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Allelopathic effects of aqueous leaf leachates and root exudations of *Conocarpus erectus* L. trees against the germination and growth of Some ornamental plants

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ABSTRACT

There are many efforts being made to improve the green space in urban streets of Iraq. Recently, interest has increased considerably in planting *Conocarpus erectus* L. trees all over Iraq. A laboratory experiment based on factorial Randomized Complete Blocks Design (RCBD) with three replicates, was conducted in College of Science University of Al-Qadisiya to investigate the Allelopathic potential of leaf leachates and root exudations of *Conocarpus erectus* L. trees against four ornamental plants including *Dimorphothica aurantiaca* L. , *Chrysanthemum carinatum* L., *Calendula officinalis* L. and *Gaillardia pulchella* L. Results showed that both Leaf leachates solution and root exudates solution of *C. erectus* , inhibited significantly the seed germination percentage (G%) , coefficient of velocity (CV), plumule length (PL) , radicle length (RL) and Vigor index(VI) of the experimental species under study. The data revealed that the reduction in all parameters measured was maximum when treated with Leaf leachates solution. It was also found that the seeds of *Calendula officinalis* L. was more resistant to the Allelopathic effects of *C. erectus* L. solutions, while *Chrysanthemum carinatum* L. Was the most vulnerable among all the seeds of other species. The total phenolics were measured using Folin–Ciocalteu’s assay, and the results revealed that leaf leachates solution scored the highest value in total phenolic content reached (120.46 mg gallic acid /g plant dry weight) in comparison to the root exudates content of total phenolics (89.12 mg gallic acid /g plant dry weight).

Keywords: *Conocarpus erectus* L., Allelopathy, leaf leachates, root exudations, ornamental plants

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INTRODUCTION

Conocarpus erectus L. (family Combretaceae) commonly known as button wood trees are widely planted all over Iraq. It was introduced into Iraq as a part of the Afforestation of Urban streets Program and has become the dominant perennial ornamental plant. The first idea that comes to mind about urban afforestation is woody trees and ornamental shrubs, but it isn't like that precisely. It is a complex system of trees, smaller ornamental plants and all the abiotic factors that surrounds them (Mare and Fikirte, 2015).

Although Buttonwood trees has set official aims for afforestation in Iraq, through being a drought resistant and fast growing plant that can produce large amounts of biomass specially at high temperatures (Suleman, et.al.,2013, Ibraheem and Abed.,2017), but it's existence is not linked to any environmental benefits. On the contrary, it doesn't encourage the biodiversity in the urban streets, the areas under the stand of *C. erectus* trees doesn't enhance growth of other plants. In addition to that, it has been reported that there is no serious herbivores or insect attack the tree and it appears to be totally free from plant diseases (Little, 1983). These observations leads to a conclusion that *C. erectus* is Allelopathic.

Allelopathy, a biological phenomenon that refers to the chemical inhibition of one plant species by another (Rizvi and Rizvi, 1986), has been implicated as an important factor in the spread of invasive plants, and urban areas are especially susceptible to invasion by exotic species (Oldfield, et.al., 2013). Allelopathic roles has been evaluated in agricultural systems and many studies addressed the Allelopathic impact of trees or crop residues in other crops or in the bushes (Attiq Ullah et al., 2013; Sirawdink, et.al., 2011; Motwani et al., 2013) without paying attention to the Allelopathic influence of urban trees in the germination and growth of other plants specially ornamental plants. Therefore, The present study aims at evaluating the effect of *Conocarpus erectus* L. tree leaf leachates and root exudes on seed germination and early seedling growth of four ornamental plant species That are abundant in public parks and home gardens in Iraq today, especially under trees.

MATERIALS AND METHODS

Experimental species

Four types of winter ornamental plants were chosen, all of which belong to the family Compositaceae and are cultivated by seeds including *Dimorphothica aurantiaca* L., *Chrysanthemum carinatum* L., *Calendula officinalis* L. and *Gaillardia pulchella* L.

The above-mentioned plants are cultivated in Iraq in parks and home gardens, especially under trees such as *C. erectus*, their seeds are grown in late summer and early autumn, blooming in late winter and spring and dying during the summer months.

The seeds were collected during 2015 and before germination test, seeds were washed with hypochlorite solution and distilled water, then stored at room temperature until use.

Experimental material

Fresh leaves of *C. erectus* were collected and rinsed thoroughly with sterile distilled water, then stored at room temperature until use.

Preparation of allelopathic solutions

Preparation of Leaf leachates

50g Fresh leaves of *C. erectus* were hand collected from one year old living trees, and rinsed thoroughly with sterile distilled water. The fresh leaves were chopped and suspended in 1L deionized distilled water at 25°C and left for 24 hours until a noticeable color change was present in the solution. This is equivalent to 5% tissue concentration, common for allelopathic bioassays (Motwani et al., 2013; Rua, et.al., 2008; Nilsson et.al. 2000; Stowe, 1979). Leaf leachates solution were stored in the dark and kept at 4°C for further studies.

Preparation of root exudates

A modified Stair step system was used for collecting *C.erectus L.* root exudates. This system was designed from an iron bar with 1m height that stands on ground by 0.5m iron base with four legs to ensure stability of the system. On each side of the bar two arms, ended with a ring (20 cm Dia.), were welded at 15cm and 30 cm height. The ring was designed for carrying a plastic pot efficiently. Each pot has a perforated bottom which is padded with a filtration paper (Whatman NO.2). The pots had plants with a one year old *C..erectus* tree. A plastic funnel was settled under each pot and connected with pipes so that the root exudates could flow into a plastic container prepared specially for collecting root exudates.

The pots were divided into two series, the treatment series and the comparison series. In the treatment series, the water passed through the pots that contain the *c.erectus* tree and then to the plastic container placed under the system. In the comparison series, water passed in a pot containing soil only and then to the container that collect the solution. After 72h from watering the pots, the root exudates of *c.erectus* tree was collected, stored in the dark at 4°C and kept for further studies.

Bioassay of allelopathic solutions

Experimental seeds (4x30 seeds of each species) were germinated at 25 °C in Petri dishes (10 cm Dia.) containing 5 ml of Leaf leachates solution, root exudates solution and Distilled water (control).

Data collection and analysis

After the start of the experiment Germination was recorded daily for 10 days. The seed was considered germinated by a visible protrusion of split seed coat with the cotyledons, hypocotyls and epicotyl.

The number of germinated seeds to the total number of seeds for each treatment was recorded daily up to 10 days and expressed as Germination percentage G%. Coefficient of velocity (CV) was calculated by the equation mentioned by Ranal and Santana (2006).

$$CV = 100 (A_1 + A_2 + \dots + A_x) / (A_1T_1 + A_2T_2 + \dots + A_xT_x)$$

where A_1, A_2, \dots, A_x : number of seedlings counted on the first day, second day, and so on until the last day (x), and T_1, T_2, \dots, T_x : number of days between sowing and the first collection, between the sowing and the second collection, and so on until the last collection (x).

After 20 days of germination experiment both radicle and plumule length (mm) were measured and Vigor index (VI) was calculated by the equation described by Dhindwal et al. (1991), as below:

$$VI = \text{Germination\%} \times (\text{radical length} + \text{plumule length})$$

Total phenolics, in Both leaf and root solutions, was measured spectrophotometrically according to the method described by Abdel-Hameed (2009) using the Folin-Ciocalteu reagent. Units of total phenolics are expressed as the number of equivalents of Galic acid per gram dry weight.

This study was conducted as a laboratory experiment based on factorial Randomized Complete Blocks Design (RCBD) with three replicates. The obtained data were analyzed using analysis of variance (two-way ANOVA) via General Linear Model (GLM) of SPSS statistical package program. and Means were compared by LSD Test (Least Significant Difference) at $P < 0.05$ and $P < 0.01$.

RESULTS AND DISCUSSION

Results

The results of ANOVA test presented in the Table below shows that all the experimental treatments and their interactions was Significant at 0.05 and 0.01 probability level. Both Leaf leachates solution and root

exudates solution of *C. erectus* inhibited significantly the germination process of the seeds of the experimental species under study.

Table : ANOVA table showing the effects of allelopathic solutions of *Conocarpus erectus* L. , ornamental plants, and their interactions on germination percentage G%, coefficient of velocity(CV),plumule length (PL), radicle length (RL) and Vigor index(VI).

S.O.V	df	G%		(CV)		PL (mm)		RL (mm)		(VI)		p	
		MS	F	MS	F	MS	F	MS	F	MS	F	<0.05	<0.01
allelopathic solutions	2	21.74	8.41	23.85	5.80	21.88	5.86	26.03	6.67	16.47	3.80	3.44	5.72
ornamental plants	3	11.33	4.24	13.36	3.25	14.02	3.75	16.79	4.30	13.32	3.07	3.05	4.82
Allelo. S. × Orna. p.	6	18.53	6.94	15.96	3.88	14.37	3.85	15.91	4.07	16.85	3.89	2.55	3.76
Error	22	2.67	-----	4.11	-----	3.73	-----	3.90	-----	4.33			

Leaf leachates solution decreased the germination percentage of *Dimorphothica aurantiaca* L., *Chrysanthemum carinatum* L and *Gaillardia pulchella* L. by 60, 62.5, 33.3 % respectively, While root exudates solution decreased the germination percentage by 40, 37.5, 33.3% respectively, in comparison to the control treatment. However, they had no effect on the germination percentage of *Calendula officinalis* L. (figure1). The onset of germination for all experimental species under study was delayed by 3 - 4 days when treated with *C. erectus* Allelopathic solutions. Figure (2) shows that the coefficient of velocity (CV) (speed of germination) was reduced significantly by the inhibitory effect of both Leaf leachates solution and root exudates solution of *C. erectus* for all experimental species under study. Reduction in CV for Leaf leachates treatment was 31.39, 36.80, 3.02 and 14.58 %, while it was 4.97, 4.01, 3.02 and 6.66% for the root exudates treatment for *Dimorphothica aurantiaca* L., *Chrysanthemum carinatum* L, *Calendula officinalis* L., *Gaillardia pulchella* L. respectively. It Can be observed from Figure (2) that the seeds of *Chrysanthemum carinatum* L. was The most vulnerable in germination under the influence of the Allelopathic solutions, while *Calendula officinalis* L. seeds was less affected than the seeds of other species.

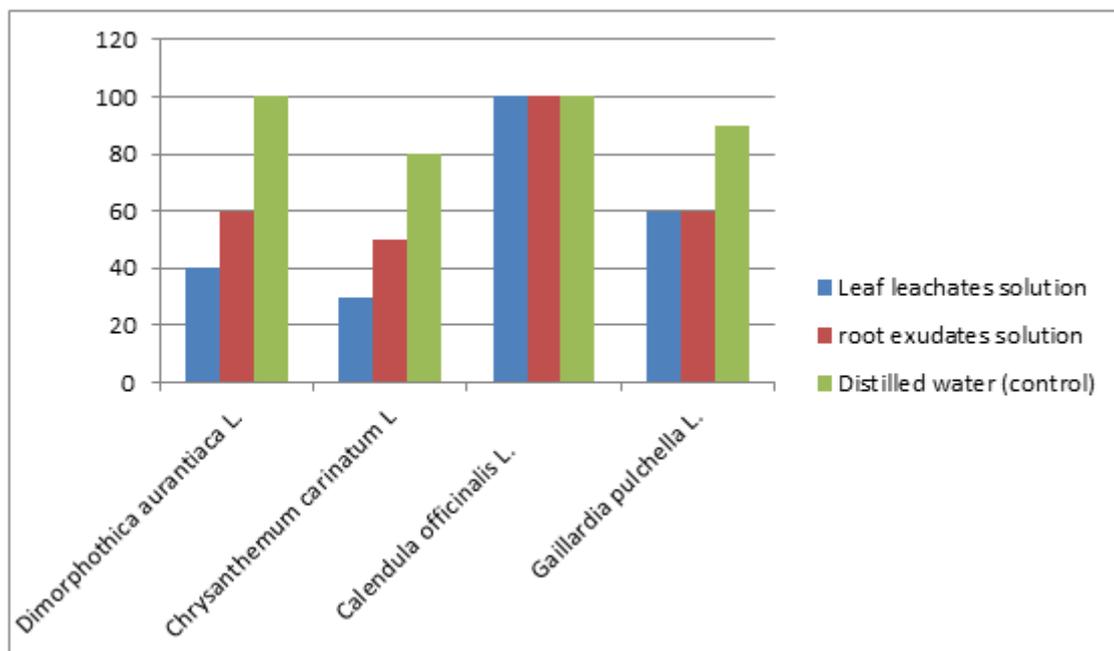


Figure 1. The germination percentage of the experimental species seeds as affected by leaf leachates and root exudations of *C. erectus*, 10 days after treatment.

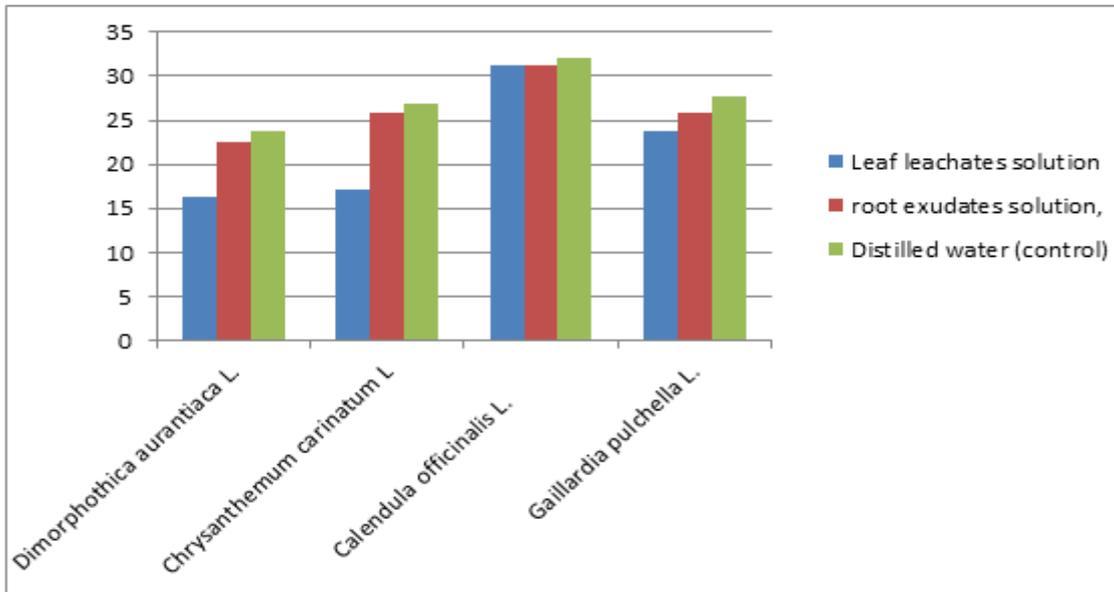


Figure 2. The coefficient of velocity (CV) of the experimental species seeds as affected by leaf leachates and root exudations of *C. erectus*, 10 days after treatment.

The plumule length, radicle length and seed Vigor index, for all experimental species under study, was found to be inhibited by both Leaf leachates solution and root exudates solution of *C. erectus*. Plumule length was reduced significantly by Leaf leachates solution and root exudates solution of *C. erectus*. The maximum valuable drop in the Plumule length was (0.74 cm) for *Chrysanthemum carinatum* L. seedlings treated with the Leaf leachates solution in comparison to the control treatment (1.75 cm), while *Calendula officinalis* L. seedling was the least affected thus it recorded the highest value for the Plumule length reached 1.82cm in comparison to the other species and to the control treatment(1.90 cm) (figure 3) .

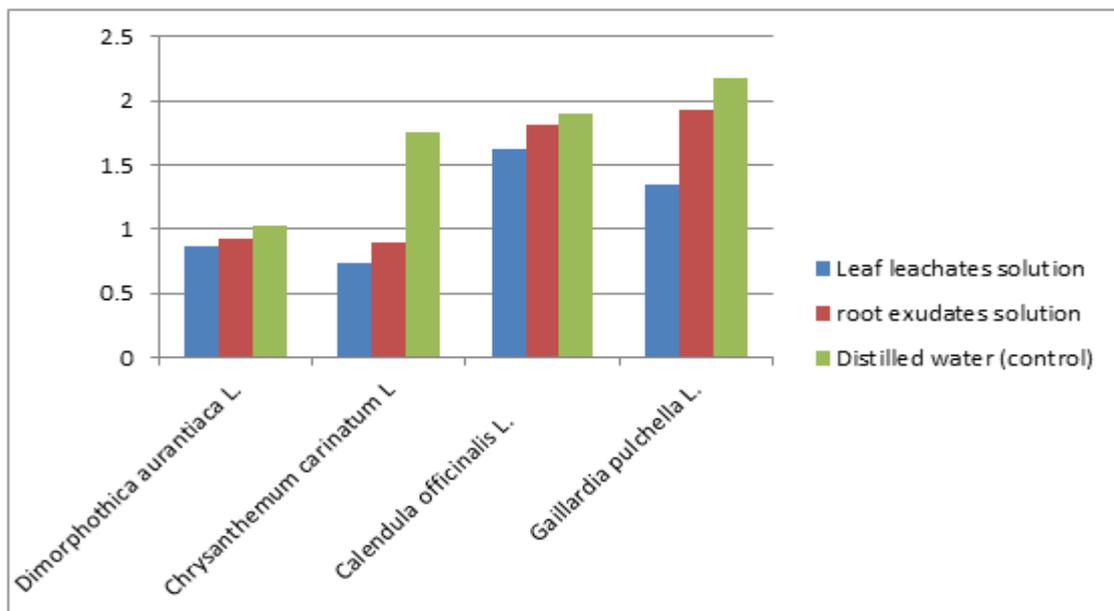


Figure 3: plumule length of the experimental species seeds as affected by leaf leachates and root exudations of *C. erectus*, 20 days after treatment.

It was also found that the seedlings treated with Leaf leachates solution and root exudates solution of *C. erectus* had a significant reduction in the radical length. The maximum reduction can be discern in the *Chrysanthemum carinatum* L. seedlings treated by Leaf leachates solution (4.24cm) in comparison to the

control treatment (5.00 cm). However the *Calendula officinalis* L. seedlings showed the minimum reduction in radical length (4.79cm) in comparison to the control treatment (5.30 cm).

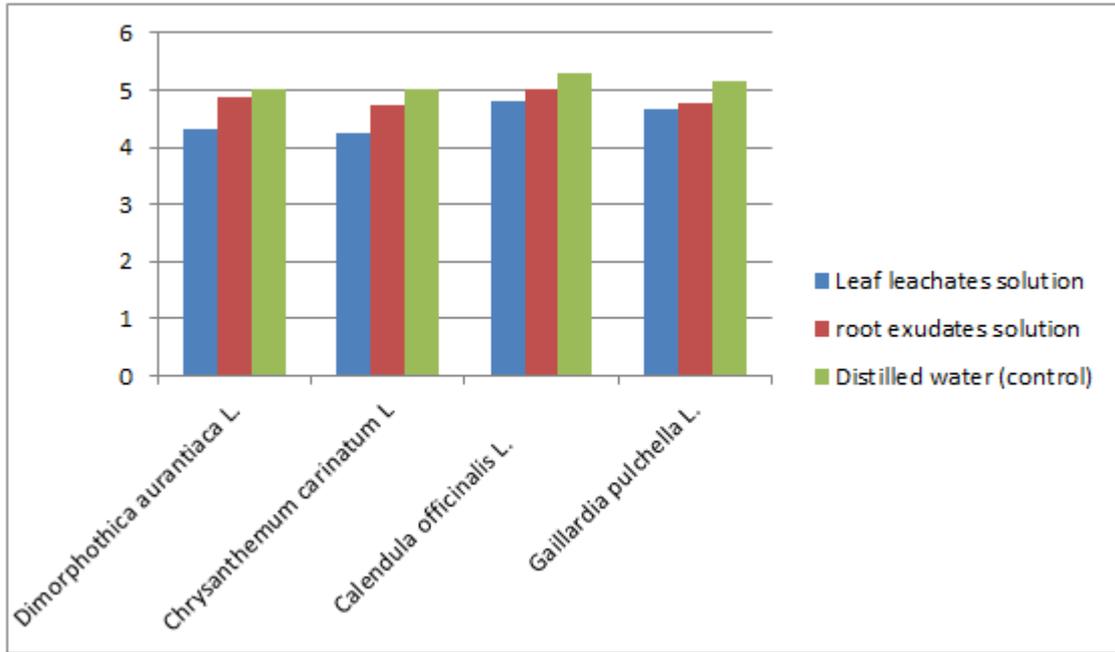


Figure 4: Radical length of the experimental species seeds as affected by leaf leachates and root exudates of *C. erectus*, 20 days after treatment.

Vigor index reflects the ability of those seeds to produce normal seedlings under less than optimum or adverse growing conditions, similar to those which may occur in the field (AOSA. 2009). Both Leaf leachates solution and root exudates solution of *C. erectus* decreased vigor index (VI) significantly at 5% probability level. Maximum VI (231.42) was recorded in control treatment, while the lowest VI (136.38) was found in *Dimorphothica aurantiaca* L. (figure 5).

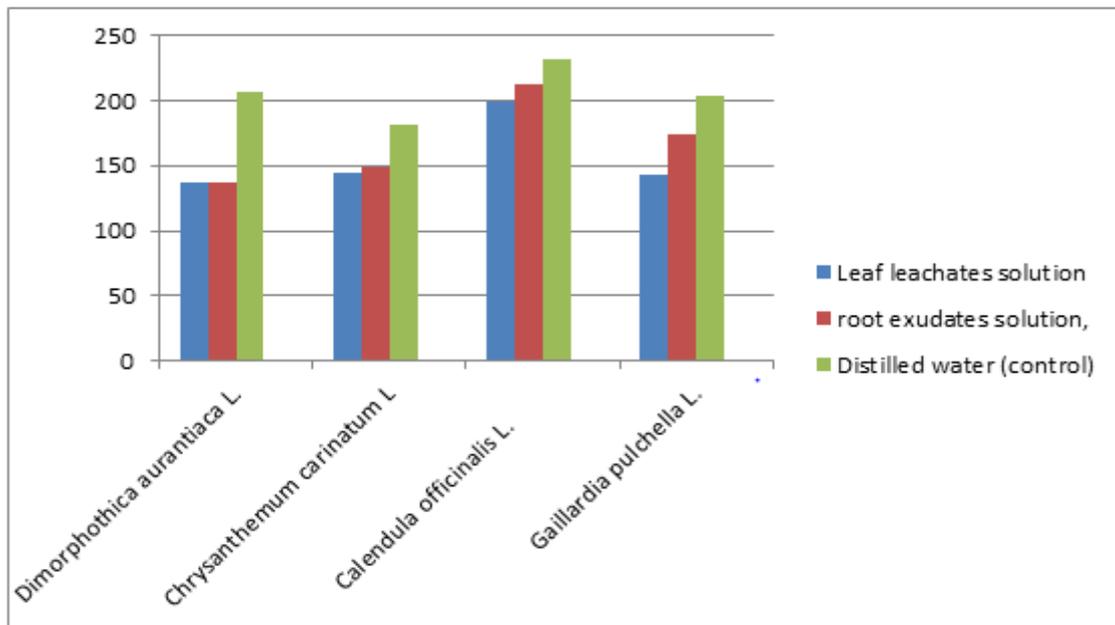


Figure 5: Vigor index for experimental species seeds as affected by leaf leachates and root exudates of *C. erectus*, 20 days after treatment.

Due to the high allelopathic activity of the Leaf leachates solution and root exudates solution of *C. erectus*, they were subjected to a bioassay screening for total phenolics. The total phenolics were measured using Folin–Ciocalteu’s assay, and The results revealed that leaf leachates solution scored the highest value in total phenolic content reached (120.46 mg gallic acid /g plant dry weight) in comparison to the root exudates content of total phenolics (89.12 mg gallic acid /g plant dry weight).

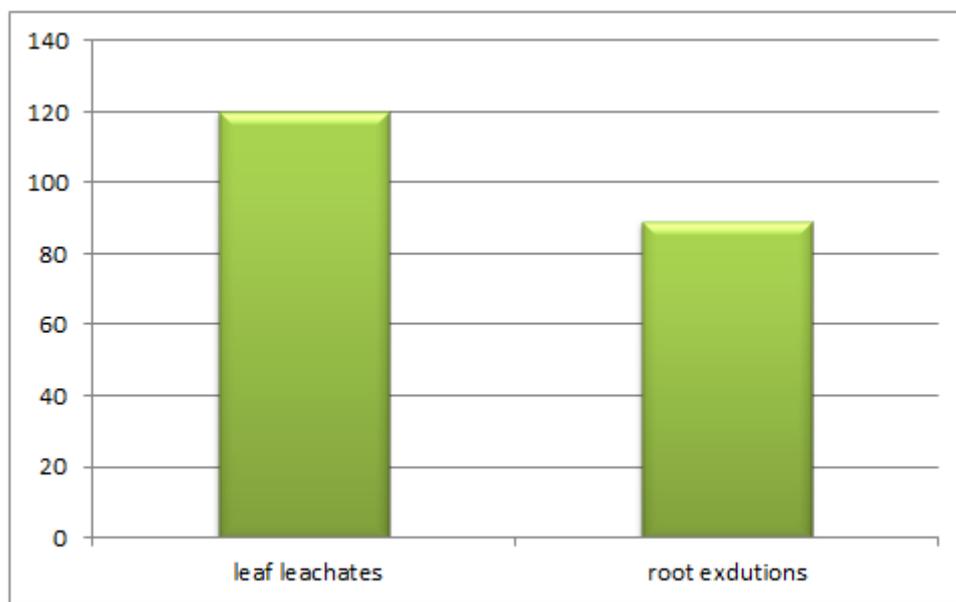


Figure 6: Total phenolics content in leaf leachates and root exudations of *C. erectus* (mg gallic acid equivalents/g plant extract)

DISCUSSION

The results of this research showed the inhibitory effects of Leaf leachates solution and root exudates solution of *C. erectus* in the germination percentage, coefficient of velocity (CV) and early growth of ornamental seedlings under study. Inhibition of seed germination is generally due to disturbance in the enzymatic activities responsible for germination and growth (Attiq Ullah et al., 2013). while The decrease in The CV is due to inhibiting water absorption (Tawaha and Turk, 2003).

Elongation of radicles and plumules were significantly inhibited by Leaf leachates solution and root exudates solution of *C. erectus* .This could be related to the inhibition of CV. whereas , Rapid or faster emergence would allow plants to initiate seedling growth earlier and accumulate more dry matter (Yu et al., 2004).

The decreased in germnation percentage, coefficient of velocity (CV), plumle length and radical length was in agreement with those obtained by Al-Shatti,et.al.(2014) who found that The leaf extract and pulverized leaf tissue of *Conocarpus lancifolius* inhibited germination, radicle and plumule lengths and caused a delay in seed germination. Zhang et.al.(2015), reported also that decomposed leaf litter from trees interplanted with crops inhibited significantly the germination speed index.

As shown in the results, the Leaf leachates solution of *C. erectus* indicated a more inhibitory effect on the germination process of the experimental species than the root exudates solution. This is evident in the inhibition of germination percentage, CV, the Plumule length, radical length and VI .these findings are in agreement with those obtained by Sirawdink, et.al., (2011) in which they had related the allelopathic effects of Eucalyptus leaf leachates to the osmotic potential of it . And it could be related to the high concentration of, phenolic acids in the allelopathic solutions (Inderjit 2006). This is confirmed by the high amount of phenolic compounds in the Leaf leachates solution of *C. erectus*, where the Screening for total phenolics revealed that the leaf leachates solution scored the highest value in total phenolic content in comparison to root exudates solution, as it reached 120.46 and 89.12 mg/g GAE respectively.

Phenolic acids are the most common compounds found in plant residues, most of which have been found to inhibit germination and growth of other plants. (Inderjit ,1996). Phenolics are known to inhibit cell division and elongation, changes membrane permeability, inhibition of mineral uptake and other biosynthetic processes such as net photosynthesis, respiration and enzymatic activities (john and sarada, 2012). Al-Shatti,et.al.(2014) found that Phenolic compounds were predominant in the leaf extract of *Conocarpus lancifolius* L. and they suggested that it may have played a major role in inhibiting germination, growth and development of corn and bean seedlings

Results showed that, among all studied species, *Chrysanthemum carinatum* L. was more sensitive to the allelopathic effect of *C. erectus*, while *Calendula officinalis* L. was the most resistant and it showed the highest values for the measured parameters. This is consistent with results obtained by Ibraheem and Sa'eed (2008) who studied the allelopathic effect of Eucalyptus and Orange Leaves on Germination and Growth of some selected Ornamental Plants.

REFERENCESES

- [1] Abdel-Hameed, E.S., 2009. Total phenolic contents and free radicals scavenging activity of certain Egyptian Ficus species leaf samples. Food Chemistry, 114: 1271-1277.
- [2] Al-Shatti, A.H., Redha, A., Suleman, P. and Al-Hasan, R. (2014) The Allelopathic Potential of *Conocarpus lancifolius* (Engl.) Leaves on Dicot (*Vigna sinensis* L.), Monocot (*Zea mays* L.) and Soil-Borne Pathogenic Fungi. American Journal of Plant Sciences, 5, 2889-2903
- [3] AOSA. 2009. Seed Vigor Testing Handbook. Contribution No. 32 to the Handbook on Seed Testing.
- [4] Attiq Ullah; Ahmad Khan, Ejaz; Baloch, Mohammad Safdar ; Nadim, Muhammad Amjad; Sadiq, Muhammad and Noor, Khaliq (2013). Allelopathic effects of herbaceous and woody plant species on seed germination and seedling growth of Wheat. Pak. J. Weed Sci. Res., 19(3): 357-375.
- [5] Dhindwal AS , Lather BPS, Singh J. 1991. Efficacy of seed treatment on germination, seedling emergence and vigour of cotton (*Gossypium hirsutum*) genotypes. Seed Res. 19:59–61
- [6] Ibraheem, Fatin K. and Sa'eed, Janan A.(2008). The Effect of Eucalyptus and Orange Leaves Added to the Soil on Germination and Growth of Four Ornamental Plants. Al-Rafidain journal of science 19(1):15-25
- [7] Inderjit (2006). Experimental Complexities in Evaluating the Allelopathic Activities in Laboratory Bioassays: A Case Study. Soil Biology and Biochemistry, 38, 256-262.
- [8] Inderjit (1996).plant phenolics in allelopathy.The Botanical Review,62(2):186-202
- [9] John, J. and sarada,S. (2012). Role of phenolics in allelopathic interations. Allelopathy journal29(2):215-230.
- [10] Little, E.L. Jr. (1983). Common fuelwood crops: a handbook for their identification. McClain Printing Co., Parsons, WV. Mohamed.
- [11] Ibraheem Lujain H. and Abed Salwan Ali. (2017), ACCUMULATION DETECTION OF SOME HEAVY METALS IN SOME TYPES OF FRUITS IN THE LOCAL MARKET OF AL-DIWANIYAH CITY, IRAQ, RASAYAN Journal of Chemistry, Vol.10, No.2 (339-343).
- [12] Mare,A. D. and Fikirte,D.T.(2015).Mapping of plantation forest in the upper catchment of Addis Ababa. international journal of environmental sciences4(3):158-165.
- [13] Motwani ,g.; Golani, N. and Solanki, H.(2013). Allelopathic Effects of aqueous leaf leachates of *Lantana camara* on *Eichhornia crassipes*. life Sciences leaflets1:83-90.
- [14] Nilsson,M. C., Zackrisson, O., Stemer, O. and Wallstedt,A. (2000). Characterization of the differential interference effects of two boreal dwarf shrub species. Oecologia 123:122-128
- [15] Oldfield EE, Warren RJ, Felson AJ, Bradford MA (2013) FORUM: challenges and future directions in urban afforestation. Journal of Applied Ecology 50:1169–1177.
- [16] Ranal, M.A. and Santana, D. G. (2006).How and why to measure the germination process. Revista Brasil. Bot.,29(1): 1-11.
- [17] Rice, E.L. (1984) Allelopathy. 2nd Edition, Academic Press, New York, 422.
- [18] Rizvi, S. J. H. and Rizvi, V. (1986). Allelopathy: Some new terminological considerations. Cur. Sci. 85:191-192.
- [19] Rua, M.A.; Nijjer, S; Johnson, A.; Rogers, W.E. and Siemann, E. (2008). experimental approaches to test allelopathy: A case study using the invader *Sapium sebiferum*. Allelopathy Journal 22(1):1-14.

- [20] Stowe, L. g. (1979). Allelopathy and its influence on the distribution of plants in an Illinois old-field. *The Journal of Ecology* 67:1065-1085.
- [21] Sirawdink, F., Zerihun, K., Amsalu, N., Nardos, Z. and Seife, B. (2011) Allelopathic Effects of *Eucalyptus camaldulensis* on Germination and Growth of Tomato. *American-Eurasian Journal of Agricultural & Environmental Sciences*, 11, 600-608.
- [22] Suleman, P.; Redha, A.; Afzal, M.; Al-hasan, R. (2013). Temperature-induced changes of malondialdehyde, heat-shock proteins in relation to chlorophyll fluorescence and photosynthesis in *Conoarpus lancifolius* (Engl.). *Acta physiologiae plantarum*. 35(4):1223-1231.
- [23] Tawaha, A. M. and Turk, M. A. 2003. Allelopathic effect of black mustard (*Brassica nigra* L.) on germination and growth of wild barley (*Hordeum spontaneum*). *Journal of Agronomy and Crop Science*. 189:298-303
- [24] Yuanhao (2015). Allelopathic effects of decomposed leaf litter from intercropped trees on rape. *Turkish Journal of Agriculture and Forestry* 39(6):898-908 ·
- [25] Yu J, Tuinstra MR, Claassen MM, Gordon WB, Witt MD. 2004. Analysis of cold tolerance in sorghum under controlled environment conditions. *Field Crops Res*. 85: 21–30.
- [26] Wang, T.S.C., Yang, T.K., and Chuang, T.T. 1967. Soil phenolic acids as plant growth inhibitors. *Soil Sci*. 103:239–246.
- [27] Zhang, Xiaoxi; Liu, Zengwen; Tian, Nan; Luc, Nhu Trung; Zhu, Bochao; Bing, Yuanhao. 2015. Allelopathic effects of decomposed leaf litter from intercropped trees on rape. *Turkish Journal of Agriculture and Forestry* 39(6):898-908 ·