbicide (phenoxy acetic acid) used to control dicotyledonous annual and perennial weeds (Gervais *et al*., 2008). There are two types of formulations of 2,4-D: amine salts and esters, high temperature and humidity play essential roles in the uptake and movement of the 2,4-D (Peterson *et al*., 2016). Low doses of 2,4-D promote plant growth while high doses promote plant overgrowth, (Grossmann, 2010).

During normal metabolic processes, reactive oxygen species (ROS) are produced when electrons are transferred to oxygen O_2, forming superoxide radicals, hydrogen peroxide (H_2O_2), and hydroxyl radicals. Alternatively, ROS can also be generated by reduction of O_2 to form single oxygen $^1O_2$ (Mittler, 2002). In low concentrations, hydrogen peroxide (H_2O_2) plays an important role in regulating gene expression related to stress responses and development (Mittler *et al*., 2011). ROS has a dual, antagonistic role in cells depending on their concentration, plants have developed a sophisticated mechanism to control ROS levels, involving glutathione, tocopherol, polyphenols, and carotenoids (Sandalio *et al*., 2009).

The cells in plants using carotenoids to neutralize toxic ROS, which act as antioxidants, detoxifying various forms of ROS (Varin and Sekhon, 2006). Carotenoids also act as an antioxidant, scavenging $^1O_2$ to inhibit oxidative damage (Edreva, 2005) and they serve as signaling molecules in response to biotic and abiotic stress (Li and Vallabhaneni, 2008). Additionally, carotenoids protect the plant against UV radiation (Young, 1991), This study aimed to assess the link between the leaf carotenoid content, mesquite mortality percentage, and the role of 2,4-dichloro phenoxy acetic acid.

MATERIALS AND METHODS

Sites of the Experiments and Plant Materials

Field experiments were carried out at the Faculty of Agriculture, University of Khartoum, Sudan. The site is located at lat.15, 40 N, long 32, 32E, in a semi-arid zone, with a wide range of temperatures, varying from 18o C to 40o C, and relative humidity ranging from 34% to 75

% (Gabbani, 2007). The experiment area was divided into three replicates, each containing thirty mesquite trees, that were classified based on their size to three categories small, medium, and large, with ten in each category.

Chemical solutions and application method

The method outlined in (Geesing et al. 2004) was utilized, to apply herbicide to the lower portion of the stem. This involved spraying the 2, 4-D herbicide at four rates (6-, 12-, 18-, and 24-grams a.i), using either diesel or water as a solvent, each weight of 2, 4-D dissolved in a liter of the solvent.

Data Collection

Extraction and estimation of carotenoid

The leaves from the outermost branches of each tree were selected two weeks after herbicide application. The collected leaves were placed in polyethylene bags and stored in a deep freeze at -20º C. The carotenoid content determined following the method described by Porra *et.al*. (1989), 0.05 grams of frozen leaves were ground into fine particles in a mortar using 2 ml of pure methanol per 0.1 gram until the tissues become colorless. The mixture was then centrifuged at 6000 rpm for 15 minutes, following which 0.5 ml of the supernatant was transferred to a new test tube, to this 4.5 ml of pure methanol was added and the absorption was measured using a spectrophotometer at 665, 650, and 470 nm. The amount of carotenoid was then calculated as mg / g green tissue using specific equation:

$$\text{Carotenoid} = \frac{1000 \times A470 - (1.63 \times A665 + 104.9 \times A650) \times V}{221 \times W}$$

A665 = Absorption at wavelength 665 nm.

A650 = Absorption at wavelength 650 nm.

A470 = Absorption at wavelength 470 nm.

V = the final volume of the pure methanol in chlorophyll extract.

W = the fresh weight (gram).

Mortality percentage

The mortality percentage is calculated one year after applying the herbicide to allow sufficient time for the herbicide to take effect in different climatic conditions. The stem appears 100 % defoliated, have no living tissue, and have not re-sprouted from ground buds was accounted. The mortality was calculated as percentage of the total number of stems.

Experimental design and data analysis

The experiments were conducted using Randomize Complete Block Design (RCBD) with three replicates. The data was analyzed using an Analysis of variance (ANOVA) test, and means were separated statistically using the least significant difference (LSD) test with a computer statistical software, "Statistix 10". Differences between means were considered significant at the (0.05) level.

RESULTS AND DISCUSSIONS

The combination of 2,4-D and diesel solvent resulted in a significant decrease in carotenoid content of the leaves and a higher mortality percentage for trees of all sizes compared to the control group. The increase in mortality percentage was linked to a *decrease* in carotenoid content as the 2,4-D application rate increased. Small, medium, and large trees treated with all rates of 2,4-D mixed with diesel showed high mortality percent, while carotenoid levels significantly decreased as the amount of 2,4-D increased. In all trees sizes, there were no significant differences in mortality percentage and leaves carotenoid content for all rates of 2,4-D dissolved in water. However, during the rainy season, the high rate of 2,4-D dissolved in water resulted in a significant increase in mortality percentage and a decrease in leaves carotenoid for medium; and large tree sizes (Tables 1 and 2).

All rates of 2,4-D dissolved in diesel solvent resulted in significantly lower leaf carotenoid content, and increased mortality percentages compared to when it was dissolved in water. In contrast, the rates of 2,4-D dissolved in water showed no significant difference. During the rainy season, the high rate of 2,4-D (24 grams a.i.) dissolved in water significantly reduced leaf carotenoid content, and increased mortality percentage, which gave (2.58 mg) and (28.79%) compared to the control, which gave 0.00 mortality percentages and 3.58 mg, of leaf carotenoid (Table 3).

Synthetic auxins 2,4-D imitate the effects of natural auxin (IAA) in plants (Pazmino *et al.,* 2012). Natural auxins IAA are quickly deactivated as they are conjugated and degraded, while 2,4-D persist in the plant for longer periods (Song, 2014). 2,4-D triggers specific peroxisomal enzymes that lead to the production of reactive oxygen species (ROS). Including analyzed glycolate oxidase (GOX) involved in photorespiration, xanthine oxidoreductase (XOD) involved in ureide metabolism, and acetyl-CoA oxidase (ACX) involved in fatty acid β-oxidation (Pazmino *et al.*, 2011).

Carotenoids are synthesized in chloroplasts and various types of plastids, but their high levels are predominantly found in the chloroplasts (Aguila 2012). Chloroplasts have been identified as the primary target of 2,4-D (Grossmann, 2009), resulting in decreasing levels of chlorophyll and carotenoids (Saygideger, 2008). The increase of ROS produced by 2,4-D may be responsible for the enhanced carotenoid degradation (Grossmann *et al*., 2001).

2,4-D activates the synthesis of 1-amino cyclopropane 1-carboxylic acid (ACC), which is the key enzyme in ethylene biosynthesis (Grossmann, 2003). This process is stimulated by Abscisic acid (ABA) biosynthesis (Raghavan, 2006). ABA accumulation causes stomatal closure, and reduces water loss (Vanderauwera *et al.*, 2011), Stomatal closure limits CO_2 assimilation leading to a reduction in photosynthetic activity and increased electron leakage from the photosystems to O_2 in the chloroplasts (Grossmann, 2010), This induces overproduction of (ROS) such as superoxide radicals (O•− 2), hydrogen peroxide (H_2O_2), and hydroxyl radicals (OH +). which, leading to oxidative damage (Eduardo *et al.*, 2017). In addition, the high accumulation of ROSs leads to oxidative damage to proteins, polyunsaturated fatty acids, and nucleic acids (Sandalio *et al.*, 2012) Furthermore, H_2O_2 production resulted in tissue damage and cell death (Grossmann, 2010).

Table 1. Effect of 2,4-D rates, solvent types and tree sizes on mortality percent and leaf's carotenoid content in the winter season.

Tree sizes	2,4-D rates (gram a.i.)	Diesel		Water	
		Mortality %	Carotenoid mg	Mortality %	Carotenoid mg
Small	Control	16.67 ef	2.49 i	0.00 f	2.84 ghi
	6	55.33 cde	2.49i	0.00 f	2.99 f g h
	12	83.33 abc	1.70 j k	0.00 f	3.23 c defg
	18	100 a	1.43 kl	0.00 f	3.18 defg
	24	100 a	1.71 j k	0.00 f	3.58 bc
Medium	Control	16.67 f	2.65 hi	0.00 f	3.30 c def
	6	56.67def	1.84j	0.00 f	3.24 c defg
	12	93.33ab	1.64jk	0.00 f	3.29 c def
	18	100 a	1.33kl	0.00 f	3.41 bcd
	24	100 a	1.17 l	0.00 f	3.75 b
Large	Control	13.33f	3.01 e f gh	0.00 f	4.17 b
	6	53.33cde	1.60 j k	0.00 f	2.75 hi
	12	56.67bcd	1.92 j	0.00 f	3.73 b
	18	93.33ab	1.13 l	0.00 f	3.40 b cde
	24	100 a	0.66 m	0.00 f	3.28 c def
SE±		16.14	0.20	16.14	0.20

Table 2. Effect of 2,4-D rates, solvent types and tree sizes on mortality percent and leaf's carotenoid content in the rainy season.

Tree sizes	2,4-D rates (gram a.i.)	Diesel		Water	
		Mortality %	Carotenoid mg	Mortality %	Carotenoid mg
Small	Control	46.67 bc	2.99 def	0.00 f	3.09 de
	6	100 a	2.81 d efg	0.00 f	3.38bcd
	12	100 a	2.61 efghi	0.00 f	2.71 d efgh
	18	93.33a	2.16 ghij	0.00 f	2.96 def
	24	93.33a	1.99 ij	0.00 f	2.34fghij
Medium	Control	48.33b	3.06 de	0.00 f	4.22a
	6	93.33a	3.13 cde	0.00 f	3.40bc
	12	93.33a	2.55 ef g hi	6.67 e f	3.11cde
	18	100 a	2.05 hij	16.67de	2.89def
	24	100 a	1.71 j	33.33cd	2.75defg
Large	Control	50 b	3.37 bcd	0.00 f	3.14 bc de
	6	100 a	2.87 defg	0.00 f	3.84 ab
	12	100 a	2.78 def g	0.00 f	3.81 abc
	18	100 a	1.96 ij	0.00 f	2.94 f
	24	100a	1.73 j	53.33b	2.66 fghi
SE±		7.00	0.35	7.00	0.35

Table 3. Effect of 2,4-D rates on mortality percent and leaf's carotenoid content in the winter and rainy seasons.

2,4-D rate (gram a.i.)	Winter season		Rainy season	
	Mortality %	Carotenoids mg/g	Mortality %	Carotenoids mg/g
Diesel only	15.56 c	2.72 d	47.22 b	3.14 a
Diesel+6 grams	64.44 b	2.25 e	97.78 a	2.93 ab
Diesel+12 grams	67.78 b	1.65 f	97.78 a	2.65 b
Diesel+18 grams	97.87 a	1.46 f	97.78 a	2.06 c
Diesel+24 grams	100 a	1.18 g	97.87 a	1.81 c
Water only	0.0 c	3.29 bc	0.00d	3.58 a
Water+ 6 grams	0.00 c	3.07 c	0.00 d	3.32 a
Water+ 12 grams	0.00 c	3.48 ab	2.22 d	3.29 a
Water+ 18 grams	0.00 c	3.33 ab	5.56 d	3.08 ab
Water+ 24 grams	0.00 c	3.54 a	28.79 c	2.58 b
SE±	9.94	0.12	3.68	0.20

CONCLUSION

The study discovered a negative correlation between the levels of carotenoids in leaves and the percentage of mesquite mortality. The research also confirmed that 2,4-D has a significant impact on strengthening this relationship.

Furthermore, the study showed that 2,4-D dissolved in diesel is more effective than when it dissolved in water. This difference in efficacy is attributed to the distinct properties of the solvents used.

CONFLICT OF INTEREST

The author here declares that there is no conflict of interest in the publication of this article.

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