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Physiological Studies on the Interactive Effects of Lead and Antioxidants on *Carum carvi* Plant

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AN EXPERIMENT was conducted to assess the responses of *Carum carvi* to lead stress and using ascorbic acid and α tochopherol as antioxidants. In this experiment, plants were grown in the nutrient medium with different levels of lead (0, 50, 100,200,500 and 1000 mM) in the form of lead nitrate $Pb(NO_3)_2$.Ascorbic acid or α tochopherol level (each 50 ppm) was used as seed presoaked for 8 h.The interactiveeffects were recorded on plant biomass, biosynthesis of photosynthetic pigments, proline, oil contents and flavonidsin *Carum carvi* plant. From our present results, itcouldbe concluded thatseed presoaked applications of ascorbic acid orα tocopherol could play a role to alleviate the harmful effect of lead stress on some metabolic and physiological processes of *Carum carvi* that reflected in, increasing fresh-dry matter of different organs, inducing a significant stimulatory effect of carotenoid contents, increasing oil, proline and flavonoid contents.

Keywords: *Carum carvi*, $Pb(NO_3)_2$, Antioxidants (ascorbic acid , α tochopherol), Proline, Oil , Flavonoids.

Introduction

Heavy metal pollution has become a global environmental threat. Among metal contaminants, lead (Pb) is a major concern because of its extensive distribution in the environment and the substantial environmental and human health problems it can cause (Dey & Mondal, 2016).

While plants need many metals such as iron, magnesium, copper, or zinc, other metals such as lead or cadmium are highly toxic(Horbowicz et al*.,* 2013). Although lead is not an essential element for plants, it is absorbed easily and accumulated in different plant parts (Sharma & Dubey, 2005). It exerts adverse effect on plant growth(Hadi *et al.,* 2010), changes in chemical composition, antioxidant enzymes system(Gupta et al*.,* 2009, 2010) and lower uptake of many minerals. Lead can cause various physiological and biochemical dysfunctions of many physiological and biochemical processes such as nitrogen assimilation and photosynthesis, (Sharma & Dubey, 2005 and Seregin & Kosevnikova, 2008). Also lead, induces oxidative stress by producing reactive oxygen species which in turn causes damage to various biomolecules like membrane lipids, proteins, chloroplast pigments, enzymes, nucleic acids, etc. (Sharma & Dubey, 2005).

Researches on certain medicinal and aromatic plants showed that they can be more resistant to some heavy metals and other pollutants than other crops (Bağdat & Eid, 2007). Aromatic plants also have a demonstrated ability to accumulate heavy metals (Zheljazkov et al*.,* 2006 and Lydakis-Simantiris et al., 2016).

Some reports demonstrated that, with planting variety of medicinal plants such as Basli, Peppermint and Anet (Dill), instead of crops in area contaminated by heavy metals like lead, copper and cadmium, although, plant's growth decreased, but there is no change oil content and without significant yield reduction (Zheljazkov et al., 2006). They suggest possible use of aromatic plants as heavy metal accumulatorsfor cleansing contaminated sites (Lydakis-Simantiris et al., 2016).

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Plants possess a sophisticated and interrelated network of defense strategies to avoid or tolerate heavy metal toxicities (Emamverdian et al., 2015). The first defense strategy is to avoid the metal entry into the cell excluding it or binding it to a cell wall (Mishra et al., 2006 and Zhou et al., 2010). The second defense systems, once heavy metal ions enter tissues and cells, plants initiate several cellular defense mechanisms constitute various antioxidants to nullify and attenuate the adverse effects of heavy metals(Reddy et al., 2005 and Wang et al., 2010).

To mitigate the harmful effects of free radicals, plant cells have developed antioxidant defense mechanism which is composed of enzymatic antioxidants and nonenzymatic antioxidants like ascorbate (AsA), glutathione (GSH), carotenoids, α-tocopherols, proline, and phenolic compounds (as flavonoids) that act as scavengers of free radicals (Michalak, 2006 and Sharma et al., 2012).

Antioxidants have synergistic effects on growth, yield and yield quality of many plant species. These compounds have beneficial effects on catching the free radicals or the active oxygen produced during photosynthesis and respiration processes (Foyer et al., 1991 and Al Qubaie, 2012).

Ascorbic acid (vitamin C) is one of the most important water soluble antioxidants in plants, having an essential role in several physiological processes, including plant growth, differentiation, and metabolism (Atharetal., 2008). It is acting as a modulator of plant development through hormone signaling and as coenzyme in reactions by which carbohydrates, fats and proteins are metabolized (Postori et al., 2003 and Nahed et al., 2009). Ascorbic acid is involved in the regulation of many critical biological processes such as photo-inhibition and cell elongation (Noctor et al.,1998) and many other important enzymatic and non enzymatic reactions (Smirnoff, 2000). Moreover, ascorbic acid is very important for the regulation of photosynthesis, flowering and senescence (Barth et al., 2006), as well as for α-tocopherol regeneration, which has been reported to act as the primary antioxidant (Bortoli et al., 1997).

Ascorbate can directly scavenge oxygen free radicals with and without enzyme catalysts by recycling tocopherol to the reduced form. Ascorbate can reactwith activated oxygen more readily than any other aqueous component, and protects critical macromolecules from oxidative damage (Foyer, 1993).

α-Tocopherol (vitamin E) is a small lipophilic antioxidant that is synthesized in all higher plants (Fryer, 1992 and Bafeel & Ibrahim, 2008). Its levelvaries in different tissues and fluctuate during development and in response to abiotic stresses. It interacts with the polyunsaturated acyl groups of lipids, stabilizes membranes, scavenges and quenches various ROS (Maeda & DellaPenna, 2007) thus protects polyunsaturated fatty acids from lipid peroxidation and modulates signal transduction (Noctor, 2006).

In cooperation with the xanthophylls cycle, vitamin E fulfills at least two different functions in chloroplasts at the two major sites of singlet oxygen production: it preserves PSII from photoinactivation and protects membrane lipids from photooxidation (Havaux et al., 2005). Plants pre-treated with α-tocopherol showed induced stress tolerance and protection against oxidative damage due to various stresses (Kumar et al.,2012).

Proline is known to occur in many plant species and normally accumulates in large quantities in responses to metal toxicity (Tripathi & Gaur, 2004), It is noted that prolineis a good indicator stress and it has an important role in osmotic adjustment. It contributes to the stability of the sub cellular structures by scavenging free radicals under stress conditions (Ashraf &Foolad, 2007 and Molinari et al., 2007).

Regarding oil content, Tarraf et al. (1999) stated that ascorbic acid treatment caused pronounced increase in lemongrass essential oil percent and oil yield per plant(Gamal El-Din, 2005 and Ayad et al., 2009).

Flavonoids are secondary plant metabolites with a vast array of possible functions, including antioxidative activity and as chelators for metals (Michalak, 2006; Keilig & Ludwig-Müller, 2009a and Ilboudo, et al., 2012). The functional diversity of flavonoids is due to their structural diversity. This diversity endows flavonoids with many biological functions in addition to their role as antioxidants in the plant (Tahara, 2007 and Symonowicz & Kolanek, 2012)

This investigation aimed to assess the efficiency of two antioxidant vitamins (ascorbic acid and α tocopherol) in alleviating lead stress on *Carum carvi* plant through their actions on fresh and dry wt., photosynthetic pigments,proline, oil contents and as well as flavonoids.

Materials and Methods

The plant material used in this investigation was Caraway "*Carum carvi*" family apiaceaeas important medicinal plant grown in sand area in Egypt. Seed were kindly supplied by the Egyptain Ministry of Agriculture. seeds of the plant were selected for uniformity of size, shape, and viability. Before sowing, the seeds were surface sterilized by soaking for 2 min in 0.1% mercuric chloride, after which they were washed several times with distilled water. 10 healthy seeds were sown in pot 30 cm in diameter filled with 10 kg air dry soil [(clay/sand 1:2) v/v]. The seeds of tested plant were divided into three groups. The first group was treated with different concentration of $Pb(NO_3)_2$ (0.0, 50, 100, 200, 500, 1000 mM). And the second group, the seeds were presoaked in 50 p.p.m ascorbic acid for 8 h before sowing them (based on preliminary studies), then treated with different concentration of $Pb(NO_3)_2$ solution (0.0, 50, 100, 200, 500, 1000 mM). Whereas the third group, seeds were presoaked in 50 p.p.mα-tocopherol acetate for 8 h, then treated with different concentration of $Pb(NO_3)_2$ (0.0, 50, 100, 200, 500, 1000 mM).

The plants were grown in a greenhouse, irrigation was carried at intervals during the experiment with sufficient tap water with 100 mL of Hogland's solution (Hewitt, 1966) to keep the soil content at field capacity. In order to prevent the accumulation of pollutants, the soil in each pot was leached every ten days with excessive amount of distilled water.

Three replicates were used for each set of experiment. Fresh and dry matter of the different organs (roots, shoots and flowers) were determined at the end of experiments (after 60 days).

Determination of photosynthetic pigments

Plants were harvested two times after exposure to lead heavy metal (30, 60 days),then The photosynthetic pigments (chlorophyll a, b, and caroteniods) were determined using the spectrophotometric method recommended by (Metzner et al., 1965) and applied for higher plants (Ahmed et al., 1980).

Determination of free proline

Free proline was determined according to (Bates et al., 1973).Approximately 50 mg of dry plant material was homogenized in 10 ml of 3% aqueous sulfosalicylic acid and the homogenate was filtered through Whatman filter paper. Two ml of the filterate was reacted with 2 ml acidninhydrin reagent and 2 ml of glacial acetic acid for 1 hat 100 ºC.After cooling the coloured reaction product was extracted with 4 mltoluene. Shaken vigorously with a test stirrer for 15- 20 sec. The chromophore layer containing toluene was separated from aqueous phase, warmed to room temperature. The absorbency was determined from standard curve and calculated as mg proline/ gm dry matter.

Determination of oil content

The method adopted for extraction of oil (the content of an oleaginous material) was that described by Meara (1955) and applied by Younis et al. (1987).

Determination of flavonoids content

Flavoniods of the plant material were extracted with 80% methanol at 60 ºC, shaken for 20 min, and filtered. The filtrate was diluted at 1:3 and 100µl of reactive solution (1% 2-amino-ethyle diphenyl borate) was added (Hariri et al., 1991) and spectrophotometric measurements were done at wavelength of 404 nm. Extract absorption was compared with that of standard (Luteolin) resulting in the calculation of total amount of flavonoids.

Statistical analyses

The experimental design was a random complete block, with three replications. The data were analyzed by the STATGRAPHICS (Statistical Graphics Corporation, Princeton USA) statistical package by the t-test and ANOVA functions to assess significant differences a many means.

Results

Growth of *Carum carvi* plants in Table 1 reaveled that fresh -dry matter of both roots and shoots origenating from seeds (none soaked with antioxidants, ascorbic or α-tocopherol acetate) were increased with the rising of Pb level when compared with the control. Whereas, the fresh and dry matter of flowers decreased with increasing the concentration of Pb levels. Also, the results indicated that plants originated from seeds soaked in ascorbic acid or α-tocopherolonly, led to highly significant increased in both fresh and dry matter of different organs.

Plants origenated from seeds presoked in ascorbic acid, and treated with different concentrations of Pb increased fresh and dry matter of shoot in respect to the control but

TABLE 1. The effect of Pb(NO3)2 and treatment with 50ppm Ascorbic acid or α -tocopherol on fresh and dry matter (g/ plant) of *Carum carvi.*

External ion conc.(mM)	Con. (mM)	Fresh matter				Dry matter			
		Shoot	**Root**	**Total**	**Flower**	**Shoot**	**Root**	**Total**	**Flower**
	0	5.576 ±0.36	1.04±0.092	6.61 ± 0.45	0.250±0.003	1.73 ±0.001	0.39 ±0.029	2.12±0.029	0.053±0.002
	50	8.58**±0.45	1.12 n.s.±0.22	9.70** ± 0.23	0.168***±0.003	2.45*** ±0.087	0.410n.s. ±0.046	2.87**±0.13	0.045**±0.001
	100	9.67**±0.48	1.21 n.s.±0.14	10.88** ±0.34	0.191***±0.002	2.55* ±0.26	0.430n.s. ±0.012	2.98*±0.27	0.047 n.s.±0.003
$Pb(NO_3)_2$	200	10.43***±0.006	1.49**±0.006	11.92*** ±0.006	0.180***±0.001	1.73 n.s.±0.098	0.420 n.s.±0.035	2.15 n.s.±0.064	0.047*±0.001
	500	11.66***±0.20	1.79**±0.12	13.45** ±0.32	0.170***±0.002	2.62* ±0.33	0.450 n.s.±0.098	3.07 n.s.±0.43	0.038***±0.001
	1000	12.48**±1.5	1.52**±0.006	14.00** ±1.5	0.130***±0.001	2.69***± 0.055	0.380 n.s.±0.023	3.07***±0.032	0.031***±0.002
	0+Asc	10.30***±0.40	1.19 n.s.±0.11	11.49***±0.29	0.300**±0.010	1.95** ±0.029	0.300*±0.006	2.25* ±0.029	0.059*±0.001
	50	9.04 n.s.±0.023	1.07 n.s.±0.25	10.11 n.s.±0.23	0.350***±0.021	2.03** ±0.017	0.370 n.s.±0.081	2.40* ±0.064	0.057**±0.002
	100	7.686* ±0.18	0.990 n.s.±0.052	8.67**±0.13	0.290*±0.026	1.86* ±0.023	0.260*±0.058	2.12*±0.081	0.068**±0.002
$Pb(NO_3)_2$+Asc	200	7.51***±0.010	0.960***±0.052	8.47***±0.053	0.190 n.s.±0.015	2.05 n.s.±0.14	0.250**±0.006	2.30 n.s.±0.14	0.037***±0.007
	500	7.15***±0.38	0.860**±0.052	8.01***±0.43	0.185**±0.002	1.07** ±0.092	0.250 n.s.±0.012	1.32**±0.041	0.049***±0.001
	1000	6.91*±0.34	0.670***±0.046	7.58** ±0.29	0.147 n.s.±0.007	1.67 n.s.±0.42	0.210**±0.006	1.88*±0.41	0.042*±0.002
	0+α toco	9.91***±0.12	1.840**±0.092	11.75*** ±0.21	0.380*±0.040	2.69** ±0.18	0.450 n.s.±0.036	3.14**±0.21	0.083***±0.002
	50	9.52 n.s.±0.53	2.14*±0.21	11.66**±0.32	0.360***±0.006	1.87** ±0.13	0.610*±0.53	2.48 n.s.±0.080	0.072***±0.001
$Pb(NO_3)_2$+α -tocopherol	100	9.15 n.s.± 1.6	1.54 n.s.±0.21	10.69 n.s.±1.9	0.300***±0.001	1.93 n.s.±0.040	0.410 n.s.±0.12	2.34 n.s.±0.053	0.057*±0.001
	200	9.10***±0.12	1.04***±0.023	10.14***±0.14	0.303***±0.007	1.65 n.s.±0.029	0.280*±0.023	1.93*±0.006	0.066**±0.003
	500	8.60**±0.40	0.940**±0.035	9.54***±0.37	0.253*±0.023	2.20 n.s.±0.35	0.320 n.s.±0.003	2.52 n.s.±0.35	0.048*±0.003
	1000	7.22*±0.33	0.700***±0.029	7.92**±0.35	0.220**±0.015	1.19***±0.18	0.150***±0.006	1.34***±0.18	0.043**±0.001
L.S.D. at 0.05 %		1.696	0.343	1.715	0.481	0.105	0.497	0.126	0.004
L.S.D. at 0.01 %		2.276	0.460	2.302	0.645	0.141	0.668	0.169	0.006

Non significant (n.s.) at $P > 0.05$
Significant (*) at $P \leq 0.05$
Highly significant (**) at $P \leq 0.01$
Very highly significant (***) at $P \leq 0.001$

decreased the fresh and dry weight of both shoot and roots with increasing the cocentrations of Pb.Where as the fresh and dry matter of the flowers increased at lower concentrations of Pb (up to 100 mM). However, plants origenated from seeds presoked in α-tocopherol and treated with different concentrations of Pb concentrations were increased fresh and dry matter of shoot and flowers with increasing the cocentrations of Pb but in roots increased up to 100 mM of pb.

Photosynthetic activity (Tables 2 and 3) is one of the highly sensitive responses of stress in plants. And most of the heavy metals are known to inhibit this process to varying levels. The biosynthesis of Chl a, Chl b as well as total pigments in *Carum carvi* leaves decreased gradually with increasing the levels of Pb ions at 30 and 60 days. But, at 60 days, the content of Chl b in plants treated with Pb increased with increasing metal levels up to 200 mM above that decreased. In case of *Carum carvi* plants originated from seeds soaked in ascorbic acid and treated with different levels of Pb at 30 or 60 days, the biosynthesie of Chl a, Chl b, and carotenoids decreased the values of Chl a and Chl b, while the carotenoid at 30 days increased up to 200 mM when compared with corresponding levels of lead metal. In case of plants treated with α tocopherol, the photosynsetic pigments increased with increasing the concentrations of pb to 100 mM above that decreased while at 60 days the value of Chl a and Chl b increased up to 500 and 200 mM of lead metal.

Seed-presoaked applications with either ascorbic acid or α-tocopherol acetate not only alleviated the inhibitory effect of Pb on the biosynthesis of photosynthetic pigments but also induced a significant stimulatory effect.

Proline content, which is a significant stress indicator, increases in the different organs of test plant, with increasing treatment concentrations of lead (Table 4). Proline content in the plants originated from treated seeds with ascorbic acid showed increment pattern in shoots of *Carum carvi* significantly, but decreased in roots. Also increase in proline content was observed along with increasing lead concentrations at 50 mM in roots and up to 500 mM in shoot above that decreased. However, the plants originated from seeds soaked in α- tocopherol significantly increased proline content in both roots and shoots of plants. Whereas, the proline content in roots and shoots of these plants treated with pb increased up to 100 mM above that decreased.

Table 5 shows clearly that the trend of oil contents of *Carum carvi* roots, shoots and flowers were considerably increase with rising of pb levels up to 200 mM above that decreased. It was observed that ascorbic acid and α-tocopherol application led to a significantly increasing in oil contents in different organs of *Carum carvi* plants especially at α-tocopherol treatments. Oil contents, in plants originated from seeds soaked in ascorbic acid were increased with rising pb levels in roots up to 200 mM, while shoots and flowers up to 500 mM , above these two concentrations, oil contents was decreased. Also, the soaked application in α-tocopherol in *Carum carvi* plants then treated with Pb levels increased in oil contents in roots, flowers up to 200 mMand in shoots at all levels. Generally, soaked application in ascorbic acid or α-tocopherol and treated with Pb metal ions increased the oil content in different organs of organs of plants when compared with the corresponding treatments with Pb.

The flavonoids contents (Table 6) increased non-significantly with the rising of the pb levels up to 200 mM in roots, 50 mM in shoots and 100 mM in the flowers and above these levels these values decreased. The plants originated from seeds soaked in ascorbic acid or α-tocopherol showed an increase in their flavonoids contents when compared with control in different organs in tested plants. However, *Carum carvi* plants originated from seeds soaked in ascorbic acid or α-tocopherol and treated with pb exhibited increased in flavonoid contents in roots, shoots and flowers up to 200 mM.

Discussion

The accumulation of fresh-dry matter of roots and shoots of *Carum carvi* was mostly enhanced with increasing metal levels (Pb). This indicates that *Carum carvi* has an ability to exhibit variable responseto heavy metal (Pb) stress. The same conclusion was recorded by Zheljazkove *et al.* (2006). In contrast , the value of fresh-dry matter of flowers generally decreased with increasing the heavy metals stress (Pb). The same conclusion was recorded by Chauhan *et al.* (2004).

The inhibitory effect of heavy metals stress induced by Pb growth parameters of *Carum carvi* is in agreement with the results obtained by some authors using different plants (Chatterjee *et al.,* 2004; Mishra *et al.,* 2006; Sharma & Dubey, 2005; Hadi *et al.,* 2010; Zhou *et al.,* 2010 and Gupta *et*

TABLE 2 . The effect of $Pb(NO_3)_2$ and treatment with 50 ppm Ascorbic acid or α-tocopherol on photosynthetic pigments (mg/ fresh matter) of *Carum carvi* leaves after 30 days of treatment.

External ion conc.(mM)	Con. (mM)	Chll A	Chll B	Chll (A+B)	%	Carotenoids	%	Total Pigments	%
Pb(NO3)2	0	3.070 ±0.046	1.531 ±0.31	4.601 ±0.35	100.02	1.610 ±0.17	100.000	6.211 ±0.19	100.02
	50	2.799 ns ±0.12	1.615 ns ±0.11	4.411 ns ±0.23	95.96	1.32 ns ±0.001	82.298	5.295 ns ±0.51	92.42
	100	1.779 *** ±0.13	2.301 ns ±0.17	4.077 ns ±0.3	88.70	0.594 ** ±0.012	36.894	4.671 ** ±0.31	75.27
	200	1.650 *** ±0.058	1.060 ns ±0.041	2.710 ** ±0.017	58.91	0.956 * ±0.027	59.379	3.666 *** ± 0.01	59.03
	500	1.126 *** ±0.055	0.595 * ±0.006	1.735 *** ±0.052	37.41	0.842 ** ±0.04	52.298	2.577 *** ±0.013	41.49
	1000	0.835 *** ±0.041	0.675 * ±0.043	1.510 *** ±0.084	32.83	0.765 ** ±0.027	47.516	2.275 *** ±0.057	36.63
Pb(NO3)2+Asc	0+Asc	2.066 *** ±0.038	1.359 ns ±0.14	3.424 * ±0.1	74.46	1.239 ns ±0.21	76.957	4.663 ** ±0.31	75.10
	50	1.817 ** ±0.15	1.125 ns ±0.36	2.942 ** ±0.21	63.96	1.200 ns ±0.064	74.534	4.142 ns ±0.15	66.70
	100	1.419 ns ±0.18	1.114 ** ±0.14	2.533 ** ±0.2	55.07	1.578 * ±0.28	98.012	4.109 ns ±0.48	66.20
	200	1.952 * ±0.059	0.823 ns ±0.11	2.775 ns 0.16	60.33	1.300 ns ±0.17	80.745	4.075 ns ±0.34	65.62
	500	1.117 ns ±0.56	0.575 ns ±0.014	1.602 ns ±0.049	36.78	0.980 ns ±0.15	60.870	2.582 ns ±0.17	43.03
	1000	0.881 ns ±0.081	0.495 * ±0.012	1.376 ns ±0.07	29.91	0.551 ** ± 0.028	34.224	1.927 ** ±0.042	31.03
Pb(NO3)2+α –tocopherol	0+α toco	4.277 ns ±0.48	0.992 ns ±0.004	5.267 ns ±0.47	114.54	2.158 ns ±0.37	134.037	7.434 ns ±0.84	119.60
	50	4.008 ** ±0.26	0.992 ** ±0.082	5.000 ns ±0.34	108.70	2.164 * ±0.73	134.410	7.164 * ±0.24	115.36
	100	2.753 * ±0.22	1.555 ns ±0.25	4.313 ns ±0.039	93.65	1.280 * ±0.18	79.503	5.593 ns ±0.22	89.98
	200	2.540 ns ±0.44	1.432 ns ±0.25	3.972 ** ±0.2	86.35	1.417 ns ±0.22	88.012	5.529 ** 0.41	86.78
	500	1.802 * ±0.17	0.860 ns ±0.095	2.662 * ±0.19	57.87	0.990 ns ±0.085	61.491	3.462 ** ±0.12	58.81
	1000	0.814 ns ±0.021	0.419 ns ±0.16	1.284 ns ±0.18	26.80	0.558 ** ±0.031	34.658	1.842 ns ±0.21	28.84
L.S.D. at 0.05 %		0.792	0.553	1.022		0.221		1.073	
L.S.D. at 0.01 %		1.064	**0.742**	**1.373**		**0.297**		**1.440**	

Non significant (n.s.) at $P > 0.05$
Significant (*) at $P \leq 0.05$
Highly significant (**) at $P \leq 0.01$
Very highly significant (***) at $P \leq 0.001$

TABLE 3. The effect of $Pb(NO_3)_2$ and treatment with 50 ppm Ascorbic acid or α-tocopherol on photosynthetic pigments (mg/ fresh matter) of *Carum carvi* leaves after 60 days of treatment.

External ion conc. (mM)	Con. (mM)	Chll A	Chll B	Chll (A+B)	%	Carotenoids	%	Total Pigments	%
	0	2.124 ±0.072	1.4±0.023	3.52±0.16	100.00	0.760 ±0.15	100.00	4.283 ±0.009	100.00
	50	2.096 ns±0.11	1.751 ns±0.29	3.847 ns±0.36	110.61	0.450 ns ±0.029	59.21	4.297 ns ±0.34	101.39
	100	2.005 ns±0.57	1.751 ns±0.43	3.757 ns ±1.0		0.520 ns ±0.001	68.42	4.277 ns ±1.0	100.90
$Pb(NO_3)_2$	200	1.896 ±0.52	1.623 ns±.33	3.520 ns ±0.18	101.18	0.410 ns ±0.058	53.95	3.930 * ±0.058	92.71
	500	0.827***±0.10	0.743 ns±0.09	1.570 *** ±0.19	45.14	0.228 * ±0.01	30.00	1.798 *** ±0.2	42.43
	1000	0.707***±0.12	0.527 * ±0.032	1.233 *** ±0.15	34.50	0.393 ns ±0.08	51.71	1.626 *** ±0.067	37.59
	0+Asc	2.048 ns±0.61	1.397 ns±0.18	3.447 ns ±0.78	99.05	0.703 ns ±0.12	92.50	4.150 ns ±0.9	97.88
	50	2.247 ns±0.14	1.47 ns±0.25	3.717 ns ±0.11	106.87	0.513 ns ±0.008	69.47	4.230 ns ±0.11	100.17
$Pb(NO_3)_2$+Asc	100	1.55 ns±0.26	1.25 ns±.14	2.800 ns ±0.4	80.51	0.509 ns ±0.005	66.97	3.309 ns ±0.4	78.08
	200	1.37 ns±0.21	0.795 ns±0.25	2.167 ** ±0.1	62.25	0.509 ns ±0.001	66.97	2.676 ** ±0.1	63.10
	500	1.327 ns±0.19	0.802 ns±0.006	2.127 ns ±0.19	61.21	0.327 ns ±0.055	47.37	2.453 * ±0.14	58.73
	1000	0.952 ns±0.029	0.577 ns±0.015	1.527 ns ±0.043	43.96	0.234 ns ±0.019	35.13	1.760 ns ±0.038	42.38
	0+α toco	3.312**±0.18	1.906 ns±0.049	5.223 ** ±0.23	150.03	0.742 ns ±0.022	101.97	5.965 ** ±0.24	141.41
	50	1.546 ns±0.26	1.885 ns±0.18	3.433 ns ±0.081	98.65	0.777 *** ±0.015	102.24	4.210 ns ±0.68	99.29
$Pb(NO_3)_2$+α – tocopherol	100	1.815 ns±0.13	1.474 ns±0.25	3.290 ns ±0.38	94.57	0.836 * ±0.079	110.00	4.126 ns ±0.45	97.33
	200	1.896 ns±0.061	1.112 ns±0.006	3.007 ns ±0.055	86.49	0.838 ns ±0.19	110.26	3.845 ns ±0.14	90.75
	500	0.856 ns±0.084	0.473*0.012	1.333 ns ±0.095	38.21	0.947 *** ±0.091	124.61	2.280 ns ±0.005	53.23
	1000	1.002 ns±0.006	0.547 ns±0.026	1.547 ns ±0.026	44.54	0.443 ns ±0.025	58.29	1.990 ** ±0.051	47.00
L.S.D. at 0.05 %		0.568	0.472	0.635		0.454		0.898	
L.S.D. at 0.01 %		0.763	0.634	0.852		0.610		1.206	

Non significant (n.s.) at $P > 0.05$
Significant (*) at $P \leq 0.05$
Highly significant (**) at $P \leq 0.01$
Very highly significant (***) at $P \leq 0.001$

TABLE 4. The effect of $Pb(NO_3)_2$ and treatments in ascorbic acid or α -tocopherol on proline contents (mg/ g dry matter) of *Carum carvi* palnt.

External ion conc.(mM)	Con. (mM)	Root	%	Shoot	%
$Pb(NO_3)_2$	0	0.611 ±0.003	100.00	1.010±0.005	100.00
	50	0.615 ns ±0.001	100.65	1.015 ns ±0.001	100.50
	100	0.633* ±0.006	103.60	1.027 * ±0.003	101.68
	200	0.659*** ±0.001	107.86	1.056 ** ±0.003	104.55
	500	0.669*** ±0.002	109.49	1.043 ** ±0.002	103.27
	1000	0.691*** ±0.005	113.09	1.020 ns ±0.001	100.99
$Pb(NO_3)_2$+Asc	0+Asc	0.608 ns ±0.004	99.51	1.019 ns ±0.002	100.89
	50	0.620 *** ±0.001	101.47	1.121 *** ±0.001	110.99
	100	0.594** ±0.002	97.22	1.123 *** ±0.002	111.19
	200	0.576*** ±0.004	94.27	1.113 *** ±0.001	110.20
	500	0.551*** ±0.001	90.18	1.090 *** ±0.001	107.81
	1000	0.520 *** ±0.004	85.11	0.969 *** ±0.004	95.94
$Pb(NO_3)_2$+α -tocopherol	0+α toco	0.620 ns ±0.004	101.47	1.022 ns ±0.003	101.19
	50	0.623 *** ±0.001	101.96	1.021 *** ±0.001	101.99
	100	0.619 ns ±0.001	101.31	1.010 ** ±0.003	100.00
	200	0.586 *** ±0.004	95.91	0.962 *** ±0.004	95.25
	500	0.587*** ±0.002	96.24	0.940 *** ±0.001	93.07
	1000	0.530*** ±0.003	86.74	0.882 *** ±0.003	87.33
L.S.D. at 0.05 %		0.008		0.007	
L.S.D. at 0.01 %		0.011		0.009	

Non significant (n.s.) at $P > 0.05$
Significant (*) at $P \leq 0.05$
Highly significant (**) at $P \leq 0.01$
Very highly significant (***) at $P \leq 0.001$

TABLE 5. The effect of Pb(NO3)2 and treatment with 50 ppm Ascrbic acid or α -tocopherol on oil content (mg/ g dry matter) of *Carum carvi* plants.

External ion conc. (mM)	Con. (mM)	Root	%	Shoot	%	Flowers	%
$Pb(NO_3)_2$	0	0.215 ±0.009	100.00	0.311±0.013	100.00	0.064±0.005	100.00
	50	0.23 ns±0.003	106.98	0.344 ns±0.017	110.61	0.064 ns±0.005	100.00
	100	0.277**±0.006	128.84	0.343 ns±0.033	110.29	0.071 ns±0.004	110.94
	200	0.235 ns±0.032	109.30	0.360	115.76	0.045 ±0.006	54.69
	500	0.132**±0.018	61.40	0.248 ns±0.028	79.74	0.048 ns±0.006	75.00
	1000	0.101***±0.001	46.98	0.2**±0.006	64.31	0.031**±0.001	48.44
$Pb(NO_3)_2$+Asc	0+Asc	0.216 ns±0.009	100.47	0.39 ns±0.064	125.40	0.093**±0.004	145.31
	50	0.246 ns±0.027	114.42	0.419*±0.018	134.73	0.095**±0.001	148.44
	100	0.311**±0.002	144.65	0.461*±0.032	148.23	0.092*±0.005	143.75
	200	0.301 ns±0.063	140.00	0.453 ns±0.021	145.66	0.091**±0.005	142.19
	500	0.195*±0.009	90.70	0.342 ns±0.044	109.97	0.071*±0.001	110.94
	1000	0.153ns ±0.020	71.16	0.269*±0.018	86.50	0.055***±0.001	85.94
$Pb(NO_3)_2$+α -tocopherol	0+α toco	0.300***±0.006	139.53	0.501***±0.001	161.09	0.096**±0.002	150.00
	50	0.306***±0.006	147.44	0.513**±0.021	164.95	0.099**±0.001	154.69
	100	0.311*±0.008	144.65	0.511*±0.051	164.31	0.083 ns±0.009	129.69
	200	0.267 ns±0.021	124.19	0.48 ns±0.021	154.34	0.071ns±0.017	110.94
	500	0.205*±0.003	95.35	0.436**±0.025	140.19	0.057 ns±0.02	89.06
	1000	0.195***±0.003	90.70	0.319 ns±0.057	102.57	0.028 ns±0.009	43.75
L.S.D. at 0.05 %		0.059		0.094		0.168	
L.S.D. at 0.01 %		0.075		0.126		0.022	

Non significant (n.s.) at $P > 0.05$
Significant (*) at $P \leq 0.05$
Highly significant (**) at $P \leq 0.01$
Very highly significant (***) at $P \leq 0.001$

TABLE 6. The effect of $Pb(NO_3)_2$ and treatments with ascorbic acid or α -tocopherol on flavonoids content (mg/ g dry matter) of *Carum carvi* plants.

External ion conc. (mM)	Con. (mM)	Root	%	Shoot	%	Flowers	%
$Pb(NO_3)_2$	0	0.065 ±0.003	100.00	0.310 ±0.006	100.00	0.611 ±0.08	100.00
	50	0.068 ns ±0.005	104.62	0.317 ns ±0.033	102.26	0.621 ns ±0.012	101.64
	100	0.071 ns ±0.005	109.23	0.300 ns ±0.029	96.77	0.620 ns ±0.021	101.47
	200	0.077 ns±0.008	118.46	0.286 ns ±0.008	92.26	0.605 ns ±0.05	99.02
	500	0.060 ns ±0.001	92.31	0.271 ns ±0.03	87.42	0.553 ns ±0.037	90.51
	1000	0.051 * ±0.004	78.46	0.253 ns ±0.024	81.61	0.528 ns ±0.016	86.42
$Pb(NO_3)_2$+Asc	0+Asc	0.069 ns ±0.005	106.15	0.318 ns ±0.01	102.58	0.636 ns ±0.021	104.09
	50	0.072 ns ±0.005	110.77	0.330 ns ±0.017	106.45	0.648 ns ±0.017	106.06
	100	0.083 ns ±0.005	127.69	0.347 ns ±0.036	111.94	0.703 ns ±0.098	115.06
	200	0.081 ns ±0.007	124.62	0.341 ns ±0.024	110.00	0.684 ns ±0.038	111.95
	500	0.059 ns ±0.001	90.77	0.290 ns ±0.043	93.55	0.574 ns ±0.032	93.94
	1000	0.055 ns ±0.004	84.62	0.237 ns ±0.008	76.45	0.493 ns ±0.043	80.69
$Pb(NO_3)_2$+α -tocopherol	0+α toco	0.071 ns ±0.009	109.23	0.350 ns ±0.035	112.90	0.640 ns ±0.012	104.75
	50	0.073 ns ±0.004	112.31	0.338 ns ±0.013	109.03	0.676 ns ±0.023	110.64
	100	0.080 ns ±0.005	123.08	0.296 ns ±0.045	95.48	0.611 ns ±0.004	100.00
	200	0.088 ns ±0.007	135.38	0.360 ns ±0.035	116.13	0.721 ns ±0.001	118.00
	500	0.064 ns ±0.007	98.46	0.281 ns ±0.008	90.65	0.601 ns ±0.02	98.36
	1000	0.057 ns ±0.001	87.69	0.270 ns ±0.034	87.10	0.520 ns ±0.001	85.11
L.S.D. at 0.05 %		0.004		0.014		0.012	
L.S.D. at 0.01 %		0.006		0.019		0.016	

Non significant (n.s.) at $P > 0.05$
Significant (*) at $P \leq 0.05$
Highly significant (**) at $P \leq 0.01$
Very highly significant (***) at $P \leq 0.001$

al. 2016).

Soaking the seeds in ascorbic acid or α-tocopherol solutions significantly increased fresh-dry matter of different organs of *Carum carvi* plants. Ascorbate has been shown to have an essential role in several physiological processes in plants, including growth, differentiation and metabolism (Foyer, 1993 and Gamal El-Din, 2005). Ascorbic acid is cofactors for enzyme activity, and effects on plant antioxidation capacity, heavy metal evacuation and detoxification and stress defense (Zhang, 2012). Also, the same conclusion by El-Quesni *et al.,* (2009)who mentioned that application of α-tocopherol increased fresh weight of shoots and roots in *Hibiscus rosasineses* L. plants. The antioxidant properties of α-tocopherol are the result of its ability to quench both singlet oxygen and peroxides (Fryer, 1992). Ascorbic acid plays an important role in α-tocopherol regeneration which has been reported to act as the primary antioxidant (Bortoli *et al.,* 1997).

Soaking application of ascorbic acid or α-tocopherol together with Pb effectively increased the fresh-dry matter of shoots in *Carum carvi* plants. In contrast, the values of fresh-dry matter of roots and flowers generally decreased with increasing Pb except under 50 and 100 mM of Pb were increased.These results are consistent with the studies of Foyer, (1993) who reported that exogenous application of ascorbic acid or α-tocopherol can overcome the harmful effect of heavy metal stress.

The inhibitory effect of heavy metal on the pigment biosynthesis, in the present investigation, is in agreement with the results obtained by some other authors (Chatterjee et al., 2004; Odjegba & Fasidi, 2006; Liu et al*.,* 2008; Singh *et al.,* 2010; Cenkci et al., 2010 and Rossato et al*.,* 2012). Pb has reduced the photosynthetic pigments (chlorophyll a and b) significantly because Pb prevents the incorporation of Fe (iron) in phytoporphyrin ring of chlorophyll molecule, so cause reduction in chlorophyll contents (Jaleel et al., 2009 and Kumar et al. 2012) and also, may be attributed to increase in activity of chlorophyll-degrading enzyme chlorophyllase under stress conditions (Liu et al., 2008).

The decrease in photosynthesis might be due to striking change in fine structure of chloroplasts (Sadak & Dawood 2014) and also due to destruction of marginal membranes of meristematic cells when Pb is present at high concentrations. Also, the inductions of oxidative stress by heavy metals might be due to blockage of electron flow in photosystem II (Kato & Shimizu, 1987).

Dela Rosa-Ibarra & Maiti (1995) assumed that the tendency to decrease in the chlorophyll content might be due to the synthesis of nitrogen compounds acting as osmotic regulators *e.g* proline consumes a large amount of nitrogen. These observation are in good agreement with our present results of *Carum carvi* plants which showed that the decrease in the biosynthesis of photosynthetic pigments under heavy metal stress (Pb) was accompanied by an increase in the biosynthesis of nitrogen compounds (proline).

The content of carotenoid when treated with Pb at 30 days their pigments increased up to 200 mM above that decreased. In terms of its antioxidant properties carotenoids can protect the photosystems is one of four ways by reacting with lipid peroxidation products to terminate chain reactions (Burton & Ingold, 1984), by scavenging singlet oxygen and dissipating the energy as heat by reacting with triplet or excited chlorophyll molecules to prevent formation of singlet oxygen or by the dissipation of excess excitation energy through xanthophylls cycle (Havaux et al., 2005).The inhibitory effect of heavy metals stress on the carotenoids is in agreement with the results obtained by Chatterjee et al. (2004).

After 30 or 60 days the content of Chl a and Chl b of heavy metals (Pb) *Carum carvi,* plants treated with ascorbic acid were decreased, while α-tocopherol increased up to 100 mM of Pb above that decreased. Ascorbic acid may be have some phytotoxic effect on chlorophyll function. In other word, decreased chlorophyll content (Kiani et al., 2008). The enhancement roles of α tocopherol on photosynthetic pigments are in agreement with those reported by Kumar et al. (2012) on stressed wheat and Al Qubaie (2012) on sunflower, since, α tocopherol may be protected the organization of the chloroplast thus minimize chlorophyll loss.

Generally carotenoid contents in *Carum carvi* plants increased under moderate level of Pb when treated with ascorbic acid or α-tocopherol. The increasing in carotenoids may be a strategy adopted by plants to alleviate the toxic effects

of free radicals generated under heavy metal toxicity (Azooz et al., 2011).Carotenoids prevent the formation of singlet oxygen by quenching the triplet state of the chlorophyll molecules as they arise (Havaux et al., 2005).

With respect to proline biosynthesis, the different organs of *Carum carvi* plant promoted action of accumulation of proline with increase in metal (Pb) levels in roots and shoots. Proline content is a significant stress indicator, thus it could be suggested that heavy metals (Pb) tolerance of *Carum carvi* was manifested via the activated proline accumulation in the different organs. The accumulation of proline in response to heavy metals of many plants was also recorded by other investigators under laboratory conditions (Tripathi & Gaur, 2004; Odjegba & Fasid, 2006 and Dey & Mondal, 2016).

There are different opinions regarding mechanisms by which proline alleviate metal toxicity effects within the cell. It has been shown that free proline acts as an osmoprotectant (Ashraf & Foolad, 2007 and Molinari *et al.,* 2007), proteinst abilizer (Sha rma & Diez,2005), metal chelator (Sharma & Dubey, 2005), inhibitor of lipid peroxidation (Mehta & Gaur, 1999), free radical scavenger (Ashraf & Foolad, 2007 and Molinari *et al.,* 2007), prevent enzyme destruction, and decrease the toxic effects of lead (Tripathi & Gaur, 2004). Therefore, it may be concluded that the accumulation of proline could be regarded as one of the major physiological mechanisms of heavy metals stress tolerance in the experimental plant.

However, Proline content in the different organs of test plant was induced by soaking plant seeds in ascorbic acid or α-tocopherol. The proline content of plants whose their seeds pretreated with ascorbic acid increased significantly in shoots of *Carum carvi* but decreased in roots of the plants. Whereas, soaking application in α-tocopherol increased significantly the proline content in both roots and shoots of the plants.

There is much evidence that ascorbic acid or α-tocopherol plays an essential role in several physiological processes in plants, including growth differentiation and metabolism (Foyer, 1993). Ascorbate functions as a reductant for many free radicals, thereby minimizing the damage caused by oxidative stress. Ascorbate can directly scavenge oxygen free radicals with and without enzyme catalysts and can indirectly scavenge them by recycling α-tocopherol to reduced form (Asada, 1992). Tocopherol is actually a family of antioxidants (Hess, 1993). The antioxidant properties of tocopherol are the results of its ablitiy to quench both singlet oxygen and peroxides (Fryer, 1992 and Maeda & Della Penna, 2007).

A response of significance in connection with the role of soaking application of ascorbic acid or α-tocopherol as antioxidant in modifying the heavy metal stress induced changes was also revealed in the present investigation with respect to the biosynthesis of, proline.Soaking application in ascorbic acid and treated with Pb ions increased the proline content at 50 mM in roots and up to 500 mM in shoot of *Carum carvi* above that decreased. However, proline content in roots and shoots of *Carum carvi* plants originated from soaked in α-tocopherol and treated with Pb increased up to 100 mM above that decreased. The application of ascorbic acid or α-tocopherol mitigate to variable extent the adverse effect of lead stress on plant growth, may be due to, the enhanced proline accumulation.

According to these results, Schat et al. (1997) reported that the application excess metals, the responses of *V.cespitosa* and *S. vulgaris* population essentially resembled each other in that the sensitive lines of both species accumulated high proline contents but tolerant ones did not. The decreased relative proline accumulation in the tolerant ecotype could be due to a slower development of water deficit under metal stress (Schat *et al.,* 1997).

Increasing Pb metal enhanced the oil contents in different organs of *Carum carvi* up to 200 mM and decreased at higher levels of pb. This is in accordance with the results obtained by Dahdoh & Moussa (2000).

Generally soaking application in either ascorbic acid or α-tocopherol increased the oil contents in roots, shoots and flowers of *Carum carvi.* These results are in agreement with the results obtained by Gamal El Din (2005) who observed that ascorbic acid significantly increased oil percentage of sunflower seeds and Also, α-tocopherol treatments significantly increased essential oil percent and yield of

Pelargonium graveolens L. (Ayad et al., 2009).

Soaking application in ascorbic acid and treated with Pb increased in oil content in roots, shoots and flowers up to 200, 100 and 500 mM, respectively. These results are also consistent with Sadak & Dawood (2014). Contrary to the present results, Dolatabadian et al. (2010) mentioned that, the highest corn oil percentage was achieved from stressed plants while ascorbic acid treatments decreased it.

Soaking application in α-tocopherol and treated with Pb increased oil content in roots and flowers of *Carum carvi* up to 200 mM but in shoots at all levels used. These increases might be due to a pronounced enhancement of α tocopherol on synthesis and accumulation of oil (Ayad *et al.,* 2009).

The flavonoids content in roots , shoots and flowers of *Carum carvi* were accumulated at lower and moderately levels of Pb (50, 100 and 200 mM). As a result of Pb stress, the enhanced content of flavonoids probably is responsible for binding of metal ions, due to the specific chemical structure, it can chelate metal ions like Pb^{2+} and form complexes (Ilboudo, et al., 2012 and Symonowicz & Kolanek, 2012). In addition to direct free radical scavenging properties and form chelate formation compex, flavonoids have antioxidant activity, in the prevention of free radicals induced by pb which damage target biomolecules (Tahara, 2007 and Keilig & Ludwig-Müller, 2009). Their antioxidant properties are related with hydroxyl group. (Symonowicz & Kolanek, 2012). However, it was observed that several classes of flavonoids showed antioxidant activity towards a variety of easily oxidizable compounds, many of those play an important physiological and ecological role as they are involved in resistance to different types of stress (Ali & Abbas, 2003 and Ali et al.,2007).

Soaking application in ascorbic acid or α-tocopherol generally increased the level of flavonoids in different organs of test plant. Also, soaking application in ascorbic acid or α-tocopherol in *Carum carvi* plant and treated with Pb up to 200 mM, induced some changes in flavonoids synthesis. Ascorbic acid or α-tocopherol counter balanced the adverse effects of heavy metals Pb on growth and content of oil and flavonoids in *Carum carvi.*

It can be concluded that presoaking seeds of *Carum carvi* in ascorbic acid or α-tocopherol could be considered of great importance for counter balanced the adverse effects of heavy metals Pb on growth and content of oil and flavonoids. The tendency of the observed heavy metal Pb induced changes leads to an assumption that the medicinal plant tested in this investigation have some variable abilities not only to tolerate moderate heavy metals Pb but also to grow well and to produce the same amount or even more oil and flavonoids than the control plants.

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دراسات فسيولوجية علي التأثيرات المتداخلة للرصاص ومضادات الأكسدة لنبات الكراوية

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أجريت تجربة لتحديد استجابات نبات الكراوية لإجهاد الرصاص ، واستخدام حمض الاسكوربيك والفا توكوفيرول كمضادات للاكسدة. في هذه التجربة، تنمي النباتات في وسط غذائي مع مستويات (تركيزات) مختلفة من الرصاص (0، 50، 100، 200، 500 و 1000 مليمول) في صورة نترات الرصاص $Pb(NO_3)_2$. وتركيز واحد لحمض الاسكوربيك أو لالفا توكوفيرول (50 جزء في المليون لكل) ، استخدم لنقع البذور فيه لمدة 8 ساعات. وتم تسجيل التأثيرات المتداخلة على الكتلة الحيوية النباتية، التخليق الحيوي لأصباغ البناء الضوئي، البرولين، الزيوت والفلافونيدات في نبات الكراوية. ومن النتائج الحالية، يمكن أن نستنتج أن البذور المعاملة بحامض الاسكوربيك أو بألفا توكوفيرول يمكن أن تلعب دورا في تخفيف الآثار الضارة لإجهاد الرصاص على بعض العمليات الأيضية والفسيولوجية لنبات الكراوية و ينعكس ذلك في، زيادة الوزن الطازج – الجاف لأعضاء النبات المختلفة ، وزيادة معنوية لمحتوي الكاروتينويدات ، و الزيوت، والبرولين و الفلافونويدات.

دراسات فسيولوجية علي التأثيرات المتداخلة للرصاص ومضادات الأكسدة لنبات الكراوية.

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كقواعد امينيه لتطعيم التوصيل سليلوز الذى (a-c)استخدمت مشتقات الأمينو إندوليل نيكوتينونيتريل (1 تم تحضيره بتفاعل الميكروكريستالين سيليلوز و مركب التولويين سلفونيل كلوريد فى وجود التراى ايثيل امين واستبدالها بمجموعة الأمينو إندوليل نيكوتينونيتريل (Ts) عن طريق الإحلال الجزئى لمجموعة التوصيل .(a-c)معطيا مشتقات جديدة من الامينو سيليلوز(2

و من ناحية اخرى تم اكسدة الكربوكسى ميثيل سليلوز الى -3,2ثنائى ألديهيد كربوكسى ميثيل سليلوز باستخدام الصوديم ميتابيرايودات (NaIO4) بدرجة أكسدة 22%. ثم تفاعل الـ-3,2ثنائى ألديهيد كربوكسى ميثيل سليلوز مع الـ-2أمينو6--إندوليل نيكوتينونيتريل (1a-c) باستخدام التراى إيثل أمين كمحفز قاعدى معطيا مشتقات جديده من قواعد شيف (a-c3). تم اثبات التركيب البنائى للمواد السليلوزيه المحضرة باستخدام الرنين النووى المغناطيسى , الاشعه تحت الحمراء, المسح الاكترونى الميكروسكوبى, و الاشعة السينيه.

اختبرت فاعلية المشتقات السليلوزيه المحضره (a-c2) و (a-c3) كمضادات لنمو البكتريا. مشتقات الامينو سليلوز (a-c2) لم تكن لها نشاطا مضادا للبكتيريا. بينما أظهرت النتائج ان مركب 3 (a, b) لهما القدره على تثبيط نمو البكتريا (جرام موجب و جرام سالب) و كذالك الكنديدا عند تركيزات 100-300) مج/ مل(و فقدت فاعليتها كمضادات بكتريه عند تركيز 50 مج/ مل, على عكس مشتقات الـ-6إندوليل نيكوتينونيتريل (a-c1) التى لم تكن لها فاعليه ضد بكتريا جرام سالب.