Original Article

Biostimulation effects of linseed and citrus oils on growth, antioxidant enzymes activity, metabolic changes and water relations of Khaya senegalensis seedlings under drought stress

Dina M. Soliman[1], Azza A.M. Mazhar[2], Rawia A. Eid[3], Nahed G. Abd El Aziz[4]
[1,2,3,4]Ornamental Plants and Woody Trees Department, National Research Centre, Dokki, Cairo, Egypt
Email: dina123soliman@hotmail.com
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Abstract

This study aim to use environmentally friendly material (Linseed and citrus oils) to reduce the harmful effect of water deficit on Khaya senegalensis plant due to its economic importance as a source of wood in addition to many uses of alternative medicine. Therefore, Khaya senegalensis plants were grown in green house with three irrigation intervals (5, 7 and 9 days) and oils foliar spray as linseed oil (Lin) at (0,5, 7%) and citrus oils at (0, 1, 2%). The results indicated that the 9 days irrigation interval gave the lowest values of the most studied growth parameters, water relations (relative water content (RWC%), water retention capacity (WRC) and membrane stability index(MSI%), photosynthesis pigments, chlorophyll stability index (CSI%), minerals content, total free amino acid while increased values of electrolyte leakage (EL%), total sugar, total phenol, and antioxidant enzymes activities(Catalase (CAT), Peroxidase (POX), Superoxide dismutase (SOD)). Both oils treated especially linseed oil, at 7% increased values of all parameters and chemical composition compared with untreatedplants plus different irrigation intervals, except EL% was decreased. The data provided evidence that linseed and citrus oils treatment reducesthe adverse effect of water deficit on Khaya senegalensis plants and can play a role in providing stress tolerance.

Keywords: Khaya senegalensis; linseed oil; citrus oil; irrigation interval; drought..

1. INTRODUCTION

Drought is the important key to environmental stress due to temperature, light, and low rainfall of many regions of the world. Despite this, its cumulative effect severely affects the plant's physiological, biochemical functions and hampers biomass production and energy [1].

Khaya senegalensis (Desr.) A. Juss., also known as African mahogany or dry–zone mahogany, is an exotic species of the Meliaceae family, native to Africa. Is an evergreen tree, but in dry climates it can be deciduous. Its wood is considered to be hardwood with excellent commercial value [2,3]. And numerous traditional medicinal uses, such as anti-sickling, antimicrobial, anthelmintic, and malaria treatment. In some areas of West Africa, seed oil provides economic value. It contains 67% oil by weight, is very rich in oleic acid (66%), and is used for culinary and cosmetic purposes [4].

Most natural oils of plants in recent years have received more and more attention as alternative, potentially beneficial compounds that are convenient for organic agriculture because they are non-residual, easy to prepare and apply, non-toxic to humans and animals, and cheap and effective [5, 6, 7]. However, their role in the growth of plants remains unclear. In this regard, among the essential oils of plants, citrus and linseed oils have dragged more attention because of their broad-properties as antibacterial, antifungal, and insecticide [6]. Citrus oil was obtained from cells within the rind of Citrus sinensis fruit. The oil consists of several biologically active compounds such as monoterpene aldehyde/alcohol, sesquiterpene group and mono terpenes like d-limonene (greater than 90%) , α/β- pinene, β-myrcene, , sabinene, linalool, α-humulene and α-terpineol, these compounds possess different beneficial properties such as anti-inflammatory, antioxidant, antimicrobial, etc. [8]. Linseed oil (flaxseed oil or flax oil) is extracted from the dried, ripened seeds of the Linum usitatissimum plant. Linseed oil chemically consists of linolicacid, oleic acid, and stearic acid [9]. These compounds have anti-oxidant properties [10]. Additionally, a few studies have been conducted on the utilisation of natural oils to promote plant growth under water deficit stress.

Therefore, this study aims to use natural oils (linseed oil and citrus oil) to reduce the harmful effects of deficit water on Khaya senegalensis plants by influencing water relations, chemical composition, enzyme activity, and growth of the plant.

2. Materials and Methods

Experiment trails were carried out at the National Research Centre (NRC) greenhouse, Giza, Egypt during two successful seasons that began in March 2020 and ended in September of the same year and were replicated during the same period in 2021.

One year seedlings of Khaya senegalensis (African mahogany) were obtained from the nursery of woody trees, Horticulture Research Institute, Agriculture Research Centre. The seedlings of plants grow in plastic pots at 30 cm filled with 10 kg soil of clay and sand at a ratio(1:1v/v) with a 15% field capacity (F.C.), (one plant/pot, the average height of seedlings is 20-25 cm with 5-7 leaves). Fertilization with krystalone (NPK at 18:18:18) was applied 3 times in the soil every 21 days after transplanting at the rate of 3 g/pot. During both seasons, Khaya senegalensis plants were grown under three different irrigation intervals (5, 7 and 9 days) and sprayed with Linseed oil (Lin) at 0, 5and 7% and Citrus oil (Ci) at 0, 1 and 2% was sprayed three times, starting after 21 days from transplanting and repeated every 21 days (Essential oils were obtained from the unit by squeezing and extracting natural oils, National Research Center).

2.1. Soil properties

Physical and chemical analysis of the experimental soil is in Table (1), according to Jackson [10].

Table 1 Physical and chemical analysis of the experimental soil

Physical properties	Clay	Silt	Sand					Texture		
	2.29	15.42	82.29					Sandy Loam		
Chemical analysis	pH	CE dS/m	Soluble Cations mmol/L					Soluble Anions(mmol/L)		
			Ca^{++}	Mg^{++}	Na^{+}	K^{+}	Co_3	HCO_3	Cl^{-}	SO_4^{--}
	7.17	1.28	5.02	1.79	3.64	1.37	nd	3.10	4.06	0.64

Electrical conductivity (EC); deciSiemens per meter (dS/m).

2.2. Growth parameters:

Growth parameters recorded (plant height (cm), number of leaves/plant, number of leaflet/plant, total leaf area/plant (cm2), stem diameter (cm), root length(cm), fresh and dry weight of leaves, stem and roots(g).

2.3. Water relations:

Relative water content (RWC %) in leaves: relative water content according to the equation described by Schonfeld et al. [12], Water retention capacity (WRC): were calculated as follows Sangakkara et al. [13], Electrolyte leakage (EL%): leaf membrane damage was determined by the recording of electrolyte leakage as described by Valentovic et al. [14], Membrane stability index (%): was determined according to the method of Sairam et al. [15]

2.4. Determination of photosynthesis pigments: Chlorophyll a &b and total carotenoids were estimated in fresh leaf samplesaccording to Saric et al. [16]. Chlorophyll stability index (CSI) was determined according to Sairam et al., (1997) [17].

2.5. Determination of total sugars, total free amino acid and total phenol:

Total sugars were estimated using ethanol extract, phenol, and sulphuric acid reagent method described by Dubois et al. [18]. Free amino acid was measured by a spectrophotometer (JWNWAY 6315) at 570 nm, using glycine as a standard described by Moore and Stein [19]. Total phenols were measured by colorimetric method, using a folien-ciocalteau reagent calculated by spectrophotometer at 650 nm and using gallic acid as the standard described by Swain and Hillis [20].

1.1. Determination of proline: Proline content was measured by using the method determined by Bates et al. [21].

1.2. Determination of minerals: N% was described by the modified Kjeldahl method as described by Cottenie et al. [22]. P% was estimated using the ammonium molybdate method according to Snell and Snell [23]. K% was measured in the digested solution by a flame photometer according to Chapman and Pratt [24]. Ca%, Mg%, and Fe ppm were determined by using an atomic absorption spectroscopy (PerkinElmer 100 B, US) using the method of Cottenie et al. [22].

1.3. Extraction and determination of antioxidant enzymes activities: Enzyme extraction was done in fresh leaves as described by Mukherjee and Choudhuri [25]. The activities of Catalase (CAT) EC 1.11.1.6 and Peroxidase (POX) EC 1.11.1.7 were determined according to the method of Kar and Mishra [26]. Superoxide dismutase activity (SOD) EC 1.15.1.1 using the method was determined by Marklund and Marklund [27].

1.4. Statistical analysis: The layout of the experiment was run in a factorial based on a randomized complete block design with 3 replications and 2 factors (irrigation intervals and oil treatments). An analysis of variance was conducted and all data were analyzed using CoStat version 6.3.1.1 for windows and the statically analysis was done by using two-way of variance (ANOVA) analysis followed by the Least Significant Difference (LSD) test was applied p = 0.05, according to Snedecor and Cochran [28].

2. Results and Discussion:

2.1. Growth parameters:

The effects of irrigation interval treatments for various growth parameters of Khaya senegalensis are presented in Tables (2-4). Plants with 9 days treated caused a significant reduction of all growth parameters in two seasons (plant height (cm), number of leaves/plant, number of leaflets/plant, total leaf area (cm2), stem diameter (cm), root length (cm), fresh weight and dry weight (g) of leaves, stems, and roots) as compared to 5 days. These results harmony with Yousaf et al. [29] On Eucalyptus citriodra and E. camaldulensis, they found decreased shoot fresh weight and root length with stress intervals. Rahmati et al. [30] showed a decreased in leaf area/shoot which can have an impact on gas exchange, relations of water, vegetative growth and development. Bangar et al. [31] suggested the reduced leaf area of plants under drought stress may be an avoidance technique, because it results in less water loss by transpiration. And loss of cell turgidity results from a reduced cell division rate, so decreased leaf area. Shahriari [32] suggested the drought reduced the amount of nutritional absorption in plants, resulting in shorter stems. As well as the shoot length of Lathyrus sativus L. plant [33]. Similarly, Battaglia et al. [34], Rehman et al. [35] , and Hura et al. [36]).

Table 2 Effect of linseed oil and citrus oil on plant height, number of leaves/plant, number of leaflet/plant, total leaf area and stem diameter of Khaya senegalensis plant under irrigation interval in seasons (2020-2021).

	(A) Irrigation intervals (day)							
(B) Treatment	**5**	**7**	**9**	**Mean(B)**	**5**	**7**	**9**	**Mean(B)**
	1st		**Plant height(cm)**			**2nd**		
control	57.71	51.70	45.48	51.63	54.26	49.14	42.62	48.67
Ci 1%	66.29	55.14	51.42	57.61	62.85	53.07	49.15	55.02
Ci 2%	64.50	50.42	47.18	54.03	60.55	50.74	46.20	52.49
Lin 5%	72.12	60.86	59.64	64.20	65.16	58.62	53.64	59.13
Lin 7%	83.86	69.08	62.01	71.65	72.88	60.44	56.33	63.21
Mean(A)	68.89	57.44	53.14		63.13	54.40	49.58	
LSD at 0.05	A	B	A*B		A	B	A*B	
for	2.00***	2.58***	4.61*		1.21***	1.57***	2.79*	
			No. of leaves/plant					
control	13.10	10.11	8.82	10.67	10.50	8.66	7.55	8.905
Ci 1%	15.50	13.40	11.38	13.42	14.13	12.10	14.50	13.58
Ci 2%	12.20	11.80	9.15	11.05	12.74	11.30	10.38	11.47
Lin 5%	17.66	15.50	13.00	15.38	15.13	13.50	12.20	13.61
Lin 7%	19.73	17.50	15.61	17.61	16.63	15.50	13.50	15.21
Mean(A)	15.63	13.66	11.59		13.82	12.21	11.62	
LSD at 0.05	A	B	A*B		A	B	A*B	
for	0.31***	0.39***	0.71**		0.49***	0.64***	1.11***	
			No. of leaflets/plant					
control	53.25	48.23	42.13	47.87	51.05	44.52	40.71	45.42
Ci 1%	56.70	55.30	50.18	54.06	55.47	52.17	46.25	51.30
Ci 2%	54.65	53.13	45.17	50.98	53.77	49.12	43.67	48.85
Lin 5%	60.79	57.63	54.28	57.56	57.98	54.10	51.81	54.63
Lin 7%	62.73	60.24	58.16	60.37	61.05	57.67	54.38	57.70
Mean(A)	57.62	54.90	49.98		55.86	51.51	47.36	
LSD at 0.05	A	B	A*B		A	B	A*B	
for	0.97***	1.25***	2.17**		0.82***	1.05***	1.83**	
			Total leaf area(cm^2)					
control	1052.29	781.58	641.62	825.17	1260.63	759.78	504.52	841.64
Ci 1%	1735.66	1192.59	1334.41	1420.90	1659.96	1171.53	911.20	1247.56
Ci 2%	1481.38	1036.19	922.46	1146.68	1245.60	968.84	683.70	966.05
Lin 5%	1900.55	1454.98	1213.31	1522.95	1985.25	1444.25	1109.91	1513.13
Lin 7%	2171.17	1832.87	1485.73	1829.92	2338.61	1724.86	1424.54	1829.33
Mean(A)	1668.21	1259.64	119.51		1698.81	1213.82	926.77	
LSD at 0.05	A	B	A*B		A	B	A*B	
for	57.54***	74.28***	128.7***		41.26***	53.27***	92.26***	

				Stem diameter(cm)				
control	0.70	0.61	0.55	0.62	0.66	0.56	0.52	0.58
Ci 1%	0.78	0.70	0.63	0.70	0.73	0.64	0.57	0.64
Ci 2%	0.73	0.66	0.57	0.65	0.68	0.60	0.55	0.61
Lin 5%	0.84	0.73	0.65	0.74	0.80	0.66	0.63	0.69
Lin 7%	0.99	0.78	0.70	0.82	0.86	0.68	0.65	0.73
Mean(A)	0.80	0.69	0.62		0.74	0.62	0.58	
LSD at 0.05	A	B	A*B		A	B	A*B	
for	0.017***	0.022***	0.039***		0.019***	0.024***	0.042*	

Ci =Citrus oil, Lin= linseed oil, A= irrigation interval, B=oil treatments, A*B= interaction, ****High significantly, **= Moderate significant.

Table 3 Effect of linseed oil and citrus oil on root length, fresh weight of leaves, stem and roots and dry weight of leaves of Khaya senegalensis plant under irrigation interval in seasons (2020-2021).

	(A) Irrigation intervals (day)							
(B) Treatment	5	**7**	**9**	**Mean(B)**	**5**	**7**	**9**	**Mean(B)**
		1st		**Root length(cm)**		**2nd**		
control	20.40	15.66	13.50	16.52	19.12	13.01	11.50	14.54
Ci 1%	25.11	21.06	18.66	29.98	23.50	20.50	18.50	20.83
Ci 2%	22.18	18.28	15.11	18.52	20.50	18.00	16.32	18.27
Lin 5%	29.06	23.11	20.25	24.14	27.13	21.25	18.03	22.14
Lin 7%	34.22	25.50	23.68	27.80	32.00	23.14	21.50	25.55
Mean(A)	26.19	20.72	18.24		24.45	19.18	17.17	
LSD at 0.05	A	B	A*B		A	B	A*B	
for	0.736***	0.950***	1.645**		1.018***	1.315***	2.277**	
				Fresh weight of leaves(g)				
control	9.84	7.53	6.88	8.08	8.63	6.48	4.98	6.69
Ci 1%	13.34	10.47	9.03	10.94	12.54	10.94	8.78	10.75
Ci 2%	11.06	8.65	7.40	9.03	10.83	8.37	6.09	8.43
Lin 5%	15.23	12.53	10.39	12.71	14.21	12.84	10.08	12.38
Lin 7%	18.32	15.29	12.26	15.29	16.73	13.86	12.19	14.26
Mean(A)	13.56	10.89	9.19		12.58	10.50	8.42	
LSD at 0.05	A	B	A*B		A	B	A*B	
for	0.351***	0.453***	0.785***		0.228***	0.295***	0.511**	
				Fresh weight of stem(g)				
control	53.25	48.23	42.13	47.87	51.05	44.52	40.71	45.42
Ci 1%	56.70	55.30	50.18	54.06	55.47	52.17	46.25	51.30
Ci 2%	54.65	53.13	45.17	50.98	53.77	49.12	43.67	48.85
Lin 5%	60.79	57.63	54.28	57.56	57.98	54.10	51.81	54.63
Lin 7%	62.73	60.24	58.16	60.37	61.05	57.67	54.38	57.70
Mean(A)	57.62	54.90	49.98		55.86	51.51	47.36	
LSD at 0.05	A	B	A*B		A	B	A*B	
for	0.97***	1.25***	2.17**		0.82***	1.05***	1.83**	
				Fresh weight of root(g)				
control	8.95	6.09	5.02	6.68	8.32	5.31	5.14	6.25
Ci 1%	9.66	8.11	7.19	8.32	9.23	7.39	6.84	7.82
Ci 2%	9.00	7.04	6.02	7.35	8.55	6.57	6.13	7.08
Lin 5%	11.16	9.64	8.74	9.84	11.00	8.36	8.17	9.17
Lin 7%	12.56	10.67	10.22	11.15	11.44	10.00	9.51	10.31
Mean(A)	10.26	8.31	7.43		9.70	7.52	7.15	
LSD at 0.05	A	B	A*B		A	B	A*B	
for	0.245***	0.316***	0.547**		0.254***	0.328***	0.568*	
				Dry weight of leaves(g)				

control	2.59	1.91	1.71	2.07	2.19	1.60	1.19	1.661
Ci 1%	3.83	2.84	2.35	3.006	3.44	2.94	2.27	2.885
Ci 2%	3.05	2.22	1.86	2.376	2.88	2.09	1.47	2.149
Lin 5%	4.45	3.55	2.78	3.593	4.06	3.57	2.64	3.424
Lin 7%	5.50	4.53	3.43	4.486	4.85	3.89	3.30	4.014
Mean(A)	3.884	3.01	2.426		3.49	2.82	2.17	
LSD at 0.05 for	A 0.119***	B 0.155***	A*B 0.268***		A 0.078***	B 0.101***	A*B 0.175***	

Ci =Citrus oil, Lin= linseed oil, A= irrigation interval, B=oil treatments, A*B= interaction, ****High significantly, **= Moderate significant.

Table 4 Effect of linseed oil and citrus oil on dry weight of stem and root of Khaya senegalensis plant under irrigation interval in seasons (2020-2021).

	(A) Irrigation intervals (day)							
(B) Treatment	**5**	**7**	**9**	**Mean(B)**	**5**	**7**	**9**	**Mean(B)**
		1st	**Dry weight of stem(g)**			**2nd**		
control	2.10	1.59	1.38	1.69	2.03	1.70	1.38	1.70
Ci 1%	3.03	2.30	2.04	2.46	2.62	2.21	1.60	2.14
Ci 2%	2.26	1.85	1.52	1.91	2.28	1.86	1.33	1.82
Lin 5%	3.61	2.87	2.56	3.01	3.04	2.42	1.98	2.48
Lin 7%	4.41	3.46	3.20	3.69	3.53	2.78	2.38	2.90
Mean(A)	3.08	2.43	2.14		2.70	2.19	1.73	
LSD at 0.05 for	A 0.066***	B 0.085***	A*B 0.147***		A 0.068***	B 0.088***	A*B 0.154***	
			Dry weight of root(g)					
control	3.33	2.16	1.75	2.41	3.03	1.82	1.74	2.19
Ci 1%	3.72	2.96	2.60	3.09	3.47	2.65	2.41	2.84
Ci 2%	3.39	2.52	2.11	2.67	3.17	2.30	2.12	2.53
Lin 5%	4.42	3.67	3.23	3.77	4.25	3.08	2.95	3.42
Lin 7%	5.02	4.19	3.97	4.39	4.46	3.81	3.60	3.96
Mean(A)	3.67	2.72	2.56		3.97	3.10	2.73	
LSD at 0.05 for	A 0.094***	B 0.122***	A*B 0.211*		A 0.091***	B 0.118***	A*B 0.205*	

Ci =Citrus oil, Lin= linseed oil, A= irrigation interval, B=oil treatments, A*B= interaction, ****High significantly, **= Moderate significant.

Fresh biomass of the body plant, 80-95% is the embrace of water, which plays an animated role in diverse physiological processes, including numerous aspects of plant growth, metabolism, and development [37]. Water deficit is one of the challenges that can impact the plant growth and biochemical change [38, 39, 40]. Plants produce oxidative stress directly or indirectly under drought conditions, and it is one of the master diverse of plant response, resulting in altered membrane integrity, cell membrane damage, biochemical and physiological alterations which lastly change the proactive of plant [41, 42, 43], because the strong correlation between growth and availability of water, cells are enlargement more affected by decreased watercompared to cell division [44].

The data presented in Tables (2-4) indicated that the foliar oil spray was useful for all growth parameters with or without stress. Linseed oil (as antitranpiration) foliar spray showed the highest values of most growth parameters, especially the 7% concentration dose , followed by plants treated with 5% linseed oil , followed by citrus oil (as biostimulation) at 1% and citrus oil at 2% compared with control plants (untreated with oil). Foliar spray of linseed oil at 7% significantly increased all growth parameters. The highest values in both seasons were (71.65 and 63.21cm) for plant height; (17.6 and 15.21) for No. of leaves/plant; (60.37 and 57.70) for No. of leaflets/plant; (1829.92 and 182933cm2) for total leaf area; (0.82 and 0.73cm) for stem diameter; (27.80 and 25.55cm) for root length; (15.29 and 14.26g) for F.W. of leaves; (10.72 and 8.23g) for F.W. of stem; (11.15 and 10.31g) for F.W. of root;(4.486 and4.014g) for D.W. of leaves; (3.69 and 2.90g) for D.W. of stem; and (4.39 and 3.96g) for D.W. of root.

Regarding the effect of interaction between oil spray and water deficit (5,7 and 9 day) irrigation intervals, foliar spray of linseed oil at 7% combined with 9 day irrigation intervals significantly increased all growth parameters in both seasons compared with water deficit (9 days) plus zero sprays of oil . These results agree with Souri and Bakhtiarizade [45] when they applied rosemary essential oil at 1000 ppm foliar spray to tomato plants, resulting in an increased fresh weight of shoot and root. This may be the essential oil plays as a biostimulant of growth characteristics and concentrates the chlorophyll content of leaves. Werrie et al. [46] indicated that a concentration of essential oils below that could stimulate the plant, a phenomenon referred to as biostimulation.

2.2. Water relations:

The relative water content (RWC %), membrane stability index (MSI %), water retention capacity (WRC, ratio TW/DW) and electrolyte leakage (EL %) were the important indicators of drought stress in the plant. In the present study (Table 5) showed decreased values of RWC, MSI%, WRC and deficiency rate significantly increased with increased irrigation interval (9 days), while EL% significantly increased with water deficit (9 days) compared with 5 days irrigation interval. The decrements were (17.03%) for RWC%, (16.60%) for MSI% and (8.99%) for WRC, while the increment was (63.23%) for EL% compared with 5 days intervals. During their whole growth cycle, plants' water relations under drought are disrupted, resulting in the major disorder of all metabolic pathways (physiological, molecular, and biochemical) [47].

Relative water content (RWC %) is a substantial determinate of metabolic activity and survival of the leaf. The reduced in RWC due to deficit water was also reported by Soltys -Kalina et al. [48] in potato cultivars and soybean [49]. The decrease in soil moisture causes an equivalent reduction of leaf water content, which motivates a decline in the turgor pressure of guard cells due to stomata closure [50]. The preservation of adequate leaf water statues is paramount for the proper biochemical and physiological function of plants, and electrolyte leakage (EL %) has been used to estimate cell membrane stability [51]. The increase in EL% occurs mainly from membrane damage under water deficit induced oxidative stress [52, 53].

The turgid weight/dry weight ratio (TW/DW) illustrates the water retention capacity (WRC) of plants that is determined by the cell structure [54]. Decreased values with water deficit might be due to the devastation of plant tissue, greater damage in cell structure and reduction in cell size, which is one of the most anatomical changes observed in leaves affected by stress of water [43]. The MSI % measures the percentage of damage and describes the ability of the membrane to survive in water deficit stress. The decrease in the values of MSI% in this study is in harmony with Sakya et al.[55] on tomato plants, Abid et al. [56] on Triticum aestivum L . , and Gedam et al.[57] on onion plants.

Table 5 Effect of linseed oil and citrus oil on RWC, MSI, WRC and EL of Khaya senegalensis plant under irrigation interval mean of two seasons.

	(A) Interval water (day)							
(B) Treatment	**5**	**7**	**9**	**Mean(B)**	**5**	**7**	**9**	**Mean(B)**
	Relative water content (RWC %)				**Membrane stability index (MSI %)**			
control	70.76	65.64	59.12	65.17	72.76	67.64	61.12	67.17
Ci 1%	79.35	69.57	65.65	71.52	81.35	71.57	67.65	73.52
Ci 2%	77.05	67.24	62.70	68.99	79.05	69.24	64.70	70.99
Lin 5%	81.65	75.12	70.14	75.64	83.65	77.12	72.14	77.64
Lin 7%	89.38	76.94	72.83	79.72	91.38	78.94	74.83	81.72
Mean(A)	79.64	70.90	66.08		81.64	72.90	68.09	
LSD at 0.05 for	A 1.214***	B 1.5668***	A*B 2.7138*		A 1.213***	B 1.566***	A*B 2.713*	
	Water retention capacity (WRC, ratio TW/DW)				**Electrolyte leakage (EL %)**			
control	5.460	5.278	5.047	5.262	26.22	32.35	40.18	32.92
Ci 1%	5.765	5.418	5.279	5.487	24.28	29.21	33.32	28.94
Ci 2%	5.683	5.335	5.174	5.397	25.77	31.66	36.41	31.28
Lin 5%	5.846	5.475	5.345	5.550	16.23	27.32	30.71	24.75
Lin 7%	6.121	5.543	5.433	5.698	10.71	24.46	27.82	21.16
Mean(A)	5.775	5.409	5.256		20.64	29.10	33.69	
LSD at 0.05 for	A 0.0438***	B 0.0566***	A*B 0.0979***		A 0.808 ***	B 1.04 ***	A*B 1.807 ***	

Ci =Citrus oil, Lin= linseed oil, A= irrigation interval, B=oil treatments, A*B= interaction, ****High significantly, **= Moderate significant.

As per data recorded in Table (5), the treated plants with linseed oil at 7% significantly increased RWC, MSI and WRC, the increments were (22.33, 21.67 and 8.29%) respectively, while significantly decreased EL% compared with other concentrations of oils or control plants. Our results are consistent with Ouerghi et al.[58] in wheat and barley plants, the foliar spray of linseed oil at (7 and 10%) as antitranspirant improved the plant water content as compared to control under water deficit, while there was no considerable decreased the activity of photosynthetic activity. In this study, we suggested the effect of linseed oil on plants and the possibility of using it as antitranspirant due to its high content of fatty acids such as (α-Linoleic acid , oleic acid) that also normally bring about stomata closure , follow reduce the cuticle transpiration and also gas exchanges, which results in optimum use of water content of plant under drought stress conditions. The interaction of linseed oil at 7% combined with 9 days irrigation intervals showed an increase of most relations water compared with zero oils at the same irrigation intervals, except EL% values decreased compared with other oils concentrations or untreated plant.

2.3. Photosynthetic pigments:

Photosynthetic pigments (chl a, chl b, and total carotenoid) concentration in the leaves of Khaya senegalensis and chlorophyll stability index (CSI %) were significantly decreased with treated plants at 9 days compared with 5 days (Table 6). Deficit water the decreased photosynthesis activity is related to the decreased transport of CO2 across stomatal and mesophyll limitations. And is an indicator of potential reduced stomatal mobility due to drought [59]. Reduction in plant photosynthesis under drought stress is attributed to stomatal or non- stomatal mechanisms. Many species of trees minify stomatal conductance to expend less water per carbon assimilated, so improving water use efficiency [60, 61]. Also, Deepak et al. [62] found the functioning of nitrate, rubisco activity; phosphate, sucrose synthase, and capacity to generate ribulose bisphosphate have been confirmed to be influenced by the lowering activity of photosynthesis. And the reduction of photosynthetic decreased metabolic aberrations [63]. Research indicated reduced synthesis of chlorophyll and altered chlorophyll a/b ratio by decreasing water supply during drought stress period [54, 64]. Among others cause for the decline in the amount of chlorophyll is the drought promoted O2 and H2O2, which result in lipid peroxidation and ultimately chlorophyll degradation [65]. Drought damages the rubisco enzyme and other photosynthesis related enzymes, resulting in a loss of photosynthetic pigment content [66].

Table 6 Effect of linseed oil and citrus oil on chl(a), chl (b), total carotenoid and chlorophyll stability index of Khaya senegalensis plant under irrigation interval mean of two seasons.

	(A) Interval water (day)							
(B) Treatment	**5**	**7**	**9**	**Mean(B)**	**5**	**7**	**9**	**Mean(B)**
	Chlorophyll (a) mg/g F.W.				**Chlorophyll (b) mg/g F.W.**			
control	0.756	0.645	0.53	0.644	0.2659	0.233	0.175	0.225
Ci 1%	0.830	0.693	0.610	0.711	0.2741	0.248	0.2123	0.245
Ci 2%	0.815	0.67	0.565	0.683	0.2734	0.242	0.1986	0.238
Lin 5%	0.935	0.732	0.630	0.766	0.303	0.2590	0.235d	0.266
Lin 7%	1.185	0.782	0.660	0.876	0.391	0.275	0.2509	0.306
Mean(A)	0.904	0.704	0.60		0.301	0.251	0.214	
LSD at 0.05	A	B	A*B		A	B	A*B	
for	0.049***	0.063***	0.109**		0.017***	0.022***	0.039*	
	Total carotenoids (mg/g F.W.)				**Chlorophyll Stability index (CSI %)**			
control	0.340	0.290	0.239	0.290	100	85.41	70.33	85.24
Ci 1%	0.376	0.312	0.274	0.321	109.89	91.71	80.74	94.11
Ci 2%	0.367	0.301	0.254	0.307	107.96	88.61	74.78	90.45
Lin 5%	0.421	0.329	0.283	0.344	123.91	96.83	83.38	101.4
Lin 7%	0.533	0.352	0.297	0.394	157.06	103.40	78.39	115.9
Mean(A)	0.407	0.317	0.269		119.76	93.19	79.32	
LSD at 0.05	A	B	A*B		A	B	A*B	
for	0.022***	0.028***	0.049**		6.94***	8.97***	15.53*	

Ci =Citrus oil, Lin= linseed oil, A= irrigation interval, B=oil treatments, A*B= interaction, ****High significantly, **= Moderate significant.

Table 7 Effect of linseed oil and citrus oil on different elements of Khaya senegalensis plant under irrigation interval mean of two seasons.

	(A) Interval water (day)							
(B) Treatment	**5**	**7**	**9**	**Mean(B)**	**5**	**7**	**9**	**Mean(B)**
		N%			**P%**			
control	1.30	1.12	0.94	1.12	0.24	0.21	0.18	0.21
Ci 1%	1.74	1.34	1.16	1.41	0.33	0.25	0.22	0.27
Ci 2%	1.52	1.24	1.07	1.27	0.29	0.23	0.20	0.24
Lin 5%	1.93	1.44	1.27	1.54	0.36	0.27	0.24	0.29
Lin 7%	2.17	1.52	1.38	1.69	0.41	0.29	0.26	0.32
Mean(A)	1.73	1.33	1.16		0.33	0.25	0.22	
LSD at 0.05	A	B	A*B		A	B	A*B	
for	0.0458***	0.0591***	0.1025***		0.1045***	0.134***	0.234***	
		K%			**Ca%**			
control	2.97	2.56	2.15	2.56	0.55	0.46	0.39	0.47
Ci 1%	3.96	3.05	2.66	3.22	0.72	0.55	0.48	0.58
Ci 2%	3.46	2.84	2.43	2.91	0.63	0.51	0.44	0.53
Lin 5%	4.40	3.28	2.91	3.53	0.80	0.6	0.53	0.64

Lin 7%	4.94	3.46	3.14	3.85	0.90	0.63	0.57	0.70
Mean(A)	3.95	3.04	2.66		0.72	0.55	0.48	
LSD at 0.05 for	A 0.0087***	B 0.0113***	A*B 0.0196***		A 0.0190***	B 0.0246***	A*B 0.0427***	
	Mg%				**Fe ppm**			
control	0.32	0.28	0.23	0.28	151.03	130.17	109.31	130.17
Ci 1%	0.44	0.33	0.29	0.35	201.63	155.28	135.19	164.03
Ci 2%	0.38	0.31	0.27	0.32	176.14	144.46	123.99	148.20
Lin 5%	0.48	0.36	0.32	0.39	223.65	166.87	147.94	179.48
Lin 7%	0.54	0.38	0.34	0.42	251.50	176.14	159.91	195. 83
Mean(A)	0.43	0.33	0.29		200.78	154.58	135.27	
LSD at 0.05 for	A 0.0116***	B 0.0149***	A*B 0.0259***		A 5.3129***	B 6.859***	A*B 11.880***	

Ci =Citrus oil, Lin= linseed oil, A= irrigation interval, B=oil treatments, A*B= interaction, ****High significantly, **= Moderate significant.

Table 8 Effect of linseed oil and citrus oil on total sugar, total free amino acid, total phenol, proline, CAT and POD of Khaya senegalensis plant under irrigation interval of mean two seasons.

	(A) Interval water (day)							
(B) Treatment	**5**	**7**	**9**	**Mean(B)**	**5**	**7**	**9**	**Mean(B)**
	Total sugar (mg/g F.W.)				**Total free amino acid (mg/g F.W.)**			
control	7.456	9.283	6.337	7.692	10.93	9.93	7.69	9.35
Ci 1%	9.132	10.53	11.36	10.34	13.00	11.31	10.37	11.56
Ci 2%	8.335	9.645	10.52	9.502	11.60	10.22	8.92	10.25
Lin 5%	10.19	11.18	13.13	11.50	15.30	12.16	11.32	12.92
Lin 7%	12.21	11.42	14.26	12.63	18.54	16.06	12.45	15.69
Mean(A)	9.466	10.41	11.12		13.87	11.84	10.15	
LSD at 0.05 for	A 0.304***	B 0.393***	A*B 0.680***		A 0.576***	B 0.744***	A*B 1.28**	
	Total phenol (mg/g F.W.)				**Proline (mg/g F.W.)**			
control	0.293	0.433	0.722	0.483	0.743	1.095	1.828	1.222
Ci 1%	0.381	0.754	0.822	0.653	0.965	1.908	2.081	1.652
Ci 2%	0.311	0.629	0.783	0.575	0.789	1.592	1.983	1.455
Lin 5%	0.570	0.810	1.056	0.812	1.443	2.051	2.673	2.055
Lin 7%	1.002	1.510	1.741	1.420	2.536	3.828	4.416	3.590
Mean(A)	0.512	0.830	1.025		1.295	2.095	2.594	
LSD at 0.05 for	A 0.0277***	B 0.0357***	A*B 0.0619***		A 0.070***	B 0.090***	A*B 0.175***	
	CAT (mg/g F.W. minute)				**POX (mg/g F.W. minute)**			
control	16.53	18.29	21.76	18.86	1.879	2.769	4.624	3.091
Ci 1%	18.26	23.80	24.33	22.13	2.442	4.827	5.264	4.178
Ci 2%	17.15	21.47	22.22	20.28	1.995	4.027	5.015	3.679
Lin 5%	19.32	23.75	25.06	22.71	3.648	5.186	6.761	5.198
Lin 7%	20.90	25.87	26.89	24.55	6.415	9.681	11.145	9.081
Mean(A)	18.43	22.64	24.05		3.276	5.298	6.562	
LSD at 0.05 for	A 0.554***	B 0.716***	A*B 1.239**		A 0.177***	B 0.229***	A*B 0.397***	

Ci =Citrus oil, Lin= linseed oil, A= irrigation interval, B=oil treatments, A*B= interaction, ****High significantly, **= Moderate significant.

Table 9 Effect of linseed oil and citrus oil on SOD activity (mg/g F.W. min.) of Khaya senegalensis plant under irrigation interval of mean two seasons.

	(A) Interval water (day)			
(B) Treatment	**5**	**7**	**9**	**Mean(B)**
	SOD (mg/g F.W. minute)			
control	6.814	7.237	7.074	7.042
Ci 1%	7.957	8.385	8.654	8.132
Ci 2%	7.051	8.161	7.673	7.628
Lin 5%	8.406	8.935	10.34	9.228
Lin 7%	9.022	9.813	11.72	10.18
Mean(A)	7.850	8.506	8.973	
LSD at 0.05 for	A 0.268***	B 0.347***	A*B 0.601***	

Ci =Citrus oil, Lin= linseed oil, A= irrigation interval, B=oil treatments, A*B= interaction, ****High significantly, **= Moderate significant.

Foliar spraying of linseed oil (Table 6) at 7% significantly increased chl a, b, total carotenoids and CSI%. The increments were (24.53, 36.0, 24.27, and 36.03%), respectively, compared with control plants. The interaction between spraying oil and water deficit (5,7 and 9 day irrigation intervals) showed the linseed oil spray at 7% followed by 5%combined with 5 days (irrigation intervals) significantly increased chl a, b, carotenoids and CSI% compared with untreated plants with oil plus irrigation intervals. These results may be explained by the fact that the use of antitranspirant (linseed oil in this study) preserved the higher amount of water under deficit stress, and the films from it on the leaf surface could act as physical barrier between plants and foliar fungal pathogens that suggested agreement with Ghebrial and Kenawy [67] on celery plants. Several studies of essential oils (such as lemon and sweet orange), reported varying antimicrobial activity at different concentrations and decreased biochemical deteriorations [68, 69].

2.4. Mineral content in leaves:

Data obtained indicated that the water irrigation at 5 day intervals significantly increased the content of N, P, K, Ca , Mg, and Fe in the leaves of Khaya senegalensis plant (Table 7), the increments were (49.14, 50.0, 48.5, 50, 48.3 and 48.4%) respectively, compared with 9 day irrigation intervals . Usually, the water deficit reduces soil nutrient and root nutrient translocation and decreased ion content in different plant tissues at the latest. Qi et al. [70] in Malus hupehensis plant indicated a decreased amount of K with drought stress; this may be caused by the scarcity of water which disturbs stomata movement and guards cell turgidity, resulting in decreased photosynthesis and finally production of plant biomass [71]. In addition, Tadayyon et al. [72] on Ricinus communis plants, found decreased Fe and Mg content with stress water conditions, while Gharibi et al.[73] found the lowest values of N and P of Lavandula latifolia and Thymus mastichina plants with a deficit of water.

The highest content of different elements was determined in the leaves of plants (Table 7) which were treated with different oils; especially the linseed oil at a high concentration (7%) followed by another concentration of the same oil, and followed by the plant treated with citrus oil at the lowest concentration (1%). On the contrary, the lowest content of elements was recorded with plants without oils applied. The interaction between water deficit and oil spray showed the efficiency of oil treatment to reduce the stress of water, which the data found the highest content values of different elements with plants under 5 days plus sprayed linseed oil at 7%, followed by plants under 5 days plus linseed oil at 5%, the lowest content values were obtained with plants under 9 days plus zero oil treated. The effect of oils on elements content may be due to their effect on stoma. Stomata regulation is very important to increas water use efficiency (WUE) by decreasing evapotranspiration [74]. WUE and sufficient K content can improve the total dry mass of the plant, rate of photosynthetic and also regulate SOD activity by K^+ to mitigate the cell membrane injury that is caused by drought-triggered.

2.5. Total sugar, total free amino acid, total phenol and proline (mg/g F.W.):

Table (8) showed reduced content of total free amino acid, but increased total sugar, total phenol and proline content in leaves with 7 and 9 days interval watering, but increased total amino acid(mg/g F.W.) with 5 days, and indicated the lowest phenol and proline accumulation with the same condition. Biochemical limitations might have a greater impact on plant performances, despite drought confirmed morpho-anatomical traits. At the biochemical level, key molecules such as carbohydrates, amino acids, and polyamines play decisive roles in the tolerance mechanism of stress and meliorative the capability of plant adaptation by osmoregulation, altering their membrane stabilization, promoting root development, ROS(Reactive oxygen species) scavenging, and reducing ion leakage [75,76]. Sugars play an important function in maintaining the overall structure and growth of plants, total sugar in general decreased in different varieties with drought conditions [76]. On the other hand, in several of higher plants commence the accumulation of proline, soluble sugar and numerous secondary metabolites to protect against drought stress [77, 78, 79, 80].These results harmony with Rayhan Ahmed et al. [81] decreased total amino acid in Chinese cabbage with drought. Gharibi et al. [73] on Achillea pachycephala plant under water deficit, showed improved phenolic content while declining flavonoids. After stress, amino acid biosynthetic enzymes were significantly decreased [82].

Proline accumulation is an important signal against drought stress to alter cell proliferation, stimulate mitochondria functioning, and help to maintain membrane integrity via detraction lipids peroxidation by defending cell redox potential and declining level of ROS [83, 84, 85]. In rice plant, antioxidant enzymes activities and proline content increased with drought stress, it may be suggested that accumulation were correlated with the growth of the plant and consequently with the drought tolerance mechanisms [86].

The content of total sugar, total free amino, total phenol, and proline (Table 8) increased in Khaya senegalensis leaves with oils spray treated ,especially the high concentration of linseed oil (7%), and the lowest content found in control plants (without treated oils). Plant essential oils (EOs) can improve not only antimicrobial properties attributable to their chemical compounds, mainly phenolic compounds, terpenes, ketones, and other, but also relieve biochemical deteriorations [87].

Data presented in Table (8) indicated that the interaction between linseed oil at 7% combined with 9 days significantly increased the content of total sugar, total phenol, and total proline, compared with un-spraying oil with 9 days irrigation intervals, while the combination with 5 days and linseed oil at 7% significantly increased total free amino acid compared with plants un-spraying oil under 9 days conditions.

2.6. Antioxidant enzymes activity:

The activity of antioxidant enzymes (CAT, POD, and SOD) increased with water deficit, especially at 9 days, followed by 7 days (Tables 8, 9). Efficient and powerful antioxidant system is importance to provide drought tolerance by reducing and repairing injury triggered by ROS [88]. Under water deficit stress, numerous studies showed enhanced activity of antioxidant enzymes namely, POD, SOD, and CAT [89, 64] on vigna mungo plant, Hosseini et al. [90] on Glycyrrhiza glabra and [91] on Coleus plectranthus plant. Gao et al. [92] and Kapoor et al. [39] found that Adonis plants under drought stress, increased CAT, SOD, POD, and APX activities, indicating that improved functioning of these enzymes to decrease the level of ROS.

Treated essential oils (linseed and citrus), showed a positive effect of antioxidant enzyme activity, found increased activity with all concentrations of oils foliar spray applied especially linseed oil at 7% followed by 5%. The essential oils (EOs) performance is attributed to the existence of natural phenolic compounds (Laranjo et al., 2017[6]) and recently have been used EOs in the film on plants to improve physicochemical antioxidant and antimicrobial estate [93, 94]. The interaction between oils treated plus irrigation interval, found the highest values of antioxidant enzymes activity (CAT, POD, and SOD) with linseed oil at 7% concentration +9 days interval of irrigation compared with plants untreated with oil.

3. Conclusion:

It can be concluded that essential oil (linseed oil and citrus oil) can be induction the water deficit tolerance of Khaya senegalensis plant, especially linseed oil at 7% concentration by the effect of growth characteristics, chemical composition, water relations, and antioxidant enzymes activity.

Conflicts of interest:
There are no conflicts to declare.

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