Caspian Journal of Environmental Sciences
Online ISSN: 1735-3866
Print ISSN: 1735-3033

Allelopathic effect of *Nerium oleander* L. alcohol extract on growth and development of *Arabidopsis thaliana* (L.) Heynh.

Inas Sabah Mohammed Shareif [1*], Sahar A.A. Malik Al-saadi[1], Hussein K. Al-Kaaby[2]

1. Department of Biology, College of Science, University of Basrah, Iraq
2. Department of Biology, College of Education for Pure Sciences, University of Basrah, Basrah, Iraq

**Corresponding author's email: inas.sabah@uobasrah.edu.iq*

ABSTRACT

The allelopathic interactions of different concentrations (0, 2, 4, 6, 10 g in 100 mL^{-1}) of alcoholic extract of *Nerium oleander* flower on *Arabidopsis thaliana* (Columbia-0) were studied. The results showed that leaf area, plant height and inflorescence emerge period decreased by elevation in the treatment concentration. The highest value of leaf area (1.176 cm^2) was recorded in control group, while it was 0.63 cm^2 in treatment 2 g in 100 mL^{-1} (T_2) and 0.5 cm^2 in T_{10}. Chlorophyll concentrations were decreased by the upraised concentrations. The highest value (4.15 mg g^{-1}) was recorded in control, while the lowest (3.3 mg g^{-1}) in T_{10}. Anatomical study revealed that the internal structure of stem and root undergone numerous character changes. The thickness of the stem varies between treatments. The control exhibited the highest average (109.37 µm), while T_{10} had the lowest average (81.25 µm). Collenchyma layer increased in all exposure treatments, compared to control. There was a change in the internal structure of the roots between the control and the alcoholic extract treatment. The root shape exhibited a lot of modification: it seemed circular in control, semi-circular in T_4, while elliptic or irregular shape in T_{10}. The root thickness reached 95 µm in T_2 and T_4, whereas the lowest thickness in T_{10} (70.33 µm). Vascular bundle in the control was cylindrical. However, there was some irregular growth on one side of the root in T_4. In T_{10}, larger vascular bundles and random growth were observed, while the root shape appeared to be irregular, due to the abnormal growth of vascular bundles or irregular growth.

Keywords: Allelochemicals, allelopathy, *Nerium*, *Arabidopsis thaliana*, anatomy, stem, root.
Article type: Research Article.

INTRODUCTION

Allelopathy is a biological phenomenon in which chemical compounds produced by living or dead plants influence the growth, as well as the development of other organisms (Cheng & Cheng 2016; Willis 2007). Allelopathy can have a direct or indirect effect, which can be beneficial or detrimental (Rice 1984). Whittaker (1971) called these chemicals allelochemicals, and majority of them are secondary products (Gross 2009). Allelopathy depends on the type of these chemical compound (Einhelling 2004), which could be released by any part of plant from root, stem or flowers by leaching, volatilization, residue decomposition, or other mechanisms (Mishyna *et al*. 2015; Ferguson *et al*. 2003). Allelopathy is important in many aspects as insecticide (Xuan *et al*. 2004), weed management (Kung *et al*. 2008), eliminatation of pollutants as nitrogen pollution (MA

Caspian Journal of Environmental Sciences, Vol. 20 No. 1 pp. 155-163 Received: April 23, 2021 Revised: June 15, 2021 Accepted: Aug. 12, 2021
DOI: 10.22124/CJES.2022.5411

Publisher: University of Guilan, Iran

2005) as well as plant growth induction and suppression (Duke 2004; Patterson 2003). *Nerium oleander* is important plant for its active chemical compound and medicinal use (Dey 2020; Dey & Chaudhuri 2004). It is very common in Iraq environment, found in most types of house gardens and orchards. *N. oleander* is a poisonous plant that is recognized for its glycosides and flavonoids. Oleandrin is the most important glycoside in this plant (Mojarad *et al*. 2013). Allelopathic effects of *N. oleander* extract on several plant species have been observed in some studies (Mojarad *et al*. 2013; Uslu *et al*. 2018; Begam *et al*. 2020). Atypical plant, *A. thaliana* has been used in studies around the world for its features (Padole & Ingle 2017), since it has short life cycle, small size, easy to breed and self-pollination (Koorn Neef & Meinke 2010). Schulz *et al*. (2007) reported that the aerosol smoke inhibits germination of *A. thaliana* seeds. Chemical component found in *N. oleander* extract contain phytochemicals (flavonoids, triterpenoids and steroids) which can interred with metabolism process (Al-Saadi *et al*. 2017b). The aim of this study was to assess the allelopathic effects of *N. oleander*, on the seed germination and growth of *A. thaliana*, as well as the anatomy of internal structure including stems and roots.

MATERIALS AND METHODS

Sample collection and extract preparation

Nerium oleander was used in allelopathic experiment. Sample was prepared from pink flower collected in the morning, then washed and dried in shed, and ground into powder. Ethyl extract of *Nerium oleander* flower was made by adding 20 g flower powder to 250 mL ethyl alcohol (70%) for 24 hours on magnetic stirrer, then filtering and drying. The treatments were prepared by dissolving the solid alcohol extraction in distilled water as 0, 2, 4, 6 and 10 g in 100 mL ($T_{0,}$ $T_{2,}$ $T_{4,}$ $T_{6,}$ T_{10}).

Pots preparation

Arabidopsis thaliana (ecotype Columbia-0) seeds were acquired from Carolina Biological Supply in USA, were germinated and grown in growth chamber for two months during January and February 2020. Using cylindrical pots (4 replicates for each concentration with control), which were 12 cm in length and 12 cm in diameter, we mixed soil containing sand and peat (in ratio 1:1). After washing the mixture with distilled water (100 Cº) for three times, we left it to dry under sun light. Experiment was conducted under controlled condition in growth chamber 20-22 ºC, light period 18:6 (light:dark), LED lamps of 6000 lux, humidity 50-70%, irrigated with Hogland solution (1.6 g L^{-1}) to field capacity. Each pot planting with 10 seeds. After 4 weeks only 6 similar plants were remained and the others were removed. After four weeks, extraction was added and After four other weeks, the *A. thaliana* was supplied by a *Nerium oleander* flower ethyl extract.

Morphological and biochemical characters

Morphological characteristics were measured after 8 weeks from the treatments including leaf area (cm^2), plant height (cm), number of siliques, inflorescence stem emerges (day) according to Saieed (1990). Total chlorophyll was measured by the method of Arnon (1949). So that, 1 g fresh leaf material was taken and ground with 20 - 30 mL of 80% acetone and then centrifuged at 5000 rpm for 5 minutes. Supernatant was transferred to the volumetric 100-mL flask and the procedure was repeated till the residue becomes colorless. The absorbance of the extracted solution was measured at 480, 510, 645 and 663 nm against the solvent (acetone) blank. Estimation of total chlorophyll content were calculated using the following formula/equation:

Total Chlorophyll: 20.2(A645) + 8.02(A663)

Anatomical examinations

Fresh material of *A. thaliana* was collected after 8 weeks from the treatments. For sectioning, fresh materials of stems and roots were fixed 24 hours in formalin acetic acid alcohol solution (FAA), and preserved in 70% alcohol, then dehydrated in ethyl alcohol series. Samples sectioned on a rotary microtome and then stained in safranin and fast green, then mounted in Canada balsam (Johansen 1940). The samples were examined using Olympus CH4 light microscope and photographed with Digital camera type DCE-2.

Statistical analysis

All the data were analyzed according to the L.S.D (Least Significant Difference) test at a significant level of $p < 0.05$ using One-Way ANOVA followed by calculating the means using computer software SPSS 17.

RESULTS AND DISCUSSION

Morphological characters

The results showed that leaf area decreased by the treatment concentration elevations. The highest value of leaf area was recorded in control sample (1.176 cm²), while 0.63 cm² in treatment 2 g in 100 mL (T_2) and 0.5 cm² in T_{10} (Fig. 1-A). The results demonstrated a gradual decrease in leaf area, which might be attributable to the antagonistic action inhibiting cell division (Hussain *et al.* 2020). Plant height average varied between control and the treatments. It was 22.97 cm in control where the highest value was 23.5 cm in T_6, then T_2 was 22.37 cm, while the least value was 17.87 cm in T_{10} (Fig. 1-B). The period of appearing the inflorescence stem was different between control and the treatments. It was appeared after 34 days in T_6, then T_{10}, while in control group was 39 days. Average number of siliques recorded the highest value in T_6 (13.4 siliques) then T_2 (13.3 silique), while in control was 4.7 and 9.62 siliques in T_{10} (Fig. 1-C and D). The control group had the highest measurement and significantly outperformed in the other treatments, since the decline was gradually observed according to concentration, which is in agreement with Nikpeyma *et al*. (2019). Cheng & Chen (2016) suggested that the positive and negative effects of chemical antagonism can be attributed to the fact that low concentrations of allelochemicals are stimulating, while high concentrations are inhibitory. In addition, plant and its development in response to stress can change gene expression, so that some genes are intensely expressed, while others are suppressed (Bray *et al*. 2000; Javaid & Anjum 2005). Chlorophyll concentrations were decreased with the elevated concentrations and recorded the highest value in control (4.15 mg g^{-1}), while the lowest value were in T_{10} (3.3 mg g^{-1}; Fig. 1-E). The results revealed that the control had the highest chlorophyll value, while T_{10} had displayed the lowest. The concentration of chlorophyll is considered evidence of plant health (Porra 2002). Healthy plant has more chlorophyll amount and more growth than the unhealthy one (Wu *et al*. 2008). Chlorophyll is an important pigment for the photosynthesis process, as it works to convert solar energy into chemical energy (Gitelson & Merzlyak 2003). Chlorophyll increases by the development of the leaf and then decays in the aging stage of the plant (Pereyra *et al*. 2014). Some studies reported that allelochemicals reduce the amount of chlorophyll by inhibiting chlorophyll a in chlorophyll content, because of finding some compounds in plants such as of phenolic, vanillic acid, o-hydroxyphenyl acetic, p-hydroxybenzoic acid, camminic acid, ferulic and p-coumaric acids (Baziramakenga *et al*. 1997; Yang *et al*. 2002; Yang *et al*. 2004; Sarkar *et al*. 2012; Singh *et al*. 2013).

Anatomical study

Transverse sections of stem

The results were depicted in Fig. 2 and Table 1. Shape, size, and the numbers of cortex layers are taxonomically significant to identify species. The stems gave significant characteristics in the variance between the concentrations and as shown in Fig. 2 and Table 1, it was found that the shape, size and number of layers of the cortex were different between the different treatments. Shape of stem varied between the treatments. It was semi-oval to circular in the control group, while circular in T_2, T_4 and T_6, and irregular in T_{10}. Thickness of stem differed between the treatments, the highest average was recorded in the control (109.37 µm), while the lowest in T_{10} (81.25 µm; Table 1). Collenchyma layer increased in all treatments compared to control group. In the case of the thickness of the epidermis, there was no difference between the treatments; the highest average was recorded in the control group (1.22 µm; Table 1). The cortex in control group consists of two types of tissues, chlorenchyma, and parenchyma tissues. Chlorenchyma layers are located after the epidermis and their number ranges from 7-9 layers. The average chlorenchyma thickness was 3.5 µm in control group 2.5 µm in T_2 and T_4 and the lowest average found in T_6 and T_{10} (2.2 µm; Table 1). Vascular bundle was collateral type, in control

treatment it was circular arrangement, regular and continuous. The xylem elements appeared separate bundles formed from long and short rows.

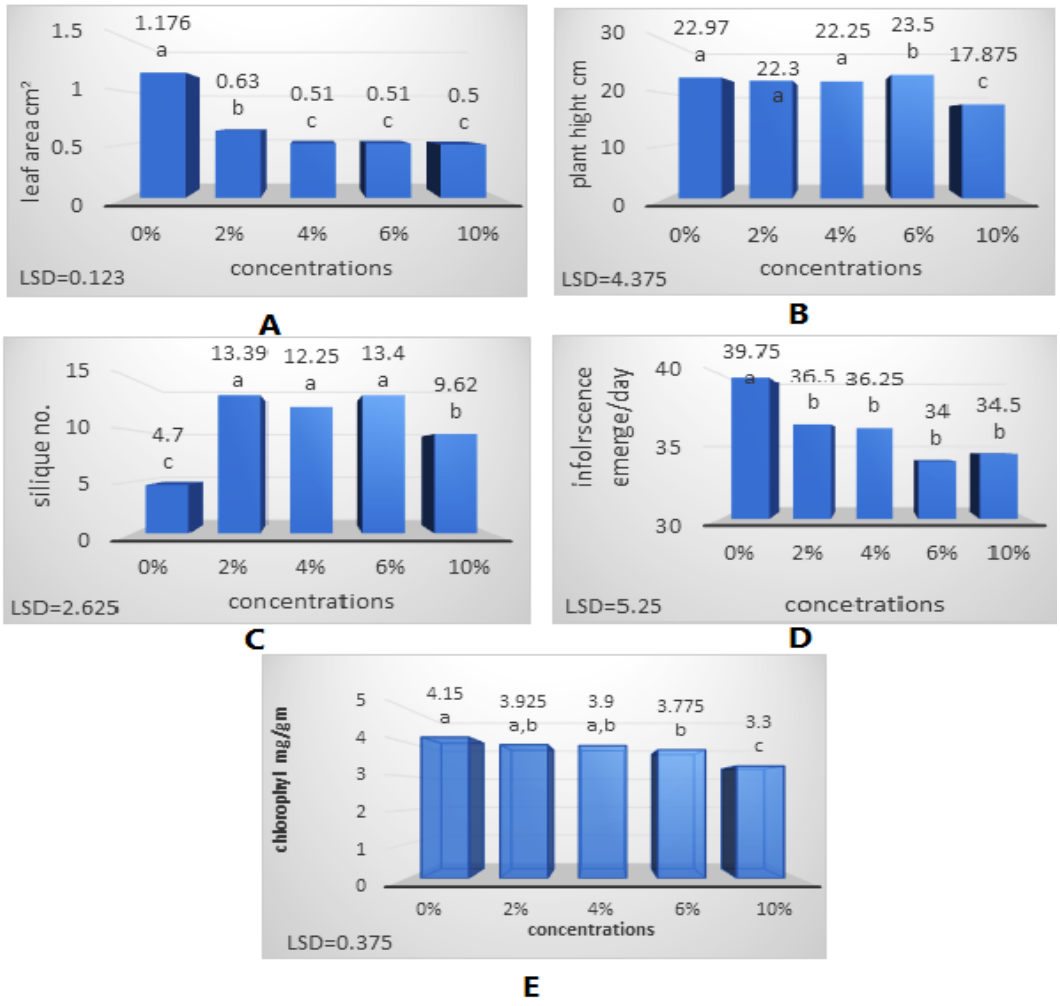

Fig. 1. Allelopathic effect of alcohol extract of *N. oleander* flowers on *A. thaliana*; (A) leaf area, (B) plant height, (C) silique number (D) inflorescence emerge/day, (E) chlorophyll concentration (mg g^{-1}).

The bundles appeared irregularly in T_2 by increased thickness. In T_{10}, the thickness of the vascular bundles was more irregularity and the vascular bundle appeared zigzag and irregular growth (Fig. 2). The changes occurred in vascular bundle were in agreement with other studies (Al-Saadi *et al*. 2013; Hamza *et al*. 2020; Sabeh *et al*. 2017; Al- Saadi *et al*. 2017a; Al-Abbawy *et al*. 2020). Thickness of the vascular bundle varied between the treatments compared to control. The lowest thickness was recorded in T_{10} (10.2 µm), while the highest in control (12.66 µm; Table 1). The pith is occupying the center of stem, consisting of circular storage cells or irregular shapes, interspersed with air spaces. Thickness of the pith increased by the elevated treatment concentration. The highest value was found in T_{10} (77.3 µm), while the lowest in control (45.2 µm; Table 1). The results of the transverse sections of stems in the control coefficient showed a similar description of the vascular bundles with most species in the cruciferous family (Mousavi & Rad 2014; Jung *et al.* 2008; Orcan & Binzet 2003; Metcalfe & Chalk 1950). According to the results, the control group exhibited the largest rate of stem diameter, and decreased gradual, with the lowest rate in T_{10}, in agreement with Khan (2017). Roots anatomy gives many

changes in th treatments, in agreement with Mojarad *et al*. (2013). Furthermore, the presence of chemical substances influences the root cell membrane and enzymes in the membrane, affecting water and element absorption (Li *et al*. 2002; Cruz-Ortega *et al*. 2007). Several allelochemical compounds influence cell size by stimulating nuclear changes and an increase in the number of vacuoles in the root, as well as inhibition of the longitudinal growth of the root leads to an increase in its thickness (Chon *et al*. 2002; Pawlowski *et al*. 2012; Chaimovitsh *et al*. 2012).

Internal structure of the root

The results of internal structure of root in different treatments are depicted in Table 2 and Fig. 3. Control treatment consists of epidermis which contains one layer with circular or rectangular cells, followed by a layer of the exodermis, then cortex consisting of several rows of cells, followed by the endodermis layer. Vascular bundle is arranged in continuous cylinder and forms the center of the root. The results between control and the treatments exposed to the alcoholic extract exhibited that there was a difference in the internal structure compared to control group. When employing the alcoholic extract of *Nerium oleander* at T_4 and T_{10}, the shape morphology displayed a lot of modifications. The shape seemed circular in the control group, semi-circular in T_4, and elliptic or irregular shape in T_{10}. The root thickness reached 95 µm in T_2 and T_4, whereas the lowest thickness in T_{10} (70.33 µm; Table 2). The results revealed that when the concentration of the alcoholic extract upraises, the number of cortical layers elevates. The primary alterations identified in root vascular bundle shape were regular shaped made continuous cylindrical shape in control group, however, it noticed some irregular growth was observed on one side of root in T_4. In this treatment, enlarged vascular bundles and random growth were found. As a result of the abnormal growth of vascular bundles or irregular growth, the root shape appeared to be irregular.

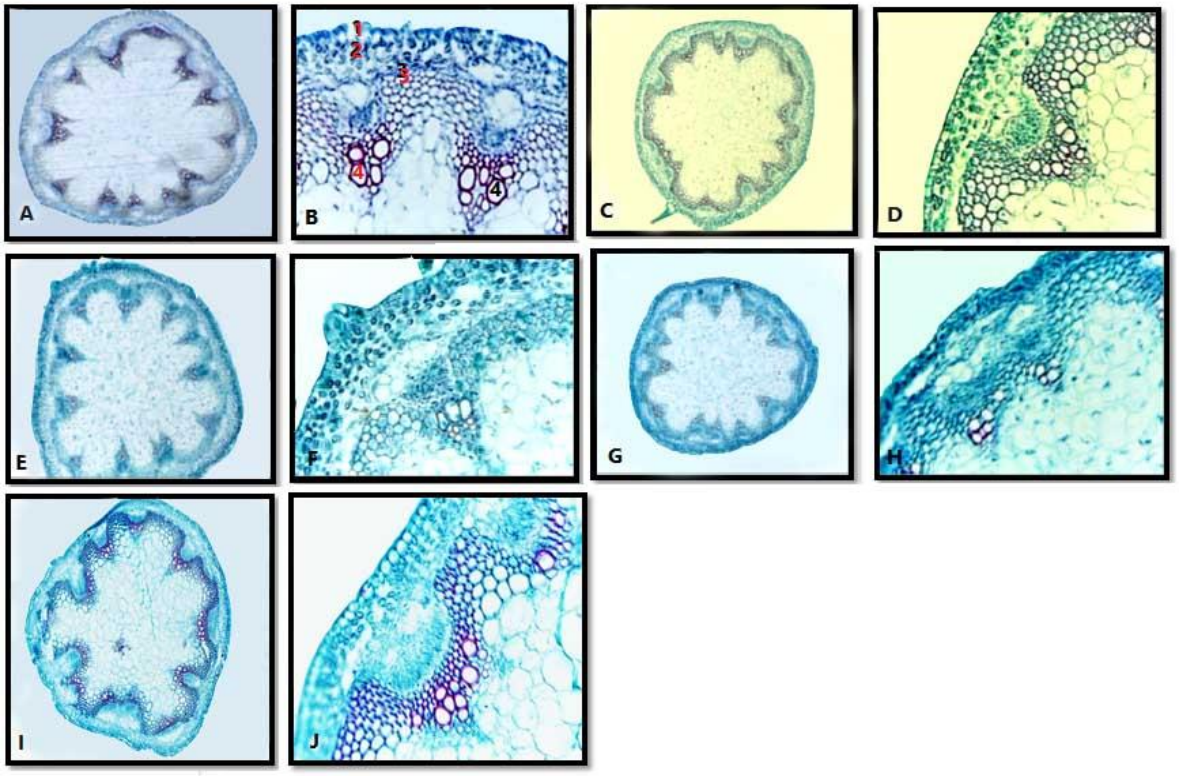

Fig. 2. transverse section of *A. thaliana* stem in control and treatment exposure with alcohol extract of *N. oleander* flowers: (A-B) control; (B-C), T_2; (E-F), T_4; (G-H), T_6; (I-J), T10.

Table 1. Anatomical measurements of the stem of *A. thaliana* after exposure to the alcohol extract of *N. oleander* flowers (µm).

Characters Treatments	Phloem	Xylem thickness	Vascular bundle thickness	Pith thickness	Cortex thickness	Chlorenchyma thickness	Collenchyma thickness	Epidermis thickness	Stem thickness
Control	(2.5_3/75) 3/2	(7.5-10) 9/16	(10-13) 12/66	(40-55) 45/2	(5-6.25) 5/5	(2/5-3/75) 3/5	(0.5-1.25) 0/8	(1-1.25) 1/22	(87.5-125) 109/37
2%	(2.5_5) 3/75	(2.5_7/5) 5.6	(10-12) 11/5	(55-60) 58/2	(3.75-5) 4/583	(1.25-3.75) 2/5	(0.75-1.25) 1/25	(1-1.25) 1.22	(75-125) 100
4%	(2.5_5) 4/5	(5_6/5) 5/6	(10-13) 10/5	(65-70) 67/08	(3.75-5) 4/375	(1.25-3.75) 2/5	(0.75-1.25) 1/25	(1-1.25) 1/2	(75-100) 95
6%	(3-5) 4/5	-3/75) (2/5 3/125	(10-11) 10/25	(70-75) 72/5	(3.75-5) 4/35	(2.5-3.75) 2/2	(1-1.5) 1/25	(1-1.25) 1/2	(75-100) 87/5
10%	(3_5) 3/75	-3/75) (2/5 2/5	(10-11) 10/2	(75-80) 77/3	(2-3.5) 3/33	(2-2.5) 2/2	(1-1.5) 1/25	(1-1.25) 1/2	(75-87.5) 81/25

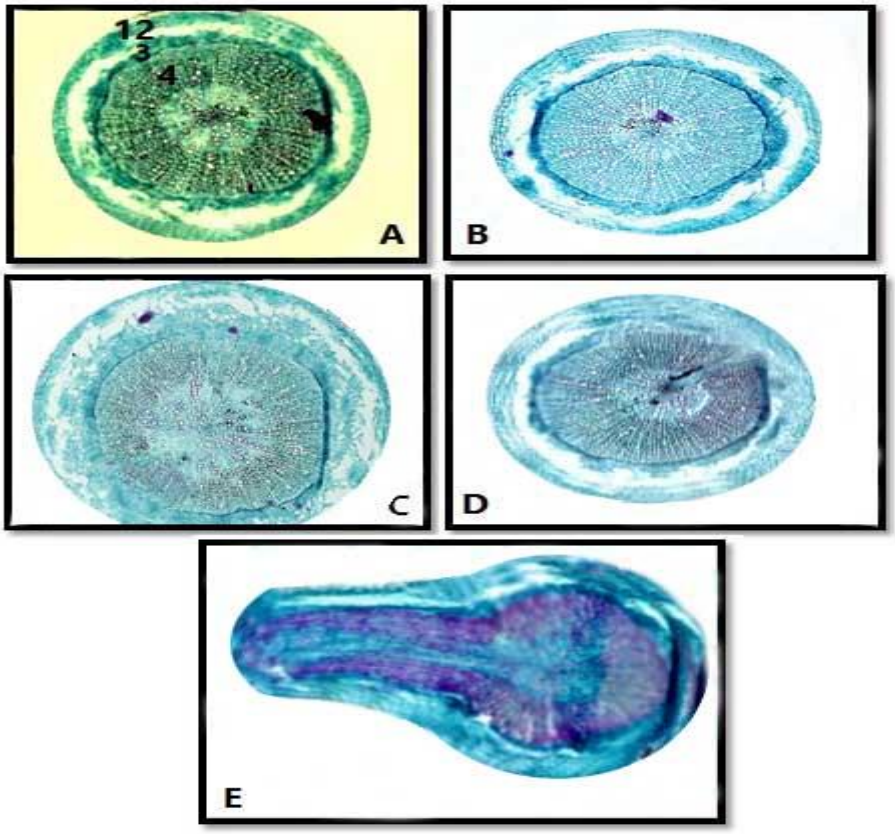

Fig. 3. Transverse section of *A. thaliana* stem in control and the treatments exposed to alcoholic extract of *N. oleander* flowers: A: control; B: T_2; C: T_4; D: T_6 and E: T_{10}.

Allelochemicals wreak havoc was found on root growth, which is characterized by a rapid metabolic rate (Cruz-Ortega *et al*., 1998). In addition to the effect on root elongation, we observed cell division and root thickness on the longitudinal growth of cells (Chon *et al*. 2002). Allelochemicals affect mineral absorption by inhibiting the absorption of calcium, iron and magnesium (El-Shabasy 2017), and also inhibit the role of antioxidant enzymes and increase free radicals and the oxidative decomposition of lipids in the lipid peroxidation. These chemicals also change the permeability of the membrane and thus reduce the disposal of free radicals ROS, leading to the destruction of the membrane system in plants (Ding *et al.* 2016).

REFERENCES

Al-Abbawy, D, Al-Sweid, Z & Al-Saady, SA 2020, Effects of salinity stress on biochemical and anatomical characteristics of *Ceratophyllum demersum* L. *EurAsian Journal of BioSciences*, 14: 5219-5225.

Al-Saadi, SAM, Al-Asaadi, WM & Al-Waheeb, AN 2013, The effect of some heavy metal's accumulation on physiological and anatomical characteristic of some Potamogeton L. plant. *Journal of Ecology and Environmental Sciences*, 4:100-108.

Al-Saadi, SA, Alutbi, AD & Madhi, ZJ 2017a, The effects of in vitro culture on the leaf anatomy of potato. *International Journal of Current Research*, 9: 54337-54342.

Al-Saadi, SAAM, Al-Derawi, KH & Abd Alrasool, S 2017b, The Effect of Steroidal Extract of *Nerium oleander* on the liver tissue structure of albino mice. *IOSR Journal of Pharmacy and Biological Sciences*, 12: 52-55.

Arnon, DI 1949, Copper enzymes in isolated chloroplasts polyphenol oxidase in Beta vulgaris. *Plant Physiology*, 24: 1-15.

Baziramakenga, R, Leroux, GD, Simard, RR & Nadeau, P 1997, Allelopathic effects of phenolic acids on nucleic acid and protein levels in soybean seedlings. *Canadian Journal of Botany*, 75: 445-450.

Begam, UJ, Srikrishnah S & Sutharsan, S 2020, Allelopathic suppressive effects of *Thevetia peruviana* L. and *Nerium oleander* L. leaf extracts on germination and seedling growth of *Cyperus rotundus* L. *AGRIEAST: Journal of Agricultural Sciences*, 14: 43-51.

Bray, E, Bailey-Serres, J & Weretilnyk, E 2000, Responses to abiotic stress. In BB, Buchanan, W, Guissem, RL, Jones (eds.) Biochemistry and molecular biology of plants, Rockville, MD: American Society of Plant Physiologists: 1158-1203.

Chaimovitsh, D, Abu-Abied, M, Belausov, E, Rubin, B, Dudai, N & Sadot, E 2012, Microtubules are an intracellular target of the plant terpene citral. *The Plant Journal*, 61: 399-408.

Cheng, F & Cheng, Z 2016, Research Progress on the use of plant allelopathy in agriculture and the physiological and ecological mechanisms of allelopathy. *Frontiers in Plant Science*, 7: 1-16.

Chon, SU, Choi, SK, Jung, S, Jang, HG, Pyo, BS & Kim, SM 2002, Effects of alfalfa leaf extracts and phenolic allelochemicals on early seedling growth and root morphology of alfalfa and barnyard grass. *Journal of Crop Protection*, 21: 1077-1082.

Cruz-Ortega, R, Lara-Núñez, A & Anaya, AL 2007, allelochemical stress can trigger oxidative damage in receptor plants: Mode of action of phytotoxicity. *Plant Signaling & Behavior*, 2: 269-270.

Devakumar, C & Parmar, BS, 1993, Pesticides of higher plant and microbialorigin. In: BS, Parmar, C, Devakumar (eds.) Botanical and pesticides. SPS Publication No. 4, Society of Pesticide Science, India and Westvill Publishing House, New Delhi, pp. 1-73.

Dey, P 2020, Review The pharmaco-toxicological conundrum of oleander: Potential role of gut microbiome. *Biomedicine & Pharmacotherapy*, Vol. 129, https://doi.org/10.1016/j.biopha.2020.110422.

Dey, P & Chaudhuri, TK 2014, Pharmacological aspects of *Nerium indicum* Mill.: A comprehensive review. *Pharmacognosy Reviews*, 8: 156-162.

Ding, HY, Cheng, ZH, Liu, ML, Hayat, S & Feng, H 2016, Garlic exerts allelopathic effects on pepper physiology in a hydroponic co-culture system. *Biology Open*, 5: 631-637.

Duke, SO, Cedergreen, N, Velini, ED & Belz, RG 2006, Hormesis: Is it an important factor in herbicide use and allelopathy? *Outlooks Pest Manag*, 17: 29-33.

Einhellig, FA 2004, Mode of allelochemical action of phenolic compounds. In: FA, Macias, JCG, Galindo, JMC, Molinilo & HG, Cutler (eds.) Allelopathy: chemistry and mode of action of allelochemicals. CRC Press, Boca Raton. pp: 217-238.

El-Shabasy, A 2017, Study on allelopathic effect of *Prosopis juliflora* on mineral content of *Acacia ehrenbergiana* in Farasan Islands, KSA. *Journal of Medicinal Plants Studies*, 5: 130-134.

Ferguson, JJ, Rathinasabapathi, B & Reichenbach, R 2003, Evaluation of allelopathic potential of wood chips used for weed suppression in sustainable fruit crops production systems. Annual meeting of the American Society of Horticultural Science, Providence, RI, pp. 3-6.

Gitelson, AA & Merzlyak, MN 2003, Relationships between Leaf Chlorophyll Content and Spectral Reflectance and Algorithms for Non-Destructive Chlorophyll Assessment in Higher Plant Leaves. *Journal of Plant Physiology*, 160: 271-282.

Gross, EM 2009, Allelochemical Reactions. *Encyclopedia of Inland Waters*, pp. 715-726.

Hamza, SM, Al-Saadi, SAM & Al-Abbawy, DA 2020, A study of physiological and anatomical characteristics of the heavy metal accumulation of *Juncus rigidus* defaunations, 1798 (family, juncaceae) in Basrah province, southern of Iraq. *Bulletin of the Iraq Natural History Museum*, 16: 63-81.

Hussein, WM, Abdul-Qadir, LH & Al-Kaaby, HK 2020, Rapid and cost-effective protocol for planting *Arabidopsis thaliana* L. in Iraq. *Journal of Basrah Researches (Sciences)*, 46: 159-169.

Javaid, A & Anjum, T 2005, Biological control of Parthenium IV: Allelopathic effect of Desmostachya bipinnata on distribution and early seedling growth of Parthenium hysterophorus L. *Pakistan Journal of Weed Sciences Research*, 11: 75-79.

Johansen, DA 1940, Plant Microtechnique, McGrow Hill, New York.

Khan, MZ 2017, Effect of allelopathic plants on morpho-anatomical feature of wheat. PhD Dissertation, *Pakistan Research Repository*, 1: 287.

Koornneef, M & Meinke, D 2010, The development of *Arabidopsis* as a model plant. *The Plant Journal*, 61: 909-921.

Li, ZH, Wang, Q, Ruan, X, Pan, CD & Jiang, DA 2010, Phenolics and plant allelopathy. *Molecules*, 15: 8933-8952.

Metcalfe, CR & Chalk, L 1950, Anatomy of Dicotyledon. Clarendon Press, Oxford, 2: 724.

Mishyna, M, Laman, N, Prokhorov, V, Fujii, Y 2015, Angelicin as the principal allelochemical in *Heracleum sosnowskyi* fruit. *Natural Product Communications*, 10: 1-5.

Mojarad, AK, Majd, A & Fahimi, H 2013, Allopathic effects of *Nerium oleander* L. on growth and anatomy structure of *Hordeum vulgare* (monocotyledon) and *Vicia sativa* (dicotyledon) seedlings. *Advances in Environmental Biology*, 7: 766-771.

Mousavi & Rad, JS 2014, Anatomical, palynological and micromorphological study of seed, trichome and stomata of *Cardaria draba* L. Desv. (Brassicaceae) in Sistan, Iran. *International Journal of Biosciences*, 5: 63-69.

Orcan, N & Binzet, R 2003, The Anatomical and palynological properties of *Alyssum obtusifolium* Steven ex. DC (Brassicaceae). *Turkish Journal of Botany*, 27: 63-68.

Pawlowski, A, Kaltchuk-Santos, E, Zini, CA, Caramao, EB & Soares, GLG 2012, Essential oils of *Schinus terebinthifolius* and *S. molle* (Anacardiaceae): Mitodepressive and aneugenic inducers in onion and lettuce root meristems. *South African Journal of Botany*, 80: 96-103.

Pereyra, MS, Davidenco, V, Nunez, SB & Argüello, JA 2014, Chlorophyll content estimation in oregano leaves using a portable chlorophyll meter: relationship with mesophyll thickness and leaf age. Review, *Agronomía and Ambiente*, 34: 77-84.

Porra, JR 2002, The chequered history of the development and use of simultaneous equations for the accurate determination of chlorophyll and b. *Photosynthesis Research*, 73: 149-156.

Rice, EL 1984, Allelopathy, Academic Press, Inc., Orlando. 2nd edition, 422 p.

Sabeh, D, Alutbi Sahar, AA, Malik Al-Saadi, Z & Madhi, J 2017, The Effect of Salicylic Acid on the Growth and Microtuberization of Potato (*Solanum tuberosum* L.) cv. Arizona Propagated in Vitro. *Journal of Biology, Agriculture and Healthcare*, 7: 64-70.

Saieed, NT 1990, Studies of variation in primary productivity morphology in relation to elective improvement of broad -leaved tree species. PhD Dissertation. Naational University of Ireland, 249 p.

Sarkar, E, Chatterjee, SN & Chakraborty, P 2012, Allelopathic effect of *Cassia tora* on seed germination and growth of mustard. *Turkish Journal of Botany*, 36: 488-494.

Schulz, M, Kussmann, P, Knop, M, Kriegs, B, Gresens, F, Eichert, T, Ulbrich, A, Marx, F, Fabricius, H, Goldbach, H & Noga, G 2007, Allelopathic monoterpenes interfere with *Arabidopsis thaliana* cuticular waxes and enhance transpiration, *Plant Signaling & Behavior*, 2: 231-239.

Singh, N, Yadav, K & Amist, N 2013, Phytotoxic Effects of Cinnamic Acid on Cabbage (*Brassica oleracea* var. Capitata). *Journal of Stress Physiology & Biochemistry*, 9: 307-317.

Uslu, ÖS, Gedik, O, Kaya, AR, Erol, A, Khan, MA, Tassever, MN, TÜrkkaya, E 2018, Allelopathic effects of flower extract of Oleander (*Nerium oleander*) on the germination of seed and seedling growth of *Lolium multiflorum*. *Iğdır University Journal of the Institute of Science and Technology*, 8: 309-317.

Whittaker, RH & Feeny, PP 1971, Allelochemics: Chemical inter-actions between species. *Science*, 171: 757-770.

Wu, C, Niu, Z, Tang, Q & Huang, W 2008, Estimating chlorophyll content from hyperspectral vegetation indices: Modeling and validation. *Agricultural and Forest Meteorology*, 148: 1230-1241.

Xuan, TD, Shinkichi, T, Hong, NH, Khanh, TD & Min, CI 2004, Assessment of phytotoxic action of *Ageratum conyzoides* L. (billy goat weed) on weeds. *Crop Protection* 23: 915-922.

Yang, CM, Chang, F, Lin, SJ & Chou, CH 2004, Effects of three allelopathic phenolics on chlorophyll accumulation of rice (*Oryza sativa*) seedlings: II. Stimulation of consumption-orientation. *Botanical Bulletin of Academia Sinica*, 45: 119-125.

Yang, CM, Lee, CN & Chou, CH 2002, Effects of three allelopathic phenolics on chlorophyll accumulation of rice (Oryza sativa) Seedlings: I. Inhibition of supply-orientation. *Botanical Bulletin of Academia Sinica*, 43: 299-300.

Bibliographic information of this paper for citing:

Shareif, ISM, Malik Al-saadi, SAA, Al-Kaaby, HK 2022, Allelopathic effect of *Nerium oleander* L. alcohol extract on growth and development of *Arabidopsis thaliana* (L.) Heynh. Caspian Journal of Environmental Sciences, 20: 155-163.