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Table: 2, Figs: 11

Allelopathic effects of *Eucalyptus camaldulensis* L. and *Nerium oleander* L. on *Phoenix dactylifera* L. (Date Palm) seeds germination and seedlings growth

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ABSTRACT

We evaluated the allelopathic effects of aqueous, acetone extracts of *Eucalyptus camaldulensis* L. and *Nerium oleander* L. leaves on seeds germination and seedlings growth of *Phoenix dactylifera* L. (Date Palm). All acetone extracts of *Eucalyptus* and *Nerium* reduced the germination percentage and rate of germination significantly. The aqueous extracts followed the similar trends to acetone ones, but were less harmful to both germination parameters. The irrigation with Eucalyptus or *Nerium* aqueous extracts also decreased the seedlings growth. The *Eucalyptus* aqueous extracts of 20 and 30 % significantly reduced the leaf length, leaf dry matter, leaf area and total chlorophyll content at (4.75, 2.59, 3.49, 11.50 and 3.09 %) and at (5.28, 4.34, 10.43, 14.10 and 3.09 %) respectively. The *Eucalyptus* acetone extracts at 20 and 30 % were more harmful to all these parameters and caused reduction of 6.72, 4.10, 11.97, 11.83 and 3.60 % and at 7.86, 6.12, 10.74, 14.43 and 3.75 %, respectively.

Nerium extracts of 30 % significantly reduced the leaf length, dry matter, leaf area and chlorophyll content by 3.82, 3.45, 7.50 and 14.10 % and 7.33, 3.87, 9.75 and 13.33 %, respectively. While its 20 % aqueous extract, significantly reduced the leaf length and total chlorophyll content only. Acetone extracts at 20 and 30 % retarded leaf length, dry matter, leaf area and chlorophyll content significantly. All extracts of Eucalyptus and *Nerium* at 30 % significantly reduced the root length and ratio of root to shoot dry weight. While 20 % Eucalyptus aqueous extracts, significantly reduced the ratio of root to shoot dry weight. The carbohydrates content in roots showed significant inhibition at 30 % *Nerium* extract, 20, and 30 % of aqueous and acetone extracts of Eucalyptus. The nitrogen, phosphorus and potassium in shoots were significantly decreased in both 30 % aqueous and acetone extracts of both plants.

Keywords: Allelopathic effects, biochemical parameters, Date palm, *Eucalyptus camaldulensis*, extracts, *Nerium oleander*, *Phoenix dactylifera* L., seeds germination, seedlings growth**.**

INTRODUCTION

The term 'Allelopathy' refers to any process involving secondary metabolites produced by plants, microorganisms, viruses, fungi that influence the growth and development of Agricultural and Biological systems. It was re-defined by International Allelopathy Society as any process involving secondary metabolites produced by plants, algae, bacteria, and fungi that influences the growth and development of agricultural and biological systems (34). In addition, John and Sarada (30) reported that allelopathy refers to one plant's beneficial or harmful effects on another plant by releasing chemicals from plant parts. It has been established that allelopathy offers great potential. In the near future, allelopathy may be applied in crop production, crop protection, agroforestry and agro-horticultural practices in developed and developing countries. Allelopathy may become one of the strategic sciences to reduce environmental pollution and to increase agricultural production and provide a basis for sustainable agriculture, hence, currently the Allelopathy

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research is being done in most countries worldwide and is now receiving more attention from agricultural and bio-scientists (35).

Eucalyptus tree (family Myrtaceae, Australian origin) an evergreen tree is widely distributed in subtropical countries. It has commercial, industrial and medicinal uses and is widely used in agroforestry. It is used to reclaim degraded landscapes (15,49). The *E. camaldulensis* L. (Red Gum) leaves ethyl acetate extract contains antimicrobial and antischistosomal activities due to the presence of biologically active metabolites. Hence its bark, leaf extracts, essential oils and plant extracts have been investigated for their antifungal, antibacterial and antiviral activity (6,21) (Figure 1).

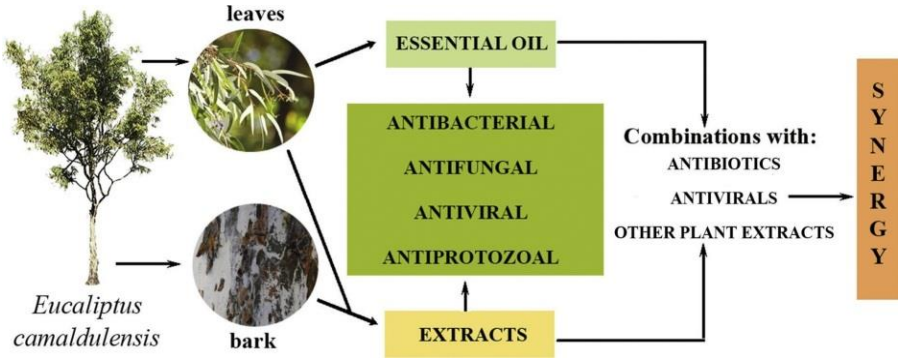

Figure 1. *Eucalyptus camaldulensis* L. (Synergy effects of *Eucalyptus* plant (6)

Figure 2. *Eucalyptus camaldulensis* L. (A); *Nerium oleander* L. (B)

Nerium oleander L. (family Apocynacae) is a tropical and subtropical ornamental evergreen shrub/small tree (Figure 2). It is grown for abundant and long-lasting flowers, different colours, and high tolerance to drought (36,45). It is used in floriculture as a flowering pot plant and in the landscape for outdoor plantings. Its leaves accumulate heavy metals (Pb, Cd, Zn and Cu), hence, may be used as bio-monitor plant to reclaim soils polluted with heavy metals.

Phoenix dactylifera L. [Date palm Arecaceae family (syn. Palmaceae)] is a flowering plant, also used as ornamental plant in landscaping projects (25) (Figure 3). Its trees are cultivated for its sweet fruits. Date palm is drought resistant, hence, requires less irrigation (3).

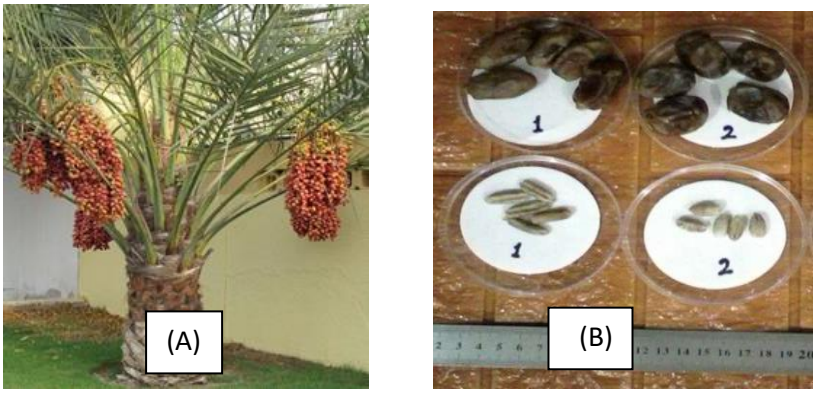

Figure 3. *Phoenix dactylifera* L. tree (A); Fruit and seeds of two Date palm cultivars as 1 Hillawi and 2 Barhi flesh and seeds (B) (1)

The paper and gas chromatography showed that 8-years old *Eucalyptus spp.* contained 8-allelopathic phenolic compounds [caffeic, coumaric, gallic, gentisic, catechol, hydroxybenzoic, syringic and vanillic acid (40)]. The leachates of bark, fresh leaves and leaf litter of *E. tereticornis, E. camaldulensis, E. polycarpa and E. microtheca* contains water soluble phenolics (coumaric, gallic, gentisic, hydroxybenzoic, syringic and vanillic acids and catechol) and are added in soil (41). The essential oils in the flowers and leaves of various plants such as mugwort (*Artemisia vulgaris*) adversely affect the seed germination and growth of *Lolium perenne* (37).

In nature, the interactions between the *Eucalyptus*, *Nerium*, and Date palm trees needs to be understood. Hence, this study aimed to (i). determine the allelopathic effects of *E. camaldulensis* L. and *N. Oleander* L. leaves aqueous and acetone extracts on *P. dactylifera* L. (Date palm) seeds germination and seedlings growth and (ii). To determine the chemical compounds in these extracts.

MATERIALS AND METHODS

These studies were conducted during March-August 2019 at Date Palm Research Centre, Basrah University, Basrah, Iraq (Longitude 47.47', Latitude 30.31', Elevation 2.4 msl, Mean maximum Temp 41.8 °C and minimum 6.8 °C, Annual Rainfall 31.07 mm (5). Four experiments were done during March-August, 2019 at Basrah Governorate to determine the effects of *Eucalyptus and Nerium* leaves aqueous, and acetone extracts concentrations 0, 10, 20 and 30 % w: v on seeds germination and seedling growth of Date Palm.

Extracts preparation

Fresh mature leaves of healthy *E. camaldulensis* L. and *N. oleander* L. trees were collected. Leaves were washed with distilled water, cut into 1 cm pieces and then air-dried under shade at room temperature for two weeks. Dry leaves were powdered in the grinder. Fifty g of this dry plant material was soaked in 200 ml of solvents (distilled water, acetone 80 %) and left for 24 h. It was first filtered using sterile muslin cloth and thereafter-through sterile Whatman filter paper (No. 2). After filtration, the crude extracts were stored in a refrigerator at 4 °C until use (33). Then extracts were concentrated with a rotary vacuum evaporator at 40 °C. Thereafter 3 concentrations (0, 10, 20, 30 %) of each were prepared and used within a week. The distilled water was used for control.

(i). Petriplate bioassays

Equal sized completely ripened date palm fruits were collected from female date palm trees of cv. Hillawi. Seeds were extracted, washed carefully and surface sterilized with 0.2 % Mercuric chloride solution for 5 min. The sterilized seeds were thoroughly rinsed with autoclaved distilled water thrice and placed in Petri dishes to evaluate seed germination. The seeds treated with extracts solutions and distilled water (control). Germination parameters were calculated by using the following formulae:

$$\text{Germination (GP \%)} = \frac{\text{Number of germinated seeds}}{\text{Total number of seeds}} \quad X \quad 100$$

Speed of Germination= n1/d1+n2/d2+n3/d3+----------

Where, n: Number of germinated seeds, d: Number of days (15).

(ii). Pot culture and Field experiments

Another group of seeds were sown in plastic pots filled with peat moss and soil (2:1 v/v) and kept in a lath house (a type of greenhouse that was popular in the 1800s and early 1900s. They are made of narrow strips of wood, as laths allow air and light to circulate, and the framework protects the plants from wind, snow, and direct sunlight).

Two weeks after germination, 0.5 g NPK (20:5:10, including micronutrients) was added to each pot and repeated one month later. Fifty ml extracts per pot were used to irrigate every week, while, control pots were irrigated with tap water. Ninety days after sowing, plants were harvested, rinsed in distilled water and dried with paper towels. The leaf length (cm) and leaf area (cm^2): were measured (19). The total chlorophyll content in leaves (mg /100 g fresh wt.) was estimated (22). Leaf dry matter (mg) and fresh weight of leaves was recorded, then dried in an oven at 70 for °C and dry matter was recorded. Total soluble

carbohydrates content in leaves and roots (%) were estimated (18). Nitrogen was estimated (29) method. In leaves Phosphorus P (%) was evaluated using spectrophotometer, while flame photometer was used to determine Potassium (%) (38).

Statistical analysis

Data was statistically analyzed by one-way analysis of variance then means were compared by RLSD (Revised Least Significant Differences) range test at ($P \geq 1\%$) For germination experiments and ($P \geq 5\%$) for the field ones as per (7). The statistical analysis was done with statistical analysis GenStat 7.2 (2007) software.

RESULTS AND DISCUSSION

(i). Petriplate bioassays

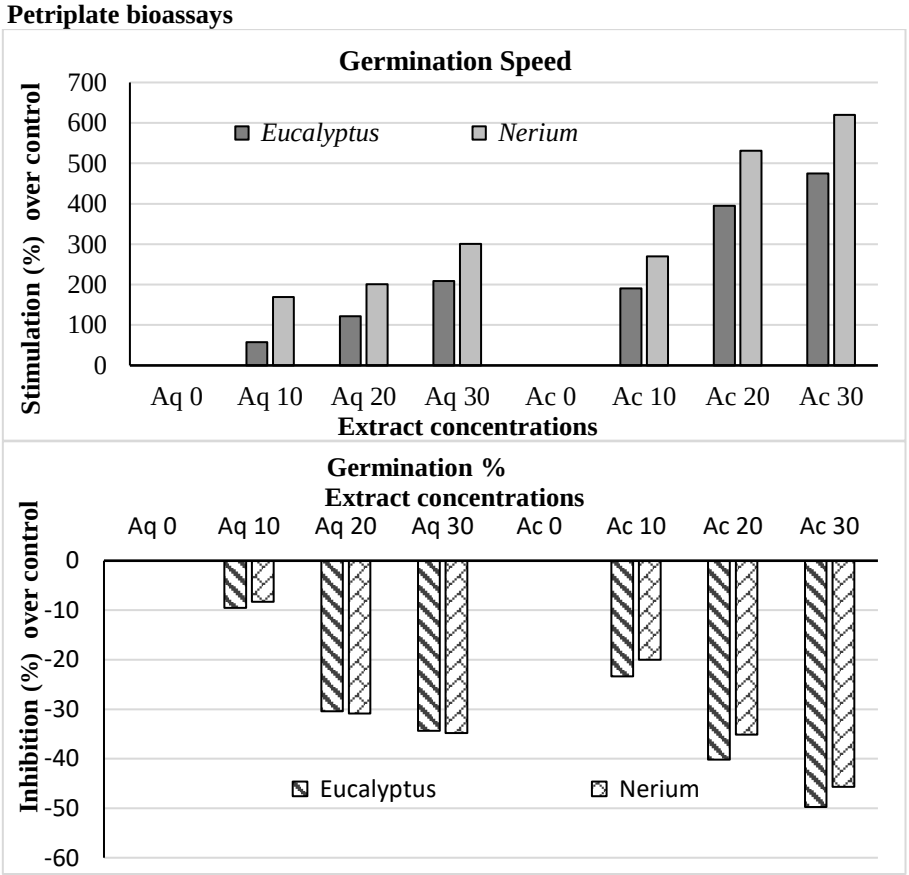

Figure 4. Effects of *E. camaldulensis* and *N. oleander* leaves extracts on Date palm seeds germination (%) and germination speed (Day). Aqueous extract treatments Aq 0 : untreated control; Aq 10 : 10 %; Aq 20 : 20 %; Aq 30 : 30 % (v/v) and Acetone extract treatments Ac 0 : untreated control; Ac 10 : 10 %; Ac 20 : 20 %; Ac 30 : 30 % (v/v).

The 10 % *Nerium* aqueous extract had non-significant inhibitory effects on seeds germination (%). While, all acetone extracts of *Eucalyptus* and *Nerium* significantly reduced the germination (%) and germination speed. The aqueous extracts also followed similar trends to acetone ones, but were less harmful to both germination parameters (Figure 4).

The inhibitory effects of *Eucalyptus* extracts on germination percentage and germination rate might be due to the presence of phenolics. The results are in agreement with the previous study, where leachates of *E. camaldulensis* affected the germination and vigour index due to the presence of phenolics in the bark, fresh leaves and leaf litter leachates resulting into allelopathic interactions (42). The aqueous extract of *E. citriodora* leaf inhibited the germination of *Vigna radiate*, *V. mungo* and *Arachis hypogaya* seeds (44). The decline in seed germination in response to allelochemicals could be attributed to the decreased water absorption causing impaired physiological processes during the seed germination (16). The terpenes along with camphor and α-pinene reported in *Eucalyptus* might be also responsible for decline in seed germination (12,17,27,23). The reduced germination (%) and germination rate followed similar pattern reported in *Lolium multiflorum* in response to *Nerium* extract (28).

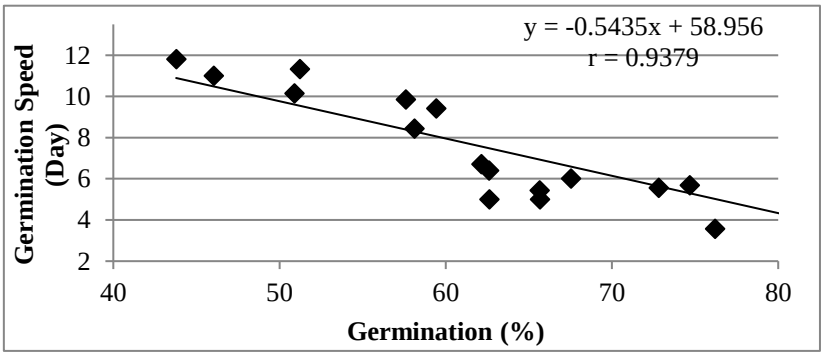

Figure 5. The relationship between germination (%) and speed of date palm seeds when treating with *Eucalyptus camaldulensis* L. leaves extracts

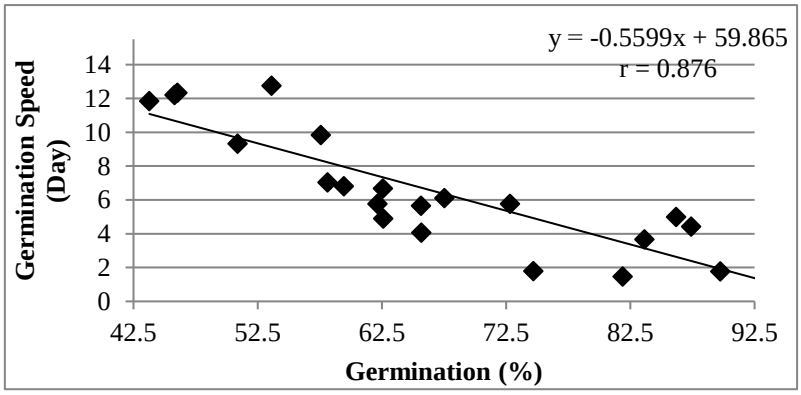

Figure 6. The relationship between germination percentage and speed of date palm seeds when treating with *Nerium oleander* L. leaves extracts.

There was a linear relationship between the germination (%) and germination speed (Figure 5) for *Eucalyptus* extracts as r = 0.937 and in Figure 6 for *Nerium* extracts (r = 0.876).

(ii). **Pot culture and Field experiments**

The 10 % *Eucalyptus* aqueous extract did not show any significant impact on the leaf length, leaf dry matter, leaf area and total chlorophyll content (Table 1). While, application of 10 % acetone extract significantly influenced the leaf growth parameters but not the chlorophyll content. The *Eucalyptus* aqueous extracts of 20 and 30 % significantly reduced all the studied parameters i.e., leaf length, leaf dry matter, leaf area and total chlorophyll content by 4.75, 2.59, 3.49, 11.50 and 3.09 % and by 5.28, 4.34, 10.43, 14.10 and 3.09 %, respectively (Figure 7). *Nerium* aqueous at 30 % significantly reduced leaf length, dry matter, leaf area and chlorophyll content by 3.82, 3.45, 7.50 and 14.10 % respectively. While, 20 % aqueous extract significantly reduced leaf length and total chlorophyll content only.

Eucalyptus acetone extracts of 20 and 30 % also significantly reduced all these parameters by 6.72, 4.10, 11.97, 11.83 and 3.60 % and by 7.86, 6.12, 10.74, 14.43 and 3.75 %, respectively. *Nerium* acetone extract of 30 % significantly reduced leaf length, dry matter, leaf area and chlorophyll content by 7.33, 3.87, 9.75 and 13.33 %, respectively. For acetone extracts at 20 and 30% retarded leaf length, dry matter, leaf area and chlorophyll content significantly. Whereas 10% extract, significantly reduced the dry matter and chlorophyll content. However, both aqueous and acetone extracts of *Nerium* did not influence the carbohydrates content in leaves and shoots of Date palm.

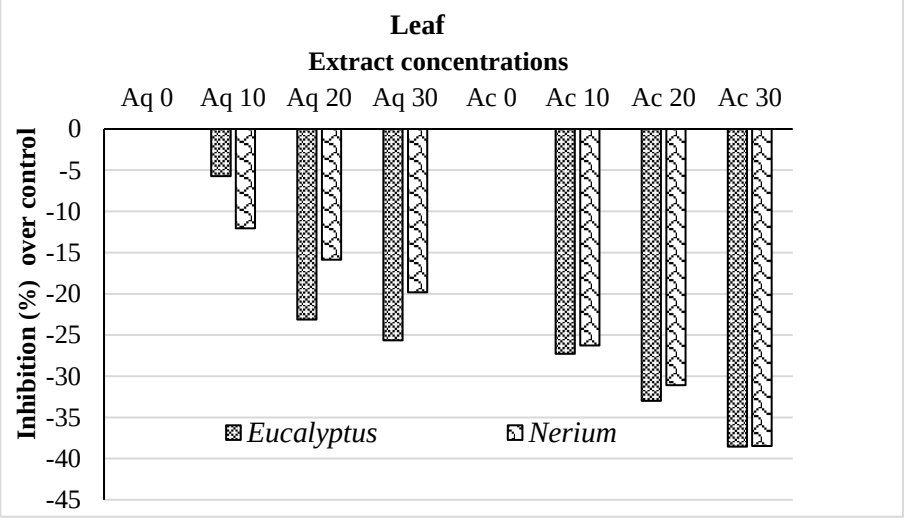

Figure 7. Effects of *E. camaldulensis* and *N. oleander* leaves extracts on Date palm seedlings leaves length. Aqueous extract treatments Aq 0 : untreated control; Aq 10 : 10 %; Aq 20 : 20 %; Aq 30 : 30 % (v/v) and Acetone extract treatments Ac 0 : untreated control; Ac 10 : 10 %; Ac 20 : 20 %; Ac 30 : 30 % (v/v).

Table 1. Effects of *E. camaldulensis* and *N. oleander* leaves extracts on Date palm seedlings leaves growth, Chlorophyll and Carbohydrates

Extracts	Conc (%) w/v	Leaf DM (%)	Leaf area (cm2)	Total chl. content in leaves (mg/ 100 g FW)	Carbohydrates in leaves (%)
		Eucalyptus camaldulensis			
Aqueous Extract	0	21.51	27.39	46.60	19.36
	10	20.60 (-0.91)	25.97 (-1.42)	43.50 (-3.10)	18.21 (-1.15)
	20	18.92 (-2.59)	23.90 (-3.49)	35.10 (-11.50)	16.57 (-3.09)
	30	17.17 (-4.34)	16.96 (-10.43)	32.50 (-14.10)	16.27 (-3.09)
CD at 1 %		2.229	2.901	3.392	1.198
Acetone Extract	0	21.59	26.08	39.63	19.60
	10	18.47 (-3.12)	21.83 (-4.25)	33.80 (-5.83)	18.49 (-1.11)
	20	17.49 (-4.10)	14.11 (-11.97)	27.80 (-11.83)	16.00 (-3.60)
	30	15.47 (-6.12)	15.34 (-10.74)	25.20 (-14.43)	15.85 (-3.75)
CD at 1 %		1.257	1.276	NS	0.785
Eucalyptus CD at 1 %		1.652	2.046	7.406	0.925
		Nerium oleander			
Aqueous Extract	0	22.29	26.50	41.40	20.40
	10	20.15 (-2.14)	23.19 (-3.31)	38.30 (-3.10)	20.19 (-0.21)
	20	20.64 (-1.65)	23.16 (-3.34)	29.90 (-11.50)	19.85 (-0.55)
	30	18.84 (-3.45)	19.00 (-7.50)	27.30 (-14.10)	19.80 (-0.60)
CD at 1 %		NS	NS	3.392	NS
Acetone Extract	0	22.61	25.10	34.43	20.50
	10	21.14 (-1.47)	16.64 (-8.46)	28.60 (-5.83)	20.28 (-0.22)
	20	19.78 (-2.83)	14.72 (-10.38)	22.60 (-11.83)	19.36 (-1.14)
	30	18.74 (-3.87)	15.31 (-9.79)	21.10 (-13.33)	18.50 (-2.00)
CD at 1 %		2.445	4.661	NS	NS
Nerium CD at 1 %		2.362	5.477	7.414	NS

+: Stimulation, -: Inhibition over control.

The significant inhibition in shoot growth might be related to the decrease in oxygen absorption capacity partly by phenolic allelochemicals, while, the reduction in chlorophyll content influenced photosynthesis (39). Reduction of chlorophyll under allelochemical stress could be due to the inhibition of chlorophyll biosynthesis or the stimulation of degradation or both (10). John and Sarada (30) reported that phenolic allelochemicals inhibited the root elongation, cell division and alter the cell ultra-structure thus subsequently interfered with normal growth and development of whole plant. Allelochemicals hinders the amino acids incorporation into proteins, therefore, decreases the rate of protein synthesis (9,11). Ayepola *et al.* (8) reported the presence of volatile oils and balsam (gum) in leaf extracts of *Eucalyptus*. The secondary metabolites screening of *E. camaldulensis* leaf extracts confirmed the presence of tannin, saponins, glycosides and phenolic allelochemicals, these influence the nutrients absorption eventually affecting the plant growth (30). In addition, phenolic allelochemicals also reduce the physiological activity of plant hormones, which consequently inhibits the normal physiological processes (24).

The higher concentration (20 and 30 %) of *Nerium* extract reduced the shoot growth, this indicate that *N. oleander* contains certain phytochemicals that retard the growth of date palm seedlings. This is in agreement with the results of previous study, as the seedling growth of ryegrass was inhibited by the increasing extract concentration of *N. oleander* (47). Furthermore, decline in seedling growth could be associated with the presence of rutin and quercetin, which suppresses the growth of seedling (32). The decline in the growth and development of plant morphological attributes such as leaf length and leaf area, consequently negatively affects the total chlorophyll content, leaf dry matter and carbohydrate content in leaves.

Leaves aqueous and acetone extracts of *Eucalyptus* and *Nerium* at 30 % significantly reduced the root length and ratio of root to shoot dry weight (Table 2, Figure 8). The 10 % aqueous extracts of both plants leaves were non-inhibitory to the root length and carbohydrates in roots. While, 20 % *Eucalyptus* and *Nerium* aqueous extracts significantly reduced both the ratio of root to shoot and their dry weight.

Table 2. Effects of *E. camaldulensis* and *N. oleander* leaves extracts on Date palm seedlings roots growth

Extracts	Conc (%) w/v	Ratio of Root: Shoot dry wt.	Root Carbohydrates (%)
	Eucalyptus camaldulensis		
Aqueous Extract	0	2.33	12.46
	10	1.93 (-0.40)	12.11 (-0.35)
	20	1.84 (-0.49)	11.80 (-0.66)
	30	1.75 (-0.58)	11.76 (-0.70)
CD at 1 %		0.093	NS
Acetone Extract	0	2.35	12.02
	10	1.97 (-0.38)	11.69 (-0.33)
	20	1.74 (-0.61)	11.54 (-0.48)
	30	1.63 (-0.72)	11.49 (-0.53)
CD at 1 %		0.106	0.135
Eucalyptus CD at 1 %		0.091	0.385
	Nerium oleander		
Aqueous Extract	0	2.36	12.23
	10	1.88 (-0.48)	12.19 (-0.04)
	20	1.76 (-0.60)	11.77 (-0.46)
	30	1.38 (-0.98)	11.69 (-0.54)
CD at 1 %		0.354	0.435
Acetone Extract	0	2.48	11.67
	10	1.65 (-0.83)	11.66 (-0.01)
	20	1.55 (-0.93)	11.57 (-0.10)
	30	1.54 (-0.94)	11.33 (-0.34)
CD at 1 %		0.691	NS
Nerium CD at 1 %		0.501	0.493

+: Stimulation, -: Inhibition over control.

The acetone extracts were more inhibitory to root growth than aqueous extracts and the decrease was concentration dependent. The 20 % of *Eucalyptus* and *Nerium* acetone extract significantly reduced the root length and ratio of root dry weight to shoot dry weight.

Maximum inhibition (37.4 and 34.3 %) in root length was recorded by 30 % acetone extract of *Eucalyptus* and *Nerium* respectively. Whereas, the carbohydrates content in roots showed a significant inhibition when applying 30 % of Nerium extract and 20 and 30 % of aqueous and acetone extracts of Eucalyptus.

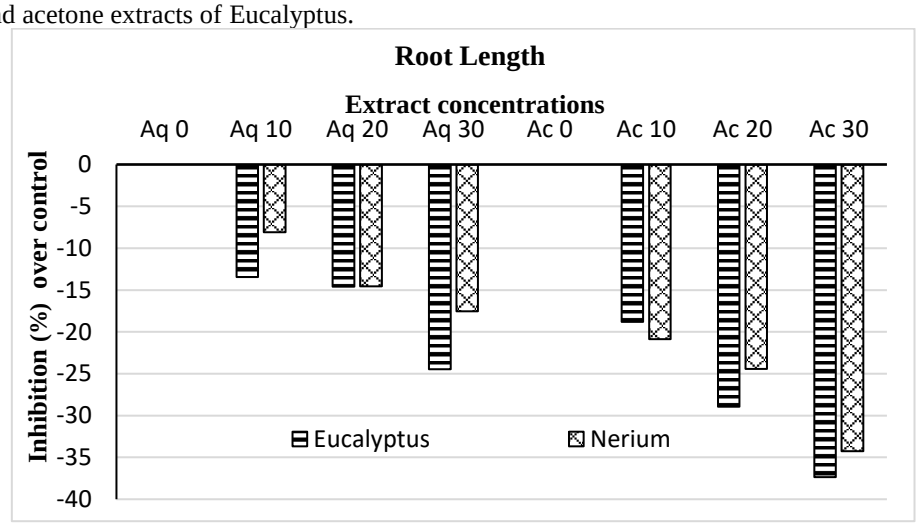

Figure 8. Effects of *E. camaldulensis* and *N. oleander* leaves extracts on Date palm seedlings root length. Aqueous extract treatments Aq 0 : untreated control; Aq 10 : 10 %; Aq 20 : 20 %; Aq 30 : 30 % (v/v) and Acetone extract treatments Ac 0 : untreated control; Ac 10 : 10 %; Ac 20 : 20 %; Ac 30 : 30 % (v/v).

Figure 9 showed the relationship between total soluble carbohydrates in shoots and in roots of Date palm as affected by Eucalyptus extracts where r = 0.874. While, Figure 10 showed the relationship between total soluble carbohydrates in shoots and in roots as affected by *Nerium* (r = 0.284).

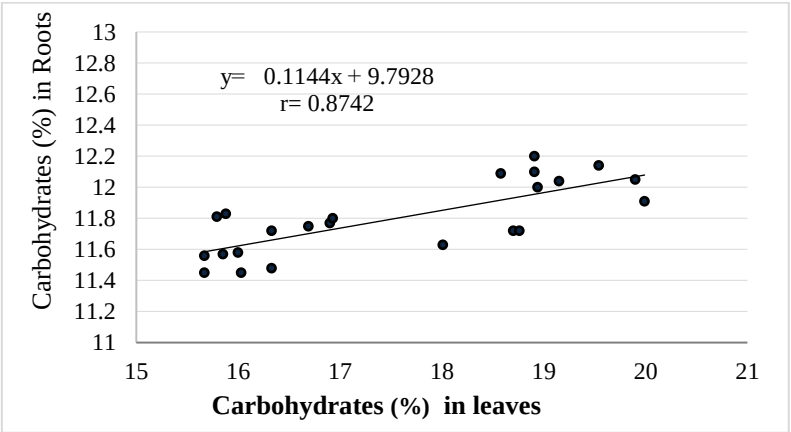

Figure 9. The relationship between total carbohydrates content in leaves and roots of date palm seedlings when treating with *Eucalyptus camaldulensis* L. leaves extracts.

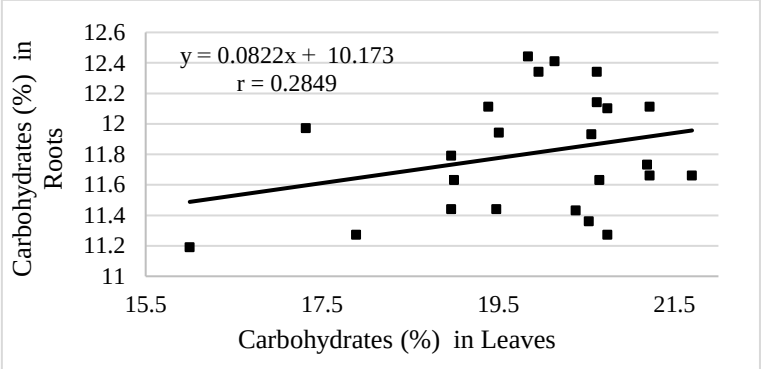

Figure 10. The relationship between total carbohydrates content in leaves and roots of date palm seedlings when treating with *Nerium oleander* L. leaves extracts.

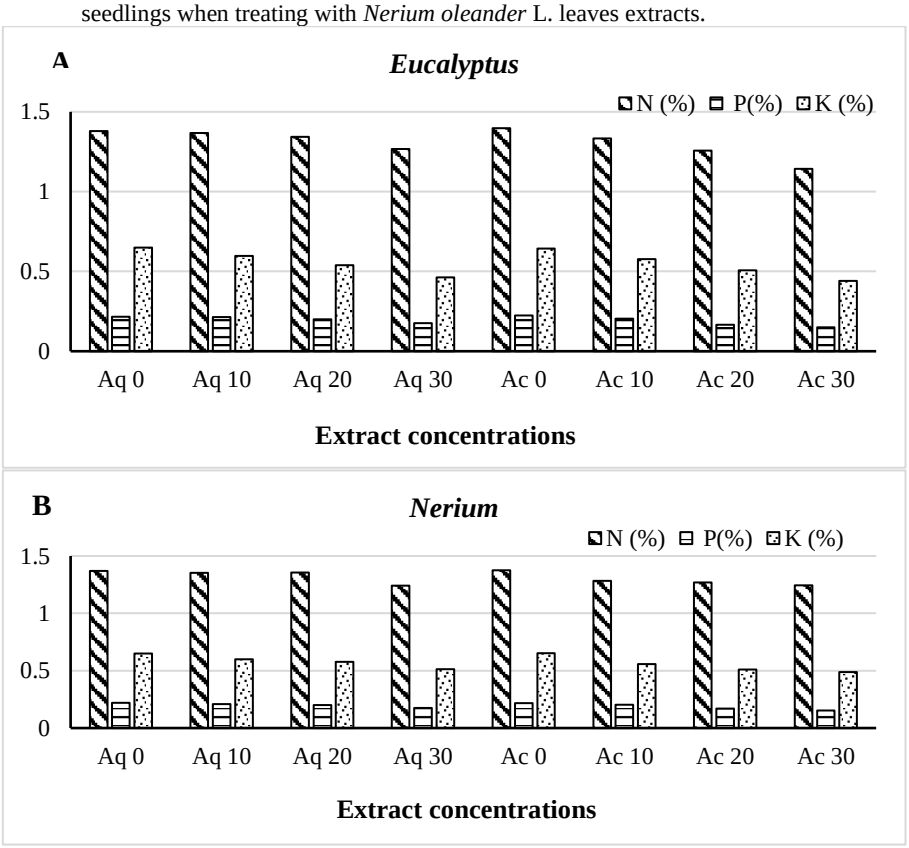

Figure 11. Effects of *E. camaldulensis* (A) and *N. oleander* (B) leaves extracts on Date palm seedlings chemical composition. Aqueous extract treatments Aq 0 : untreated control; Aq 10 : 10 %; Aq 20 : 20 %; Aq 30 : 30 % (v/v) and Acetone extract treatments Ac 0 : untreated control; Ac 10 : 10 %; Ac 20 : 20 %; Ac 30 : 30 % (v/v).

The nitrogen (N), phosphorus (P) and potassium (K) in shoots were significantly decreased ($P \geq 5$ %) over control in 30 % of both aqueous and acetone extracts of *Eucalyptus* and *Nerium* treatments. The 20 % of *Eucalyptus* aqueous extracts caused 8.21, 18.45 and 28.72 % inhibition in the N, P, K content. Whereas, 20 % of *Eucalyptus* aqueous extracts were significant in reducing phosphorus, potassium and *Nerium* aqueous extract at 20 % significantly reserved phosphorus and potassium only (Figure 11). Abdul-Sahib *et al*. (2), reported findings similar to this study on Date palm, with *Conocarpus erectus* L. leaf extract.

The significant inhibitory effects of *Eucalyptus* extracts on root parameters and macro elements contents in shoots may be due to the presence of phenolic allelochemicals that retards normal growth and development of the whole plant (30). The negative effects of extracts could due to the impairment of roots activity, as they are the first organs that contact the allelochemicals in the rhizosphere, therefore, hinders the ion uptake and hydraulic conductivity (13). The roots tip respiration might be inhibited, which eventually reduces their elongation. Besides, allelochemicals could be associated with root browning and the decrease in root hairs formation (43). Karaaltin *et al.* (31) stated that radicle length was significantly reduced due to the allelopathic effects of *N. oleander* extract. Phenolic acids are considered toxic to plant growth due to their interference with vital growth processes and many enzymes and phytohormones activities (20,26). Uslu *et al.* (48) reported that the *N. oleander* plants reduced the germination and growth of surrounding plant species.

CONCLUSIONS

The aqueous and acetone extracts of both *Eucalyptus* and *Nerium* plants leaves significantly reduced the seed germination and seedlings growth of Date palm. However, the acetone extracts were more harmful to all studied parameters than water extracts. Irrigation with *Eucalyptus* or *Nerium* extracts decreased the seedlings shoot growth, root growth and contents of macro elements in shoots. *Nerium* extracts were more effective than *Eucalyptu*s in decreasing seeds germination and seedling growth of Date palm.

DECLARATION

We declare that all authors of this manuscript made a significant contribution, and we have not excluded any author that substantially contributed. We have followed the ethical norms established by our respective institutions.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ETHICAL APPROVAL

The authors declare that the study was carried out following scientific ethics and conduct. However, this study did not involve any use of animals, hence no ethical approval has been obtained from the concerned committee.

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