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ALLELOPATHIC EFFECT OF *EUCALYPTUS* SPP. LEAVES ON SOME CHEMICAL CONSTITUENTS OF SEEDLINGS OF TWO ORNAMENTAL PLANTS

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Abstract

An experiment was conducted to investigate the allopathic effect of terpenes, alkaloids and phenols compounds extracted from leaves of *Eucalyptus* spp on some leaf chemical constituents of seedlings of two ornamental plants; *Calendula officianlis* and *Tagettes patula*. Results showed that sugar content was increased significantly as the concentrations of the terpenes increased. The increase in concentrations of terpenes, alkaloids or phenols caused significant increase in protein content in leaves although the increases were always less than that for the control. It was clear that the melenodialdehyde (MDA), glutathione and proline content in the two ornamentals positively affected when seedlings treated with the three compounds. The MDA content increases as the concentration of the three compounds used increases. The 400mg/L of the three compounds treatment recorded the highest glutathione content, *Tagettes patula* plant.

Key words : Sugar, protein, MDA, glutathione, proline.

Introduction

Allelopathy phenomena is defined as a process of secreting secondary products by some plants to the surrounding environment (Weston, 2005) and these secreted products, which called allelochemicals or phytoxins, affect plant growth nearby via several ways (Akemo *et al*., 2000). Allelopathic compounds exert their role through their effects on membrane permeability (Gniazowsk and Bogatek, 2005), interfere in mitosis of growing cells (Batish *et al*., 2004), inhibit protein synthesis (Bertin *et al*., 2007), hinder enzymes from exerting their normal functions (Muscolo *et al*., 2001), effect respiration and photosynthesis (Yu *et al*., 2003) and decline in crop yield (Zhao-Hui *et al*., 2010). Also, it has been found that phenolic allelochemicals can reduce or inactivate the normal physiological activities of several phytohormones (Zhao-Hui *et al*., 2010).

Eucalyptus, is considered as one of the strongest allelopathic trees that contain allelochemicals compounds in its leaves, bark and roots. These allelochemicals have adverse effects on other crops in the ecosystem (El-Darier, 2000, 2002; Ziaebrahimi *et al*., 2007). Several of such compounds were identified in different parts of *Eucalyptus* species trees such as champhene, cryptone, p-cymene, d-limonene, α-pinene, β-pinen, spathulenol, terpineol, citronellal and linalool (Ghafar *et al*., 2000). Some studies show that phenolics and terpenoids, particularly monoterpenes, are the major components of essential oils of *Eucalyptus* spp. trees that hinder vital processes in plants such as photosynthesis, respiration, and mineral absorption (Batish *et al*., 2004). Some phenolics (*i.e*., ferulic acid and cinnamic acid) can inhibit protein synthesis (He and Lin, 2001). It was also mentioned that *E. grandis* leaves residues has a strong allelopathic effect in causing significant reduction in protein and sugar contents in rice (Singh and Rao, 2003, and Fukoa *et al*., 2012). On the other hand, it was noted that during heavy metal stress, phenolic compounds can directly scavenge molecular species of active oxygen (Michalak, 2006). Reddy *et al*. (2004) and Bajji *et al*. (2001) have stated that the allelopathic compounds induce proline accumulation in plants. In addition, Djanaguiraman *et al*. (2005) has found that proline content in sorghum and Black gram increased due to the treatment with the extract of *Eucalyptus* leaves.

The aim of this investigation was to find out the

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allelopatheic effects of terpenes, alkaloids and phenols extracted from leaves of *Eucalyptus* spp. on some chemical constitutes, including two antioxidants of two ornamentals seedlings; *Calendula officianlis* and *Tagettespatula*.

Materials and Methods

The present experiment was conducted to investigate the allopathic effect of terpenoids, alkaloids and phenols extracted from *Eucalyptus*sp leaves on sugar, protein, malenodialdehyde, glutathione and proline content in seedling of the two ornamental plants; *Calendula officianlis* and *Tagettedpatula*.

Leaves were collected from the gardens of College of Agriculture, Al-Qadisyia University. They were cut into small pieces of less than 1cm and were shade dried under normal conditions, then in oven at 65^0c for 24 h. Dried leaves were mashed and stored in a polyethylene bags in the refrigerator until use. The dry plant materials were utilized for extraction of terpenes, alkaloids and phenols. Terpenes were extracted using 20g from the dried leaves according to Harborne (1984) method, in the same way alkaloids (Fazel *et al*., 2008) and phenols (Gonzalez *et al*., 2003) were extracted.

Seeds of *Calendula officianlis* and *Tagettes patula* were surface sterilized with 95% ethanol and then with sodium hypochloride 5.5% for 5 minutes and thoroughly washed with sterile water several times. Twenty five seeds were sown evenly between two layers of Wathmann No. 1 in 10cm diameter petri dishes. Terpenes, alkaloids or phenols solutions at concentration of 0, 100, 200, 300 or 400 µg/L were added to each petri dish. Dishes were kept at room temperature. After germination, seeds were thinned to ten in each petri dish. Two weeks after germination, seedlings were taken for chemical analysis. Sugar estimation of fresh leaves of the two plants was done using Dubois *et al*. (1956) method. Protein content was determined following the method of Herbert et al., (1971) using Bovine Serum Albumin (BSA) as standard. Proline content of leaves were determined following the Bates *et al*. (1973) protocol. Malenodiadehyde was taken according to Zacheo *et al*. (2000) procedure. Glutathione measurement was carried out following the method of Cakmak and Marschner (1992). The experiment was replicated four times. Data was analyzed statistically by analysis of variance technique and comparison among treatment means was made by LSD at 0.05 test.

Results and Discussion

It was clear from the results presented in table 1 that sugar content in seedlings of the two plants was increased significantly as the concentration of the terpenes increased. The highest concentration of terpenes gave a pronounced increase in sugar content amounted of 25.20 µg/ml in compare to 6.75 µg/ml for control treatment, with more than 3.5 fold increase. However, the 100 and 200mg/L of terpenes did not differ from each other in their effect. In comparison between the two cultivars, *Tagettes patula* had higher sugar content (25.58 µg/ml) than *Calendula officinalis* plant (7.21 µg/ml). With regard to the interaction between the concentration and kind of plant, the highest sugar content was obtained at the treatment of 400 mg/Lof the terpens on *Tagettes patula* plant (33.90 µg/ml), while the lowest was obtained at 100 mg/Lof the terpens on *Calendula officinalis*. These results indicate that terpenes concentrations play an important role in affecting sugar content. For alkaloids treatments, the 400mg/L caused significant increase in sugar content amounted of 10.00 µg/ml in compare to 6.50 µg/ml for the control, whereas the lowest content (6.40 µg/ml) was obtained at the 100mg/L of the alkaloid. With regard to phenols treatments, they behave in their effects on sugar content similarly to that of terpenes and alkaloids. The highest concentration of phenols resulted in the highest sugar content, 14.75 µg/ml compare to 7.00, 5.07 and 6.43 µg/ml for 0, 100 and 200 mg/L of the phenols, respectively. It was obvious that the two plants contain close amount of the sugar, although they were significantly different from each other. For the interactions, the highest amount of sugar was recorded at the combination of 400 mg/L of phenols and *Tagettes patula* plant (16.00 µg/ml), while the lowest amount was recorded at the combination of 100 mg/L of phenol and *Calendula officinalis* plant (4.25 µg/ml). The increase in concentrations of terpenes, alkaloids or phenols caused significant increase in protein content in leaves of the two ornamental plants studied (table 2), but the increases were always less than that for the control, which gave the highest content. The lowest values of protein content; 13.50, 9.75 and 13.40 µg/ml were obtained at 200 mg/L of terpenes, 100 mg/L of alkaloids and at 300 mg/L of phenols, respectively. Protein content in *Tagettes patula* leaves was higher than that in the other ornamental plant when treated with terpenes or alkaloids. For the interaction, the highest protein content recorded were 25.00, 24.00 and 26.50 µg/ml at the treatment with 0 mg/L of terpenes on *Calendula officinalis*, 400 mg/L of alkaloids on *Tagettes patula* and 0 mg/L of phenols on *Calendula officinalis*, respectively.

From the above results, it was clear that the three compounds had positive effect on sugar content and negative effect on protein content (in compare to control).

Table 1 : Effect of different concentrations of terpenes, alkaloids and phenols extracted from *Eucalyptus* spp. on sugar content (µg.ml^{-1}) in two ornamental plants.

Plants / Conc. (mg.L^{-1})		*Calendula officinalis*	*Tagettes patula*	Mean
Terpenes	0	5.50	8.00	6.75
	100	3.60	27.00	15.30
	200	4.70	28.00	16.35
	300	5.75	33.00	19.37
	400	16.50	33.90	25.20
	Mean	7.21	25.58	
	LSD 0.05	For Kind of plants 3.24	Concentrations 5.13	Interaction 7.25
Akaloids	0	6.00	7.00	6.50
	100	6.80	2.40	6.40
	200	7.00	7.90	7.45
	300	8.10	8.75	8.42
	400	9.60	10.40	10.00
	Mean	7.50	7.09	
	LSD 0.05	For Kind of plants N.S	Concentrations 0.98	Interaction 1.39
Phenols	0	6.50	7.50	7.00
	100	4.25	5.90	5.07
	200	6.75	6.10	6.43
	300	9.00	8.20	8.60
	400	13.50	16.00	14.75
	Mean	8.00	8.74	
	LSD 0.05	For Kind of plants 0.60	Concentrations 0.80	Interaction 1.20

Table 2 : Effect of different concentrations of terpenes, alkaloids and phenols extracted from *Eucalyptus* spp. on protein content (µg.ml^{-1}) in two ornamental plants.

Plants / Conc. (mg.L^{-1})		*Calendula officinalis*	*Tagettes patula*	Mean
Terpenes	0	25.00	13.00	19.00
	100	14.50	13.50	14.00
	200	12.00	15.00	13.50
	300	11.50	18.00	14.75
	400	10.75	21.25	16.00
	Mean	14.75	16.25	
	LSD 0.05	For Kind of plants 1.83	Concentrations 3.60	Interaction 4.49
Alkaloids	0	20.00	11.50	15.75
	100	12.00	7.50	9.75
	200	11.25	8.75	10.00
	300	8.75	11.75	10.25
	400	3.75	24.00	13.87
	Mean	11.25	12.70	
	LSD 0.05	For Kind of plants 1.42	Concentrations 2.24	Interaction 3.26
Phenols	0	26.50	12.00	19.25
	100	24.75	7.00	15.87
	200	20.00	8.75	14.37
	300	15.50	11.30	13.40
	400	11.75	23.50	17.62
	Mean	19.70	12.51	
	LSD 0.05	For Kind of plants 1.22	Concentrations 1.83	Interaction 2.65

Earlier studies showed that *E. tereticorniss* extract significantly affect sugar and protein causing an increase in their content as an adaptation mechanism for stress tolerance (El-Darier and Youssef, 2000). It was also noticed some allelopathic compounds such as caffeic, phenolic, P-hydroxybenzoic acid, vanillic and syringic and others affect protein synthesis and other biomolecules (Dudan and Lakshinarayana, 1995). Al-Shahat (2000) has indicated that higher concentrations of phenols caused inhibition of protein biosynthesis due to the presence of P-coumaric acid which cause an inhibition of treptophane and increase the activity of abscisic acid, and in turn cause protein breakdown via the activation of some enzymes such as protease and peptielase. It was also noticed that *E. grandis* leaves residues have strong allelopathic effect in causing significant reduction in protein and sugar contents in rice (Singh and Rao, 2003 and Fukoa *et al.*, 2012). The current result agrees with previous results of El-Rokiek and El-Nagadi (2011), who found that the extract of *E. citriodora* caused an increase in sugar and protein content of *P. oleracea* plant.

It was clear from table 3 that the melenodialdehyde (MDA) content in *Calendula officinalis* and *Tagettes patula* was positively affected when plants treated with

Table 3 : Effect of different concentrations of terpenes, alkaloids and phenols extracted from *Eucalyptus* sp. on malenodialdehyde (µmole.g^{-1} FW) in two ornamental plants. All values multiply by 10^{-4}.

Conc. (mg.L^{-1})		*Calendula officinalis*	*Tagettes patula*	Mean
Terpenes	0	10.26	3.01	6.63
	100	7.65	4.10	5.87
	200	8.76	5.55	7.16
	300	10.78	5.81	8/29
	400	20.78	6.66	13.72
	Mean	11.69	5.13	
	LSD 0.05	For Kind of plants 1.10	Concentrations 1.70	Interaction 2.50
Alkaloids	0	7.01	6.01	6.51
	100	6.21	4.90	5.56
	200	7.19	5.52	6.36
	300	7.32	5.55	6.44
	400	9.48	8.30	8.89
	Mean	7.44	6.60	
	LSD 0.05	For Kind of plants 0.90	Concentrations 1.50	Interaction 2.10
Phenols	0	8.20	4.35	6.27
	100	7.12	2.68	4.90
	200	7.25	4.57	5.91
	300	10.39	5.81	8.10
	400	15.94	13.90	14.92
	Mean	9.77	6.25	
	LSD 0.05	For Kind of plants 1.00	Concentrations 1.60	Interaction 2.30

terpenes, alkaloids and phenols extracted from *Eucalyptus* leaves. The MDA content increases as the concentration of terpenes increases. The highest content achieved was at 400 mg/L of terpenes (13.72*10^{-4} µmole/g) compare to 6.63*10^{-4} µmole.g^{-1} for control treatment. It was noted that *Calendula officinalis* seedlings contain higher amount of MDA (11.69*10^{-4} µmole/g) than *Tagettes patula* seedlings (5.02*10^{-4} µmole/g). For the interaction between the two factors, it was noted that the highest MDA content was in *Calendula officinalis* seedlings treated with 400 mg/L of terpenes. The lowest content was found at the combination of 0 mg/L of terpenes and *Tagettes patula* seedlings. With regard to the effect of alkaloids, increasing their concentrations resulted in more MDA in the same way as for terpenes effect. The results also showed that the MDA content in *Tagettes patula* seedlings was less (6.06*10^{-4} µmole/g) than that in *Calendula* (7.44*10^{-4} µmole/g). For phenols effect, it was clear that MDA content noticeably increased as the phenols concentrations increased. *Calendula officinalis* seedlings had higher content of MDA than *Tagettes patula* seedlings. For the interaction, the highest MDA content was recorded at the combination of 400 mg/L and *Calendula officinalis* while the lowest content was at the combination of 100 mg/L and *Tagettes patula* plant.

Glutathione content increased significantly as the concentrations of the three compounds used increased. The 400mg/L of terpens, alkaloids and phenols recorded the highest glutathione content which was 489.5, 479.0 and 571.0 µg/ml in compare to 255.0, 250.5 and 241.0 µg/ml for the control of the three compounds, respectively. Comparing between the two plants, the glutathione content in *Calendula officinalis* plant recorded values of 259.8, 175.2 and 173.2 µg/ml when treated with terpenes, alkaloids and phenols, respectively comparing to its content in *Tagettes patula*, which was 369.4, 480.6 and 442.6 µg/ml, respectively too. For the interaction, the results showed also that the highest glutathione content was recorded at 400 mg/L of terpenes, alkaloids and phenols on *Tagettes patula* plant; 667.0, 717.0 and 927.0 µg/ml, respectively. The lowest content was; 107.0, 97.0 and 77.0 µg/ml at the combination of 100mg/L of terpenes and *Tagettespatula*, 100 mg/L of alkaloids and *Calendula officinalis* and 100 mg/L of phenols and *Tagettespatula*, respectively. It was clear from the above results that the two antioxidants were significantly affected by the treatment with the three compounds extracted from *Eucalyptus*. It was reported that allelopathic substances cause deleterious effect in plant tissues leading to produce of specific substances, called antioxidants, in order to reduce injury (Li and Hu, 2005 and Singh *et al*., 2006). It was also reported that allelopathic compounds such as benzoic acid and cinnamic acid affect plant cells function resulted in lipid peroxidation and MDA production (Tanveer *et al*., 2008). This was noted in the current study and agreed with the results of Feng *et al*. (2003) in cucumber. It was reported that glutathione content increased in plants when exposed to various environmental stress such as oxidative and low temperature stress and others. Elke *et al*. (2004) noted the presence of glutathione in cells of *Brassica napus*

Table 4 : Effect of different concentrations of terpenes, alkaloids and phenols extracted from *Eucalyptus* sp. on glutathione content(µg.ml^{-1}) in two ornamental plants.

Conc. (mg.L^{-1}) \ Plants		*Calendula officinalis*	*Tagettes patula*	Mean
Terpens	0	251.00	259.00	255.00
	100	182.00	107.00	144.00
	200	285.00	387.00	336.50
	300	269.00	427.00	348.00
	400	312.00	557.00	489.50
	Mean	259.80	369.40	
	LSD 0.05	For Kind of plants 10.85	Concentrations 17.16	Interaction 24.27
Alkaloids	0	204,00	297.00	250.50
	100	97.00	125.00	111.00
	200	114.00	597.00	355.50
	300	220.00	667.00	443.50
	400	241.00	717.00	479.00
	Mean	175.20	480.60	
	LSD 0.05	For Kind of plants 4.79	Concentrations 7.57	Interaction 10.71
Phenols	0	185.00	297.00	241.00
	100	135.00	77.00	106.00
	200	142.00	187.00	164.50
	300	189.00	725.00	457.00
	400	215.00	927.00	571.00
	Mean	173.20	442.60	
	LSD 0.05	For Kind of plants 5.93	Concentrations 9.38	Interaction 13.27

Table 5 : Effect of different concentrations of terpenes, alkaloids and phenols extracted from *Eucalyptus* sp. on proline content(µg.ml^{-1}) in two ornamental plants.

Conc. (mg.L^{-1}) \ Plants		*Calendula officinalis*	*Tagettes patula*	Mean
Terpens	0	2.95	0.80	1.87
	100	3.85	1.08	2.46
	200	3.98	1.34	2.66
	300	4.10	2.05	3.07
	400	4.19	2.26	3.22
	Mean	3.81	1.50	
	LSD 0.05	For Kind of plants 0.83	Concentrations 1.31	Interaction 1.86
Alkaloids	0	3.02	0.35	1.68
	100	3.67	0.17	1.92
	200	3.85	0.43	2.14
	300	3.98	1.03	2.50
	400	4.02	1.55	2.78
	Mean	3.70	0.70	
	LSD 0.05	For Kind of plants 0.46	Concentrations 0.73	Interaction 1.03
Phenols	0	3.42	0.60	2.01
	100	3.33	0.62	1.97
	200	3.63	1.77	2.70
	300	3.83	1.86	2.84
	400	3.93	3.16	3.54
	Mean	3.62	1.60	
	LSD 0.05	For Kind of plants 0.50	Concentrations 0.80	Interaction 1.13

L. exposed to environment stress, which contributed in protecting the cellular molecules from the free radicals. It was also stated that glutathione content increased due to oxidative stress in order to reduce the damage occur to stressed cells (Noctor *et al*., 1998).

The presence of MDA in plant cells was considered as an indicator of lipid peroxidation and act to reduce the damage produced by the allelopathic substances and different other stresses (Aravind and Prasad, 2003 and Gao, 2000). The formation of MDA in stressed plants was due to the fact that these plants produce defensive substances in order to maintain the membrane integrity (Zunion and Zygadlo, 2004). In spinach, it was found that the adding of *E. maridenii* leaves residues to the soil caused an increase in MDA content (Chen *et al*., 2013). Same results were found in peascv. Azad (Dixit *et al*., 2001) and barley (Guo *et al*., 2004).

Results of table 5 indicated that proline content increased as the concentrations of terpenes, alkaloids and phenols used increased. The highest proline contents in leaves of the two ornamentals recorded were; 3.22, 2.78 and 3.54 µg/ml at the highest concentration of terpenes, alkaloids and phenols, respectively. The results also showed that *Calendula officinalis* seedlings had higher proline content than that of *Tagettespatula*. For the interaction, the combination of 400 mg/L and *Calendula officinalis* gave the highest content of proline which were; 4.19, 4.02 and 3.93 µg/mlfor the terpenes, alkaloids and phenols treatments, respectively. The lowest values of proline were; 0.80, 0.17 and 0.60 µg/mlat the combination of *Tagettespatula*and 0 mg/L of terpenes, 100 mg/L alkaloids and 0 mg/L of phenols, respectively. It was obvious that proline content noticeably increased in the two ornamentals treated the three compounds. Proline accumulation in plant cells is considered as an adaptation mechanism to ensure the stability of cellular structures under stress conditions (Najaphy *et al*., 2010). The increase in proline content under stress is due either to protein breakdown or to the conversion of nitrogenous compounds to protein as a result of decreasing cell activities in synthesizing protein (Stewart, 1983). The current results come in agreement with previous ones. Reddy *et al*. (2004) and Bajji *et al*. (2001) have stated that the allelopathic compounds induce proline accumulation in plants. Furthermore, it was noted that *Eucalyptus* extracts treatments increased proline content in chek pea (Das *et al*., 2012) and wheat seedlings (Sakhaee *et al*., 2010). Also, Dianaguiraman *et al*. (2005) has found that proline content in rice and Black gram increased due to the treatment with the extract of *Eucalyptus* leaves. Same results were obtained with maize treated with *Eucalyptus* leaves (Pawar and Chavan, 2004).

The overall results indicate that there was significant increase in sugar content in seedlings of the two plants as the concentration of the terpenes and phenols increased, while for alkaloids treatments, the highest concentration resulted in the highest amount of sugar. Also, the three allelochemicals caused an obvious decrease in protein content, but when compare between concentrations of each allelochemical, the increase in concentrations of terpenes, alkaloids or phenols caused significant increase in protein content in leaves. Melenodialdehyde and glutathione content in *Calendula officinalis* and *Tagettespatula* were positively affected when plants treated with the three allelochemicals. In addition, results regarding proline showed an increase in its content due to the treatment with terpenes, alkaloids and phenols.

Conclusion

From the above results, we can conclude that active substances extracted from *Eucalyptus* leaves had advesrse effect on protein, but they increase the amount of the two antioxidants and proline, in which means they increase plant capability to withstand environmental stress.

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