Pak. J. Bot., 45(3): 795-806, 2013.

APPLICATION OF CARROT ROOT EXTRACT INDUCED SALINITY TOLERANCE IN COWPEA (*VIGNA SINENSIS* L.) SEEDLINGS

SALWA MOHAMED ABBAS* AND SAMIA AGEEB AKLADIOUS

Biological Science and Geology Department, Faculty of Education, Ain Shams University, Roxy Cairo, Egypt
**Corresponding author's e-mail: salwa_morsy2001@yahoo.com*

Abstract

Salt stress is considered as a restricting factor for plant products. Therefore, many compounds have been applied to minimize the harmful effects of salinity. Carrot roots extract is one of the natural biostimulant compounds with growth-stimulating characteristics. Moreover, it has been used as amendments in plant growth due to the presence of a number of plant growth-stimulating compounds. This study provides a review of the effect of carrot roots extract on the growth and development of plants grown under salt stress conditions with an importance on the use of this renewable bioresource in sustainable agricultural systems. The effect of presoaking of the cowpea seeds in carrot roots extracts (25 and 50 g/100 ml) and NaCl (100 mM) were examined. The results revealed that treating the seeds with sodium chloride accompanied by carrot root extracts increased growth parameters, total chlorophyll, carotenoids and total carbohydrate of the cowpea seedlings as compared to the seeds treated with sodium chloride alone. Also, antioxidant compounds content (anthocyanins, ascorbic acid, flavonoids and phenol compounds) were increased. Furthermore, treating the seeds with carrot roots extract and salt stress lead to differential expression of the genetic information in cowpea seedlings, resulting in changes in gene products, including protein and isozymes profiles. These changes induced the synthesis of certain proteins and simultaneously decreased the expression of other protein sets. The results also demonstrated that low concentration (25 mg/ L) of carrot root extract was more effective in reducing the adverse effects of salinity through the enhancement of multiple processes.

Introduction

Salinity is one of the major environmental factors limiting plant growth; excess salt may affect plant growth either through osmotic inhibition of water uptake by roots or specific ion effects, which may cause direct toxicity (Ashraf & Harris, 2004; Saqib *et al*., 2012). The effects of salinity were determined at seedling stage of wheat range from reduction in germination percentage, fresh and dry weight of shoots and roots to the uptake of various nutrient ions (Afzal *et al*., 2006). Salt stress has toxic effects on plants, like loss of chloroplast activity, decreased photosynthetic rate and increased photorespiration rate which lead to an increasing of reactive oxygen species (ROS), which produced as a result of salt stress, lead to chlorophyll degradation, photo damage and lipid peroxidation (Apel & Hirt, 2004). The plants possess specific mechanisms include activation of antioxidant enzymes and non enzymatic antioxidants to scavenge ROS (Noreen & Ashraf, 2009; Wang *et al*., 2013). The obtained results in the study of Tayefi *et al*., (2011) suggest that, oxidative stress may play an important role in salt-stressed safflower plants by induction of specific isozymes. One mechanism that plants have developed to grow under stress conditions is the synthesis and accumulation of osmolytes (osmoprotectants). These osmolytes act as compatible solutes, they are small, uncharged, molecules and do not inhibit normal cellular processes. Carrot (*Daucus carota* L.) roots belong to Apiaceae vegetables. The analysis of carrot roots by HPLC showed that, carrot roots extract have a high content of vitamin A as Beta Carotene, protein, carbohydrates, fat and vitamins B1, B2 , B6 , C, D and E which are antioxidants that reduce free radicals cell damage (Baranska *et al.*, 2005). Vitamins considered bio-regulator compounds, which relatively in low concentrations influence plant growth by regulating many physiological processes, such as synthesis of enzymes, act as co-enzymes, and added to protect plant from harmful effects of stresses (Youssef & Talaat, 2003). In plants, carotenoids which are precursors of vitamin A, play important roles in the assembly of photo systems, in light harvesting, and in photo protection and as antioxidants in protecting cells from the damaging effects of free radicals (Paine *et al*., 2005). Also carrot (*Daucus carota* L.) roots contain anthocyanin, amino acids, proline, sugar, carotenoids, flavones, proteins and fibers. All these components of carrot rich in essential oil, caffeic acid, chlorogenic acid and gallic acid also, roots of carrots contain three activities of 3-deoxy-d-arabino-heptulosonate 7-phosphate (DAHP) synthase, this enzyme catalyzes the first step of the shikimate pathway (Alasalvar *et al*., 2001). Baranska *et al*., (2005) reported that the analysis of different colored carrots, for their content of anthocyanins , four carotenoids, phenolics, caffeic acid and chlorogenic acid which the most predominant phenolic compound in all carrot varieties, were quantified by HPLC. The endogenous hormone levels in carrot, such as indoleacetic acid (IAA), abscisic acid (ABA), gibberellins and cytokinins-zeatin were determined in callus cultures of *Daucus carota* L by Spectrophotometric and chromatographic analyses (Jim *et al.,* 2005). Puchooa & Ramburn (2004) showed that using of carrot juice in the medium increases the fresh weight, dry weight and moisture content of the explants with increasing concentration of carrot juice in the medium. Extract of carrot root also promoted the growth of carrot root explant, tobacco stem callus and sunflower crown gall tissue. These facts seem to suggest that extracts from many different kinds of plant materials contain common growth promoting substances, which may be involved in the mechanism of induction of growth in various kinds of plant tissues (Jim *et al.,* 2005; Anwar *et al.,* 2011). It was hypothesized that carrot extract-induced salinity tolerance in common bean might be attributed largely to components in the carrot extracts.

Cowpea, is an annual plant, and now grown worldwide for its edible bean, popular both dry and as a green bean. The leaf is occasionally used as a leaf vegetable, and the straw is used for fodder. Generally, because the previous constituents of carrot juice, it's obvious that, its contents can alleviate injury effects of salt stress, thus this study aimed to finding natural products which can recover salinity effects on plants growth.

Materials and Methods

Carrot roots extract exhibit growth-stimulating activities and used as biostimulants in crop production that promote plant growth (Zhang 1997). Carrot roots extraction was obtained according to Sofowora (1982). Carrot Roots obtain from the local Markets in Egypt, about 100 g of the washed fresh carrot roots were weighed. It was sliced into tiny pieces and blended in an electric blender with 160 ml sterile distilled water and 160 ml of ethanol. These were then transferred into separate flask and shaken for one hour and filtered with Whatman No. 1 filter paper and the filtrate was adjusted to pH 7.0 with 1 N NaOH and rising up to adequate volume with sterile distilled water. Main contents of carrot roots extract are presented in Table (1) which have been analyzed by Baranska *et al*., (2005), Alabran *et al*., (1975) and Alasalvar *et al*., (2001), United States Department of Agriculture Handbook).

Table 1. Chemical analysis of carrot roots extract

Vitamines (mg⁄100g DW)		Macro and Micronutrients (mg⁄100g DW)		Other Components	
Vit. A (as Beta Carotene)	11.0	Potassium	63.5	Carbohydrates	18 g⁄100g DW
Vit.C	35.00	Calcium	69.00	Protein	12 g⁄100g DW
Vit.B1	1.50	Magnesium	2.00	Fat	1.5 g⁄100g DW
Vit.B6	1.70	Phosphorus	1.45		
Vit.B2	2.00	Sulphur	0.92	Essential oil	0.59 %
Vit.E	2.10	Copper	0.80		
Folic acid	1.36	Iron	1.30		

Growth conditions: Seeds of cowpea (*Vigna sinensis* L.) were obtained from Agricultural Research Centre, Egypt. The seeds were sterilized by using 30% hypochlorite for five minutes and then washed three times with distilled water. The seeds were arranged in 15 cm Petri dishes covered with two sheets of filter paper and divided to three groups: The 1st group was moistened with 10 ml of distilled water (control). The 2nd group was moistened with 100 mM NaCl. The 3rd group was moistened with different levels (25 and 50 g/ 100 ml) of carrot roots extracts. Following sowing, germination experiment was carried by placing all groups of Petri dishes in an incubator at 27±1ºC for 7 days. After that, only 7-days seedlings of the 3rd group was divided into two sets; the 1st set of the seedlings was allowed to complete their germination until the end of the experiment (15 days) while, the 2nd set was transferred to another set of Petri dishes and moistened with 100 mM NaCl until the end of the experiment. All groups of the seedlings were collected at 15 days after sowing. There are three replications for each treatment.

Variables measured: Ten seedlings per treatment were taken at the end of the experimental period (15 days after germination) to investigate the effects of germination in carrot roots extract and/or NaCl on some growth aspects, molecular and physiological changes of cowpea seedlings as follow:

Growth parameter: Shoot length, root length, leaves number, fresh weight of seedlings, dry weight of seedlings were obtained by drying the material in oven at 80ºC until constant weight.

Photosynthetic pigments: An accurate weight (0.5 g) of fresh young cowpea leaves were homogenized in 85% acetone and used for determination of photosynthetic pigments (Chl.'a', Chl.'b' and carotenoids) using spectrophotometric method developed by Metzziner *et al*., (1965). The samples were read at 663, 664 and 452.5 nm respectively.

Anthocyanin: Anthocyanin content was estimated according to the method of Krizek *et al*., (1993). Leaf samples were homogenized in 10 mL of acidified methanol (HCl: methanol, 1:99, v/v). The homogenate was centrifuged at 18000 g for 30 min at 4°C, then the supernatant was filtered through Whatman No1 to remove particulate matter and was stored in darkness at 5°C for 24 h. The amount of anthocyanin was determined from the absorbance at 550 nm. Anthocyanin content was expressed as µmol/g FW and the concentration of anthocyanin was calculated using the extinction coefficient of anthocyanin ε = 33 000/mol2 cm.

Total carbohydrates: Total soluble sugars were extracted by overnight submersion of dry tissue in 10 ml of 80% (v/v) ethanol at 25°C with periodic shaking, and centrifuged at 600g. The supernatant was evaporated till completely dried then dissolved in a known volume of distilled water to be ready for determination of soluble carbohydrates (Homme *et al*., 1992). TSS were analyzed by reacting of 0.1 ml of ethanolic extract with 3.0 ml freshly prepared anthrone (150 mg anthrone + 100 ml 72% H_2SO_4) in boiling water bath for ten minutes and reading the cooled samples at 625 nm using Spekol SpectrocololourimeterVEB Carl Zeiss (Yemm & Willis, 1994).

Ascorbic acid: was determined as described by Mukherjee and Choudhuri (1983). 4 ml of the extract was mixed with 2 ml of 2% dinitrophenyl-hydrazine (in acidic medium) followed by the addition of 1 drop of 10% thiourea (in 70% ethanol). The mixture was boiled for 15 min in a water bath and after cooling to room temperature, 5 ml of 80% (v/v) H_2SO_4 was added to the mixture at 0°C (in an ice bath). The absorbance was recorded at 525 nm by spectrophotometer.

Determination of phenolic compound: Phenolic compound were extracted from fresh seedling samples with ethanol 80%. Shahidi & Naczk (1995) determined total phenol content.

Using Folin-ciocalteus reagent at 725 nm. Adding 1ml of each extract, 0.5ml Folin reagent and 7.5 ml distilled water then shaking for 3 minutes then 1 ml Na_2CO_3 was added, the blue color developed was determined after 1h at 725 nm against blank. The phenolic compound concentrations were determined by using the standard curve of catichol (100 ppm). The concentrations of phenolic compound were expressed as µg. g^{-1} dry weight (DW).

Determination of total flavonoids: The total flavonoids were measured by the method of Bushra *et al*., (2009). Briefly, extracts of each plant material (1 mL) were diluted with 4 mL water in a 10 mL volumetric flask. Initially, 5% $NaNO_2$ solution (0.3 mL) was added to each volumetric flask; after 5 min, 10% $AlCl_3$ (w/w) was added; and at 6 min, 1.0 M NaOH (2 mL) was added. Absorbance of the reaction mixture was read at 430 nm.

Electrophoretic of protein patterns: Electrophoretic protein profile of cowpea leaves were analyzed according to SDS-PAGE technique (Laememli, 1970) which relates polypeptide maps, molecular protein markers, percentage of band intensity using gel protein analyzer version 3 (MEDIA- CYBERNE TICE,USA).

Isozymes electrophoresis: The Isozymes used were: alcohol dehydrogenase (Adh), esterase (EST) and peroxidase (POD). Isozymes were separated in 10% native-polyacrylamide gel electrophoresis as described by Stegemann *et al*., (1985). For isozyme extraction, 0.5g of fresh leaves was homogenized in 2ml extraction buffer using a mortar and pestle; centrifuged at 3000 rpm for 10 minutes; the supernatant was kept at 20ºC until use. For electrophoresis, 50µl of extract was mixed with 25µl of treatment buffer and 50µl of this mixture was applied to the well. The staining gels were carried out according to Wendel & Weeden (1989) for Adh, Siciliano & Shaw (1976) for esterase and Vallejos, (1983) for POD respectively. Gels were washed two or three times with tap water; fixed in ethanol: 20% glacial acetic acid (9:11 v/v) for 24 hours; and photographed.

Statistical analysis: All analyses were done on a completely randomized. The results were subjected to two-way analysis of variance (ANOVA) and the mean differences were compared by the Duncan test at 5% significance level.

Results

Growth parameters: Our results revealed that salinity had a significant ($p<0.05$) effect on reducing the length of shoots and roots, leaf number, fresh and dry mass of cowpea seedlings (Table 2). Whereas carrot roots extract increased the values for both stressed and unstressed plants. Under the control conditions, both treatments of carrot extract had ameliorative effects on the growth parameters. The 25 g/100ml level was the most effective. These results indicated that the concentration (25 g/100ml) of the carrot roots extract was more effective on growth of cowpea seedlings under control and salinity conditions.

Table 2. Effect of carrot roots extract on the growth criteria of cowpea seedlings germinated under salt stress conditions.

NaCl (mM)	Carrot extract (g/100ml)	Shoot length (cm)	Root length (cm)	No. of leaves	Fresh weight of seedlings (g)	Dry weight of seedlings (g)
0 mM	0	20.00 e	7.41 c	2.03 b	0.66 b	0.06 a
	25	27.41 a	11.83 a	3.41 a	0.94 a	0.09 a
	50	25.81 b	9.65 b	2.63 ab	0.81 ab	0.08 a
100 mM	0	16.63 f	6.62 d	1.85 b	0.63 b	0.05 a
	25	24.45 c	10.16 b	2.64 ab	0.72 b	0.09 a
	50	21.62 d	8.14 c	2.36 b	0.69 b	0.07 a

Data are reported as means (n=10). Means were separated by Duncan's multiple range tests, different letters indicate a significant difference at $p\leq0.05$

Photosynthetic pigments content: Salinity caused reduction in total chlorophylls, carotenoids and total pigments contents in leaves of cowpea seedlings. On the other hand, treatment with carrot roots extract alleviates the harmful effect of salinity stress since the contents of photosynthetic pigments showed significant increases when the seeds treated with carrot roots extract and germinated under stressed conditions (Table 3) as compared with control. The maximum increase in total pigments was observed in case of the seedlings treated with 25 g/100 ml of carrot extract and grown under unstressed conditions.

Anthocyanins: Salt stress increased the content of anthocyanin in leaves of cowpea above that of the control (Fig. 1). Treating the seeds with both concentrations of carrot roots extract before germination caused an obvious increase in anthocyanin content in case of plants

germinated under normal or salt stress conditions. The highest increase (158%) in the content of anthocyanin occurred in case of plants treated with the lower concentration of carrot root extract (25 g/100 ml) and germinated under salinity conditions.

Total carbohydrates: Total carbohydrate content in the cowpea seedlings was slight increased because of salt stress, but the same content-exhibited obvious increases in the seedlings treated with carrot root extract and germinated under salt stress conditions (Fig. 2) as compared with that observed in the seedlings grown under unstressed conditions. Data also showed that, application of a lower concentration of carrot roots extract (25g/100 ml) to seeds exhibited higher total carbohydrate content than that occurred in case of application of the higher one.

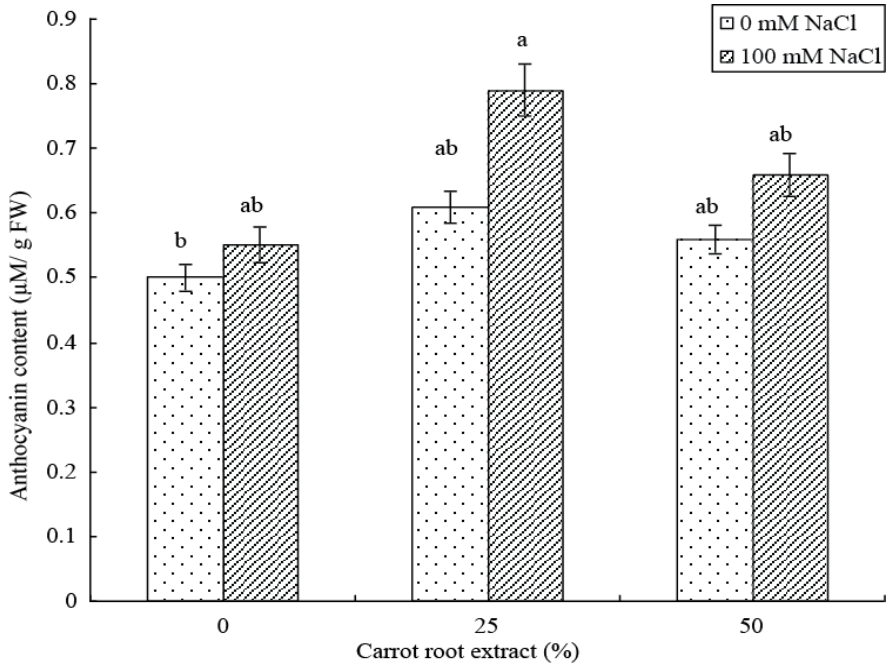

Fig. 1. Effect of carrot extract on anthocyanin content of cowpea seedlings germinated under salt stress conditions. Data presented as means of 3 replicates ± SD. Bars treatments with different letters are significant different ($p<0.05$).

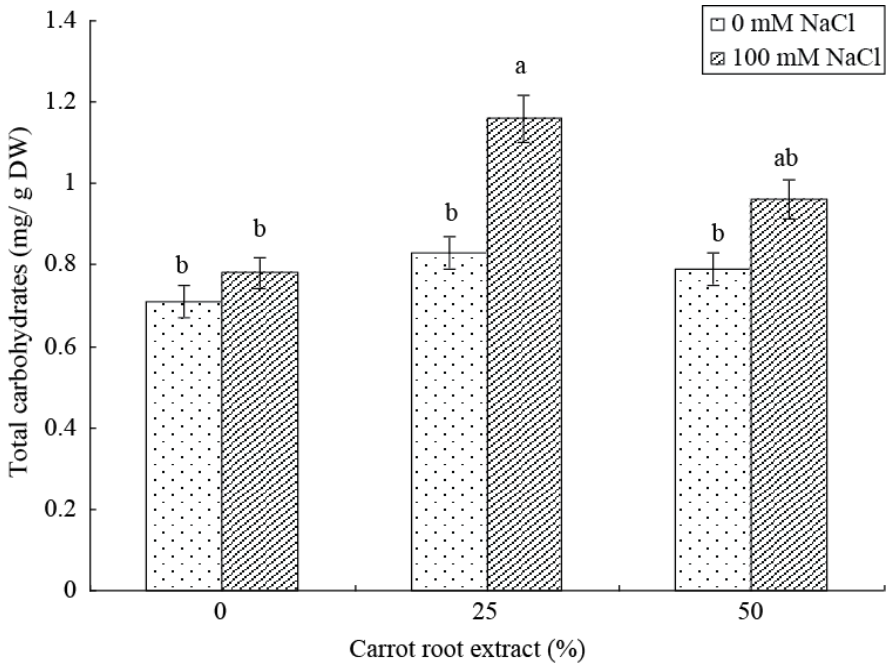

Fig. 2. Effect of carrot extract on total carbohydrates content of cowpea seedlings germinated under salt stress conditions. Data presented as means of 3 replicates ± SD. Bars treatments with different letters are significant different ($p<0.05$).

Table 3. Effect of carrot roots extract on photosynthetic pigments of cowpea seedlings germinated under salt stress conditions.

NaCl (mM)	Carrot extract (g/100 ml)	Chlorophyll (a)	Chlorophyll (b)	Chlorophyll (a+b)	Carotenoids	Total pigments
0 mM	0	1.99 c	1.02 c	3.01 d	0.43 d	3.49 e
	25	5.83 a	2.15 a	7.98 a	1.33 b	9.31 a
	50	4.01 b	2.05 a	6.06 b	1.81 a	7.87 b
100 mM	0	1.07 d	0.59 d	1.66 e	0.57 d	2.23 f
	25	3.73 b	1.42 b	5.15 c	1.13 bc	6.28 c
	50	2.05 c	1.29 bc	3.34 d	0.83 cd	4.17 d

Data are reported as means (n=5). Means were separated by Duncan's multiple range tests, different letters indicate a significant difference at $p \leq 0.05$

Ascorbic acid, total phenols and flavonoids contents: The present investigation showed that, exposing cowpea seedlings to salt stress conditions alone (100mM NaCl) caused a slight increase in ascorbic acid, total phenols and flavonoids contents as compared to control (Figs. 3, 4 & 5). Application of carrot roots extract increased significantly these contents above both unstressed and stressed conditions. The magnitude of induction was much more pronounced by applying 25 g/100 ml of carrot root extract under salinity conditions.

Protein electrophoretic pattern: The results of SDS – PAGE electrophoretic patterns of proteins extracted from the leaves of cowpea seedlings treated with carrot roots extract and germinated under normal or salinity conditions are shown in (Table 4 and Fig. 6). Application of carrot roots extract to the seeds of cowpea which grown under salinity conditions induced a considerable variation in the protein patterns of the produced leaves. In the present work, three types of proteins patterns are observed in the protein extracted from cowpea leaves, some protein bands were disappeared, other proteins were selectively increased and synthesis of new set of protein was induced. Table 4 showed that the total number of protein bands in the leaves of cowpea treated with carrot roots extract and germinated under 100 mM NaCI was increased (10 bands) as being compared with the respective control (8 bands). There were increases in the number of protein bands in the leaves of the seedlings treated with carrot roots extract and grown under both unstressed and stressed conditions. Results also showed the appearance of two novel protein bands having molecular weights 52.88 and 32.45 kDa due to treating the seeds with carrot roots extract (50 g/100 ml) plus 100 mM NaCl. In addition, protein bands of molecular weights of 69.57, 54.64 and 37.94 kDa were induced in the seedlings treated with carrot root extract and grown under unstressed or stressed conditions. On the other hand, protein band of 56.96 KDa disappeared in the seedlings treated with carrot roots extract and grown under normal or salinity conditions (0 and 100mM NaCl). In response to treating with 100 mM NaCl only, cowpea seedlings exhibited the appearance of new protein band with molecular weight 38.93 KDa. Seven protein bands of molecular weights (215.41, 1 89.50 173.31, 158.38, 94.44, 69.57 and 33.90 kDa) were de novo synthesized in cowpea leaves grown under salinity stress alone or in combination with carrot roots extract.

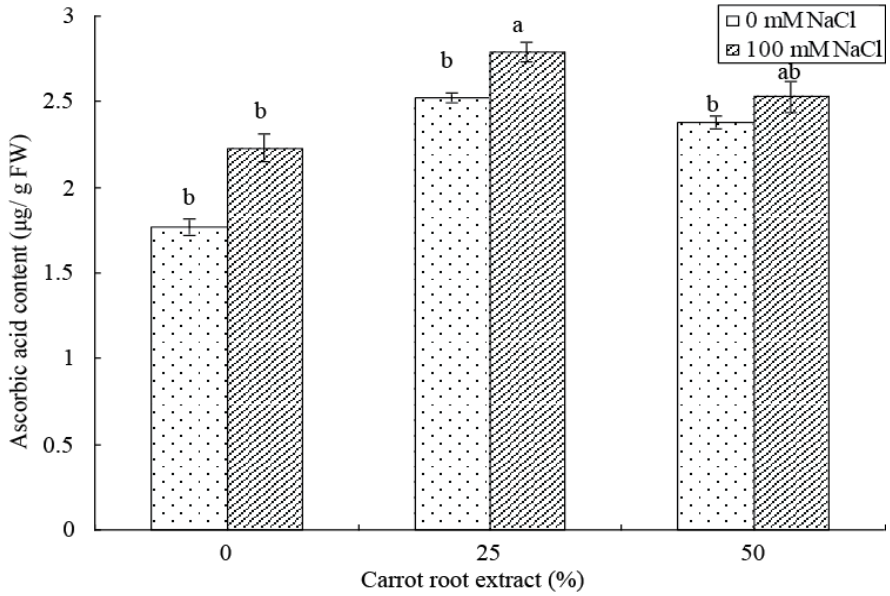

Fig. 3. Effect of carrot roots extract on ascorbic acid content of cowpea seedlings germinated under salt stress conditions. Data presented as means of 3 replicates ± SD. Bars treatments with different letters are significant different ($p<0.05$).

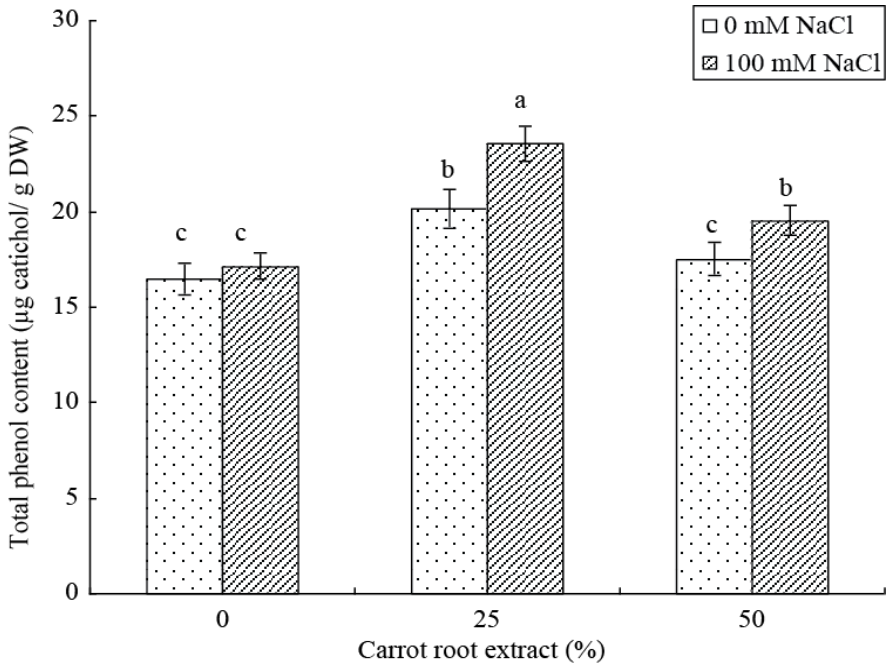

Fig. 4. Effect of carrot roots extract on total phenol content of cowpea seedlings germinated under salt stress conditions. Data presented as means of 3 replicates ± SD. Bars treatments with different letters are significant different ($p<0.05$).

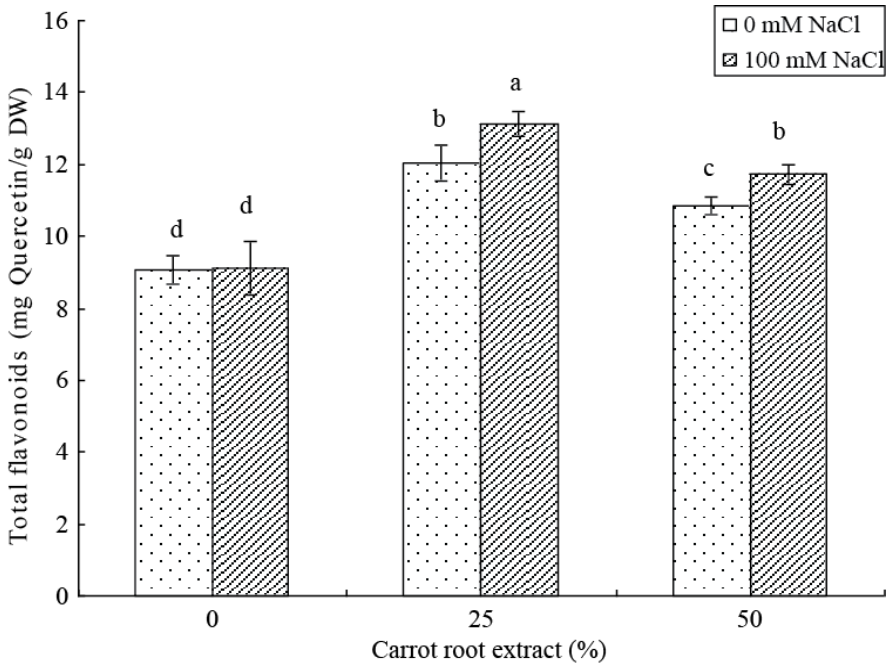

Fig. 5. Effect of carrot roots extract on total flavonoid content of cowpea seedlings germinated under salt stress conditions. Data presented as means of 3 replicates ± SD. Bars treatments with different letters are significant different ($p<0.05$).

Isoenzymes expression: The induction of new isozymes and the change in the isoenzymes profiles are considered to play an important role in the cellular defense against oxidative stress, caused by salt stress. Isoenzymes expression of cowpea seedlings treated or non-treated with salt represented in (Tables 5, 6 and Fig. 7). In the present work, three enzymes alcohol dehydrogenase (Adh), Esterase (EST) and peroxidase (POX) were studied.

Alcohol dehydrogenase (Adh) ısoenzyme: Isoenzyme profiles of alcohol dehydrogenase (Adh) in cowpea leaves demonstrated that the number of bands were increased in the seedlings grown under salinity condition (100mM NaCl) and also in others seedlings treated with carrot roots extract (25 g/100ml) compared with that of the control. There are only 2 polymorphic bands, which present in some treatments and absent in the others.

Table 4. Effect of carrot roots extract on electrophoretic pattern of cowpea seedlings germinated under salt stress conditions.

Molecular weight (kDa)	NaCl (mM)					
	0 mM			100 mM		
	Carrot root extract (g/100 ml)			Carrot root extract (g/100 ml)		
	0	25	50	0	25	50
215.41	+	+	+	+	+	+
189.50	+	+	+	+	+	+
173.31	+	+	+	+	+	+
158.38	+	+	+	+	+	+
94.44	+	+	+	+	+	+
69.57		+	+	+	+	+
56.96	+			+		
54.64		+	+		+	
52.88						+
46.77			+		+	+
45.26	+	+		+		
38.93				+		
37.94		+	+		+	+
33.90	+		+	+	+	
32.45						+
Total number of bands	8	9	10	10	10	10
Number of new bands		3	4	2	4	5

Table 5. The presence (+) and absence (-) of bands in threer isozymes, alcohol dehydrogenase (Adh), esterase (EST) and peroxidase (Px) at the effect of carrot roots extract on cowpea seedlings grown under salt stress conditions.

	NaCl (0mM)			NaCl (100mM)		
Adh	**1**	**2**	**3**	**4**	**5**	**6**
1	-	+	-	+	+	-
2	+	+	+	+	+	-
Total	**1**	**2**	**1**	**2**	**2**	**0**
			EST			
1	+	+	+	+	+	+
2	+	+	+	+	+	+
3	-	+	-	+	-	-
4	-	+	+	+	+	+
Total	**2**	**4**	**3**	**4**	**3**	**3**
			Px			
1	-	-	-	+	-	-
2	+	+	+	+	+	+
3	-	+	-	+	+	+
4	+	+	+	+	+	-
Total	**2**	**3**	**2**	**4**	**3**	**2**

Lane 1 = 0 (control), Lane 2 = carrot root extract (25 g/100ml), Lane 3 = carrot root extract (50 g/100ml), Lane 4 = carrot root extract (0), Lane 5= carrot root extract (25 g/100ml), Lane 6= carrot root extract (50 g/100ml)

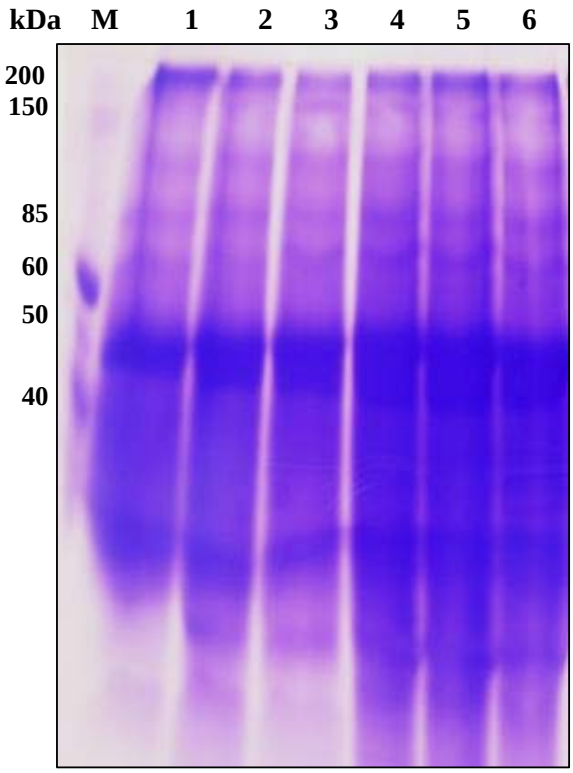

Fig. 6. Electrophoretic banding profiles of protein extracted from the leaves of cowpea seedlings germinated under salt stress conditions.
M: Marker protein
NaCl (0 mM): Lane 1 = 0 (control), Lane 2= carrot root extract (25 g/100ml), Lane 3= carrot root extract (50 g/100ml)
NaCl (100 mM): Lane 4 = carrot root extract (0), Lane 5= carrot root extract (25 g/100ml), Lane 6= carrot root extract (50 g/100ml)

Esterase (EST) ısoenzyme: Esterase electrophoretic patterns illustrated that there are four bands were appeared in all treatments. Bands No.1 and 2 were present in all treatments (common bands). The other 2 bands were present in some treatments and absent in the others (polymorphic). The application of carrot roots extract (25 g/100ml) increases Esterase (EST) activity as compared with those of corresponding control. In addition, treating the seeds with carrot roots extract alone or in combination with salinity (100 mM NaCl) increased the activity of esterase isoenzyme.

Peroxidase activity (POX): Expression of the peroxidase isoenzyme demonstrated that the total number bands were increased not only under salted stressed conditions, but also due to application of carrot roots extract (25 g/100ml). Band No.2 was present in all treatments (common band) and the other three bands were present in some treatments and absent in the others (polymorphic bands). Moreover, band No. 1 was a unique band, which characterizes the seedling grown under salinity condition alone (100 mM NaCl). Peroxidase in cowpea leaves was increased because of treating the seeds with carrot roots extract particularly by using the lower concentration (25 g/100ml) as compared with that of the control.

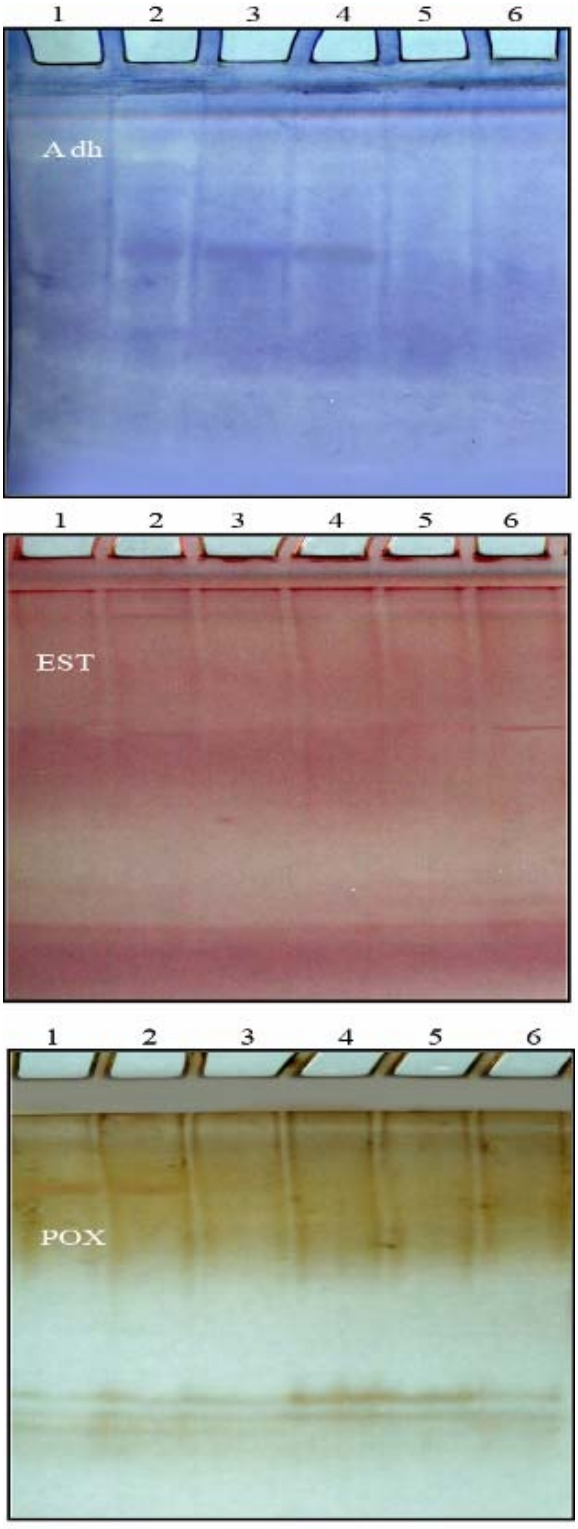

Fig. 7. Zymogram of three enzymes, Alcohol dehydrogenase (Adh), Esterase (EST) and peroxidase activities of the leaves of cowpea seedlings germinated under salt stress conditions.
NaCl (0 mM): Lane 1 = 0 (control), Lane 2= carrot root extract (25 g/100ml), Lane 3= carrot root extract (50 g/100ml)
NaCl (100 mM): Lane 4 = carrot root extract (0), Lane 5= carrot root extract (25 g/100ml), Lane 6= carrot root extract (50 g/100ml)

Table 6. Number and types of bands as well as the percentage of the total polymorphism generated by three enzymes alcohol dehydrogenase (Adh), esterase (EST) and peroxidase (Px).

Isozymes	Monomorphic Bands	Polymorphic		Total bands	Polymorphic %
		Unique	Non Unique		
Adh	-	-	2	2	100%
EST	2	-	2	4	50%
Px	1	1	2	4	75%

Discussion

Chemical analysis of carrot roots extract shows that, carrot roots extract has a high content of vitamin A as beta carotene, other vitamins, hormones, amino acids, proline, sugar, protein, carbohydrates, macro-micronutrients, phenol compounds, fat and fibers (Alasalvar *et al*., 2001). Thus, it is clear that all carrot roots extract components have defense systems are very important for the determination of plant salt tolerance and influence plant growth by regulating many physiological processes (Sairam *et al*., 2005).

Growth parameters: Many efforts have addressed growth-regulating compounds and nutrient mixes to be good for inducing the growth with low cost natural extracts. Optimum plant growth and development is important for greater final dry matter and yields. In order to achieve this, sufficient amounts of nutrients should be applied through organic and inorganic sources. The extracts from many different kinds of plant materials promote the growth of a certain tissue such as carrot roots, that may be these extracts contain common growth promoting substances involved in the mechanism of induction of growth of plants (Puchooa & Ramburn, 2004).

Abiotic stresses such as drought, salinity, and temperature extremes can reduce the yield of major crops and limit agricultural production worldwide (Zia & Khan 2002; Zahir & Farrukh, 2012) . Salinity induced reduction in overall growth might be a sign of endogenous hormonal imbalance (Iqbal & Ashraf, 2010). Our obtained results showed that carrot roots extract have a stimulatory action on the growth of cowpea seedlings. These results are in agreement with that obtained by Doco *et al*., (1997) who reported that the extract of carrot has promoting activity on the shoot and root growth of the seedlings. The increasing in roots length of the seedlings treated with carrot extract may be due to the presence of the phytohormone, auxin substances in the carrot extract, which play a key role in the initiation and emergence of lateral roots as well as regulating lateral root development. Similar results obtained by Kashyap *et al*., (2001) who found that the acid extract of carrot juice waste exhibited significant promoting effects on the root growth of Chinese cabbage. Moreover, Puchooa & Ramburn (2004). Found an increasing in fresh weight, dry weight and moisture content of the explants of *Daucus carota* with increasing the carrot juice concentration in the medium. Adnan *et al*., (2012) reported that all the seed priming treatments with extract of Mungbean stimulated the seedlings vigour in terms of germination and vigour index. Hassanein *et al*., (2009) mentioned that the using of α-Tocopherol or nicotinamide may act as growth stimulants and can play an important role in mitigating the adverse effect of salt on metabolic activities relevant to growth through increasing IAA content, these vitamins already present in carrot roots extract. The carotenoids, which present in carrot root extract modify the synthesis of essential germination promoters such as gibberellins. Riboflavin B2 as coenzymes and probably play important roles in the biochemical processes and antioxidant defense system (Athar *et al*., 2009).

Photosynthetic pigments: Decreasing the amount of photosynthetic pigments is one of the effects of salt stress in plants and has been reported in many crop species, the decreasing in chlorophylls in salinized plants could be attributed to increased activity of the chlorophyll-degrading enzyme, chlorophyllase and the decrease in carotenoids under salt stress leads to degradation of b-carotene. Carotenoid pigments protect chlorophylls from photo-oxidative destruction. The increasing in chlorophyll content observed in our study due to treating the stressed seedlings by carrot roots extract was a result of reduction in chlorophyll degradation, since this carrot extract contain different levels of minerals which improve plant mineral uptake by the roots and enhancing leaf chlorophyll content of the seedlings treated with carrot extract (Mabey, 1997). Photosynthesis is a process that is sensitive to environmental factors such as macro and micronutrients, nutrients such as N, P, K, Mg, Fe and Cu, which are readily available in carrot roots extract, are used in the formation of chlorophyll (Fang *et al*., 1995). The amount of all photosynthetic pigments was significantly enhanced when plants were treated with carrot extract, it was shown that ascorbic acid (one of components in carrot roots extract), is very important for the regulation of photosynthesis, and due to its antioxidants properties (Athar *et al*., 2008). Carotenoids, which as major components in carrot roots extract, are essential in photosynthesis where they function as energy carriers and photo-oxidation protectors because carotenoids are free radical scavengers. The effect of the carrot and its extracts can be due to many interacting factors which have chemical nature capable to increase pigments content (Branco, 2001). Also, have a role in activation of enzymes that regulate photosynthetic carbon reduction and protect chloroplast from oxidative damage. In all photosynthetic organisms, the carotenoids b-carotene and zeaxanthin and tocopherols (vitamin E) and vitamin B serve an important photoprotective role, by scavenging reactive oxygen species.

Total carbohydrates: Plants adopt several mechanisms to achieve tolerance by activating and/or accumulating several detoxification and protective molecules and compatible osmolytes as carbohydrates (Baranska *et al*., 2005). Carrot roots extract contains auxins and cytokinins which have beneficial effect on carbohydrates accumulation (Jim *et al*., 2005). The significantly higher levels of carbohydrates were observed in sunflower plants treated with α-tocopherol or nicotinamide, vitamins play a key role in alleviating salinity stress either via osmotic adjustment or by conferring desiccation resistance to plant cells (Hassanein *et al*., 2009). Application of carrot extract to the medium of *Daucus carota* grown in vitro might caused an increase in the level of a number of substances such as carbohydrates (Souci *et al*., 1994). Also phosphorous, is a component in carrot roots extract, plays a role in increasing water-use efficiency, improves leaf expansion, improved photosynthetic surface area and carbohydrate utilization (Wittenmayer & Merbach 2005). In addition micronutrients such as Fe, Cu, Zn, and Mn (which to be present in carrot roots extract) assist in the formation of chlorophyll, cell division and growth, carbohydrate formation, as well as the maintenance of the plant's enzyme system.

Anthocyanine, ascorbic acid, total phenols and flavonoids contents: The ability of higher plants to overcome the toxic effects of active oxygen seems to be very important for their tolerance to these stresses. Antioxidants are the first line of defense against free radical damage. There are several compounds which contribute to the antioxidative properties, these include anthocyanins, polyphenols, vitamin C, flavonoids and carotene (Longo & Vasapollo, 2006). Under salt stress, the level of non-enzymatic antioxidant was increased, due to their capacity to protect itself against oxidative stress. Carrots extract contain bioactive compounds, such as phenols, carotenoids, and anthocyanins, which in plants, scavenging of free radicals (Chukwu *et al*., 2012). Flavonoids are known to be synthesized as a response to different environmental stimuli; their ability to act as antioxidants depends on the reduction potentials of their radicals and accessibility of the radicals (Asami *et al*., 2003). It has been found that there is considerable increase in flavonoid levels following abiotic and biotic stresses. Ascorbic acid (vitamin C) is the most abundant, powerful and water soluble antioxidant acts to prevent or in decreasing the damage caused by reactive oxygen species in plants, it can directly scavenge superoxide, hydroxyl radicals and singlet oxygen and reduce H_2O_2 to water via ascorbate peroxidase reaction (Athar *et al*., 2008). Ascorbic acid is a key non-enzymatic antioxidant that participates in redox regulation in different cell compartments to protect plant cells from oxidative stress. In addition, it was shown that ascorbic acid is very important for the regulation of photosynthesis (Athar *et al*., 2008). It has been implicated in the regulation of the cell division and cell elongation. It is also involved in hydroxylation of proline, regulation of the cell cycle and numerous fundamental processes of plant growth and development (Noctor & Foyer, 1998). Ascorbic acid reduces O_2 and regenerates α-tocopherol, it also increases carbohydrates and total nitrogen percent (Athar *et al*., 2008). Polyphenolic compounds in carrot roots extract are the most important classes of secondary metabolites, which play an important role in the biosynthesis process of non-enzymatic antioxidants in the shoots under salt stress and having their stimulative role in plant growth and development due to their antioxidant capacity. These phenolics are including anthocyanine and flavonoids which are response to various environmental stresses (Waseem *et al*., 2006). Plant phenols are used for rising plant resistance to undesirable effects of biotic and abiotic stresses and regulate of plant physiological stages. Their increases might be due to the increase in their biosynthesis under salt stress condition. Inclusion of carrot juice in the medium of *Daucus carota* grown *In vitro* might have also caused an increase in the level of vitamins and antioxidants, which all known to be present in carrot juice (Souci *et al*., 1994).

SDS-PAGE electrophoretic patterns of proteins: New proteins are synthesized in response to interaction between environmental stress and growth substances in carrot roots extract have been reported as stress protein in plants, which suggested to protect the cell against the adverse effect of salt stress. Moreover, increase in number of protein bands in the leaves of the seedlings treated with carrot roots extract and germinate under unstressed or stressed conditions of salinity has to be preceded by activation to transcribing nuclear DNA to RNA-dependent RNA polymerase. The results revealed that salinity and/or growth regulators (in carrot roots extract) altered the protein synthesis patterns, and this might due to the presence of several osmoresponsive genes, which may be involved in adaptation to salinity (Puchooa & Ramburn 2004). The protein band with molecular weight 38.93 kDa was *de novo* synthesized in salinized cowpea seedlings, it has been suggested that this protein band was salt inducible and could be involved in plant adaptation for growth under stress condition and has an osmoprotection function (Parida *et al*., 2005). Under salt stress, the accumulation of the specific proteins may reflect the physiological reactions to a combination of ions in salt tolerance, osmotic adjustment, Na^+/K^+ homoeostasis (Parida *et al*., 2005). Protein profile of cowpea seedlings shoots indicate that carrot roots extract components (nutrients such as N, P, proteins, antioxidants, organic acids and growth regulators) may regulate the expression of salt-stress inducible proteins as well as induced de novo synthesis of specific polypeptides, which are anticipated to play an important role in salt resistance (Puchooa & Ramburn 2004**).** Application of carrot juice in the medium of *Daucus carota* grown in vitro might have also caused an increase in the level of a number of substances such as, amino acids, purines and pyrimidines, all known to be present in carrot roots extract (Souci *et al*., 1994).

Isoenzymes expression: The increase in alcohol dehydrogenase and peroxidase activities, which was observed in this study, could reflect a similar process of oxidative stress with the implication of peroxidase activity as part of the antioxidant response against H_2O_2

(El-Baz *et al*., 2003). It was found that osmotic stress increased the peroxidase activity in sorghum seedlings under osmotic stress (Vardhini & Rao, 2003). El-Baz *et al*., (2003) found that the profile of peroxidase isoenzyme was modified throughout salinity conditions; this may be due to its ability to tolerate salt stress or due to the effect of salt stress which may cause some shift in gene expression. In many plants, correlation between the increase in peroxidase expression and the resistance to stress condition has been reported.

The obtained results about the changes in esterase isozyme are similar to Mohamed (2005) who found that under salt stress conditions, 150 mM NaCl caused increasing in esterase isozyme bands in shoots of maize plants similar pattern was observed in roots. Esterase isozyme patterns in higher plants differ between distinct physiological stages. Appearance or disappearance of isoenzymes bands marks represents the increase in isoenzymes activities and change in their expression. Finally, the obtained results indicated that treatment with carrot roots extract alleviate the retarding effects of salt stress on cowpea seedlings by increasing levels of enzymatic antioxidants.

Conclusions and future perspective: The results indicated that nutritional variable components of carrot roots extract and the beneficial effects of carrot roots extract against abiotic stress could partly elicited by bioactive contents. Therefore, research into developing sustainable methods to alleviate a biotic stresses. Thus, these studies have shown that carrot extracts can be protecting plants against these stresses and offers potential for field application. Thus, this work recommended that application by carrot roots extract as presoaking was effective in overcoming salt stress on cowpea seedlings.

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(Received for publication 28 March 2012)