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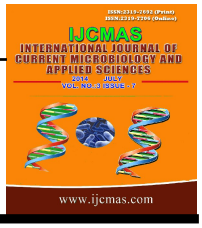

Original Research Article

Effect of the combined treatment with sodium and calcium chlorides on the growth and medicinal compounds of *Cichorium intybus*

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Keywords

Cichorium intybus, medicinal plants, alkaloids, saponin, phenolic compounds, salinity, calcium chloride

A B S T R A C T

Growth criteria, photosynthetic pigments and secondary metabolites of *Cichorium intybus* were studied under salinity by NaCl (0 - 150mM) and $CaCl_2$ (0 – 20mM) as single treatments or combined with each other through the different growth stages. The plant growth as indicated by shoot dry weight was inhibited by NaCl salinity over 50mM due to the marked inhibition in plant pigments by salinity. Plant pigments on the contrary increased by $CaCl_2$ which ameliorated NaCl inhibition to pigments when combined with NaCl treatments, especially carotenoids. The highest content of alkaloids, the major secondary metabolite, was obtained at flowering stage, but of saponin and phenolic compounds it was at the vegetative stage. Alkaloids and saponin contents increased in response to single application of NaCl or $CaCl_2$ treatments but their combinations inhibited alkaloids accumulation although 10mM $CaCl_2$ with 50 mM NaCl led to the highest alkaloids content. Phenolic compounds accumulated due to NaCl concentration over 100 mM. $CaCl_2$ singly or combined with NaCl enhanced significantly shoot phenolic compounds content. This study suggests addition of $CaCl_2$ for cultivated *Cichorium* plant in the slightly saline newly reclaimed area as $CaCl_2$ not only counteract salinity stress inhibitory effect for the plant growth but also induce metabolism of the plant important secondary metabolites those give the plant its medicinal importance.

Introduction

Cichorium intybus (Chicory) is a summer, tap rooted perennial herb of Asteraceae. *C. intybus* plants grow naturally in the Bersim (*Trifolium alexandrinum*) as a natural weed. It is harvested and eaten by animals with Bersim but there is no study for its nutritive value. *C. intybus* was reported to have many medical uses as: Anti oxidative as the water extract of *C. intybus* showed a remarkable anti oxidative effect on LDL (low density lipoproteins), and inhibitory effects on the production of thiobarbituric acid reactive substance and the degradation of fatty acids in LDL (Kim and Yang, 2001). Vitamin E and unsaturated fatty acids in LDL were protected by adding water extract of *C. intybus* from the effects of metal catalyzed LDL oxidation (Kim and Yang, 2001). Also, *C. intybus* derived beta (2-1) fructans, have been shown to exert cancer protective effects in animal models (Hughes and Rowland, 2001). The two plant-fructans,

oligofructose and long chain inulin, exerts protective effects at early stage in the onset of cancer (Hughes and Rowland, 2001). The latex in the stems is applied to warts in order to destroy them (Duke and Ayensu, 1985). The root, boiled in water is said to help in decreasing cancer of the breast and face (Hartwell, 1967). The plant root and root callus extracts of *C. intybus* were compared for their anti-hepatotoxic effects in Wister strain of Albino rats against carbon tetrachloride induced hepatic damage. The observed increased levels of serum enzymes (aspartate transaminase, alanine transaminase) and bilirubin in rats treated with carbon tetrachloride were very much reduced in the animals treated with both plant root and root callus extracts. Also, the decreased levels of albumin and proteins after treatment with carbon tetrachloride were changed into an increase in the rats treated with plant root and root callus extracts (Zafar and Mujahid, 1998).

C. intybus extract has antibacterial action against some bacterial species as *Staphylococcus aureus*, *Pseudomonas aeruginosa*,*Escherichia coli*, and *Candida albicans* (Shaikh *et al*., 2012). The root and the leaves of *Cichorium intybus* are appetizer, cholagogue, depurative, digestive, diuretic, hypoglycemic, laxative and tonic (Foster and Duke, 1990). The root extract has proved to be of benefit in the treatment of jaundice, liver enlargement, gout and rheumatism. The extract of freshly harvested plant is used for treating grave (Grieve, 1984).

The medical importance of *C. intybus* lies mainly in the photosynthetic pigments (chlorophyll a, b and carotenoids) and the secondary metabolites alkaloids, phenolic compounds and saponin of its edible parts. The content of these important metabolites evaluates its economic utilization as a complementary food and a source of many medicinal compounds. The phytochemical analysis of plant extracts indicated the presence of the major phyto-compounds, including phenolic compounds, alkaloids, glycosides, flavonoids, and tannins. The phenolic concentrations in the plant ranged from 28 to 170 mg/g of dry plant extract and there is a fair correlation between antioxidant/free radical scavenging activity and its phenolic content. These compounds in addition have an osmoregulatory role and their accumulation is considered as an adaptation mechanism to the imposed stresses as salinity (Elhaak and Wegmann, 1997 and Elhaak and Migahid, 1999).

Saponin have various effects attributed to a diverse range of properties, some of which induce both beneficial and detrimental effects on human health such as pesticides, insecticidal and molluscicidal activity, allelopathic action, anti-nutritional effects, sweetness and bitterness, and as phyto-protectants those defend plants against attack by microbes and herbivores (Tschesche, 1971; Hostettmann and Marston, 1995). Also, saponin is useful medically in controlling cholesterol, decreasing incidence of heart diseases and protection from cancer (Oakenfull and Sidhu, 1990 and Also, saponin is useful medically in controlling cholesterol, decreasing incidence of heart diseases and protection from cancer (Oakenfull and Sidhu, 1990).

Salinity stress affects the phenolic compounds content in the plant, as it induces disturbances of the metabolic processes leading to an increase in phenolic compounds (Dhingra and Varghese, 1985; Ayaz *et al*., 2000). Elhaak and Migahid, (1999) and Ali and Abbas (2003) found NaCl salinity results in the accumulation of total phenolic compounds and flavonoids in barley seedlings.

The aim of this study is to determine the physiological response of *C. intybus* to salinity stress and its effect on the medicinal compounds and the remediation role of $CaCl_2$ to salinity effects.

Materials and Methods

The seeds of *Cichorium intybus* were obtained from weed research center, Sakha, Kafr El-Sheekh, Egypt and surface sterilized with 5% Clorox for 8 minutes followed by rinsing many times in distilled water. Seeds were germinated in plastic pots of 18cm diameter and 25 cm depth, each pot was filled with quartz sand previously washed several times with tap water then with concentrated HCl and finally with distilled water. A number of 10 seeds were sown in each pot and pots were irrigated with tap water and allowed to germinate for 7 days at the greenhouse conditions. Pots were irrigated for 17 days with one-quarter strength Hogland's solution whenever needed. After that the prepared plant pots were arranged into three groups, the first group was treated with NaCl concentrations (50, 100 and 150 mM), the second group of pots was treated with the $CaCl_2$ concentrations (5, 10, 15 and 20 mM), while the third group of pots was treated with the different combinations of NaCl and $CaCl_2$, treatments.

Control pots were treated with distilled water in addition to one-quarter strength Hogland's solution. Each treatment was replicated three times, for a total of 60 pots. The plants were irrigated with the previous treatment solutions day after day, some times with distilled water to adjust salt concentration, until the end of the plant growth season. The excess solution was drained through a whole at the bottom of each pot. The pots were arranged according to the split plot design and left to grow under the natural conditions in the plant garden of Botany department, Faculty of Science, Tanta University.

Plants were harvested after 21, 90, 135 and 165 days from the treatment starting for representing seedling, vegetative, flowering and fruiting stages respectively. Fresh samples were used for the determination of photosynthetic pigments content according to Metzner *et al*. (1965). The rest of plants were weighed as fresh and dried in an air-forced oven at 60^0 C. The dried materials were then grinded using an electric mixer, and powders were kept in paper bags for further analyses.

Total alkaloids in dry shoot samples were determined according to the method described by Harbone (1973). Saponin content of the plant dry shoot was estimated by the method described by Hiai *et al*. (1975), while total phenolic content was estimated using the method described by Jindal and Singh (1975).

The obtained results were statistically analyzed using two ways analysis of variance (ANOV). The correlation coefficients between the plant pigments and its content of each alkaloids, saponins and phenolic compounds were calculated. All of the statistical methods were according to the methods described by Bishop (1983).

Results and Discussion

Dry weight

The shoot system dry weight of *C. intybus* increased with the plant age under the control and other treatments (Table 1). Shoot system dry weights during all growth stages were reduced significantly by NaCl treatments and the reduction increased by increasing NaCl concentration in comparison with the control at all growth stages. The minimum shoot system dry

weight was obtained by 150 mM NaCl in which the inhibition, compared to the control, was 41, 42, 27 and 20 % at seedling, vegetative, flowering and fruiting stages, respectively showing a decrease in the inhibition percentage with the plant age due mainly to the production of salt adaptation metabolites or absorption of osmoregultory elements by time or increased root growth. All $CaCl_2$ concentrations slightly reduced the shoot system dry weight below the control value at all growth stages.

The most observed reduction was due to 5 mM $CaCl_2$ and it was by 37, 43, 37 and 30 % at each seedling, vegetative, flowering and fruiting stages, respectively. These results are in agreement with those results obtained by Del Zoppo *et al*. (1999); Reda *et al*. (2000) and Keles (2004). Combining $CaCl_2$ with NaCl led to more inhibitory effect on the shoot system dry weight at all growth stages in comparison with control or NaCl alone. The most marked decrease in shoot system dry weight was due to the combination of the highest concentration of both compounds (150 mM NaCl with 20 mM $CaCl_2$) at seedling and vegetative stages and with 150 mM NaCl plus 10 mM $CaCl_2$ at flowering and fruiting stages. With these combinations the shoot system dry weight was reduced by 46, 39, 47 and 61 % at seedling, vegetative, flowering and fruiting stages, respectively compared with the control, indicating that the combined effect of NaCl and $CaCl_2$ was more at the fruiting stage that would affect the plant yield.

The dry weights of *C. intybus* shoot decreased significantly and linearly by the increase in NaCl concentration singly or in combination with $CaCl_2$ (Fig. 1). These results are in agreement with those obtained by many authors such as Misra *et al*. (1997) and Del Zoppo *et al*. (1999). The decrease in the water content by NaCl may be resulted from the decrease in the water potential of the growth medium which makes the water unavailable for the plant. On the other hand, the decrease in the plant water content was a normal result to the reduction in its uptake caused by the inhibition in root growth.

The shoot dry weight was more affected by salinity stresses than the root dry weight. This is in agreement with the results reported by Ghanem and Salama (1995); Reda *et al*. (2000) and Elhaak *et al*. (2001). The previous authors reported that the deleterious effect of salinity on the plant growth parameters, especially the dry weight, was attributed to the decrease in osmotic potential and hence the plant water potential. The reduction in the dry weight by increasing NaCl concentration may be also due to the passive accumulation of Na^+ ion, decrease in the osmotic potential, specific ion toxicity and nutrient ion deficiency (Greenway and Munns, 1980).

Water content

The water content of *C. intybus* shoot system under different concentrations of NaCl, $CaCl_2$ and their combinations at the plant different growth stages varied significantly ($P<0.01$). The shoot system water content of the plant increased also from seedling to vegetative stage, and then it progressively decreased until it reached its minimum value at the end of plant growth season (fruiting stage).

The shoot system water content was reduced by increasing NaCl concentrations at all growth stages. The most pronounced reduction in the shoot system water content was by 150 mM NaCl, this concentration led to a decrease in the shoot system water content by 19, 26, 10 and 7 % compared to the control at seedling, vegetative, flowering and fruiting stages respectively.

$CaCl_2$, as a single treatment, increased the shoot system water content in comparison with the control at the seedling stage. The highest water content was due to 5 mM $CaCl_2$ treatment and was higher by 12 % than the control. In comparison with seedling stage, $CaCl_2$ treatments at vegetative, flowering and fruiting stages lowered the shoot system water content below the control value, the most observed decrease was due to the highest $CaCl_2$ treatment (20 mM), which lowered the water content by 11.5, 25.5 and 21.0 % of the control value at the previous stages respectively.

Most combinations of $CaCl_2$ and NaCl increased the shoot system water content of the plant in comparison with NaCl alone. The highest water content was due to the combination of 50 mM NaCl with 10 mM $CaCl_2$ at seedling stage, 50 mM NaCl with 20 mM $CaCl_2$ at vegetative stage, 100 mM NaCl with 5 mM $CaCl_2$ at flowering stage and 100 mM NaCl with 15 mM $CaCl_2$ at fruiting stage.

The statistical analysis for the variations in the root water content of *C. intybus* at the different growth stages (Table 4) in response the effect of different concentrations of NaCl, $CaCl_2$ and their fractional combination showed that they were highly significant ($P<0.01$) by NaCl at all growth stages, and by each $CaCl_2$ and $CaCl_2$+NaCl at seedling, vegetative and fruiting stages but non-significant ($P>0.05$) at flowering stage.

Photosynthetic pigments:

In the present study, the photosynthetic pigments representing the antioxidant source of *C. intybus* (chlorophyll a, b and carotenoids) increased by plant age (Table 3). At low NaCl concentration (50 mM) chlorophyll a increased at all growth stages compared with the control, the maximum increase was by 25, 14 and 2 % at the vegetative flowering and fruiting stages, respectively. Higher NaCl concentrations (100 and 150 mM) decreased chlorophyll a, the most observed decreases were at vegetative and flowering stages by 150 mM NaCl (26 and 15%) and at fruiting stages by 100 mM NaCl (33 %). The decrease in chlorophyll content under salinity stress may be due to the suppression of specific enzymes that are responsible for the synthesis of photosynthetic pigments (Murkute *et al*., 2006) and/or through direct influence on the photosynthetic apparatus (Netondo *et al*., 2004; Ehsanzadeh *et al*., 2009). Also, the reduction in the uptake of minerals (e.g. Mg counteracted by Na) needed for chlorophyll biosynthesis reduces the chlorophyll content (Sheng, *et al*. 2008). On the contrary all $CaCl_2$ treatments stimulated increases in chlorophyll a at all growth stages, the marked ones was due to 20, 5 and 10 mM $CaCl_2$ treatments at vegetative, flowering and fruiting stages (24, 28 and 21 %, respectively). In soybean Ca^{2+} in the nutrient solution has produced a positive effect on the chlorophyll and carotenoids content (Milivojevic and Stojanovic, 2003).

The combination of $CaCl_2$ with 50 mM NaCl had less or without effect, while with higher concentrations it increased chlorophyll a content in comparison with NaCl alone. In most cases, especially at the flowering stage, these combinations increased chlorophyll a over the control value. During the growth period, most effective concentrations of $CaCl_2$ were between 5-10 mM and over that, the stimulation was mostly diminished. This result is in an agreement with the results of Abdul Jaleel and Azooz (2009) who stated that the combination of NaCl with $CaCl_2$ resulted in the increase of chlorophyll and

carotenoids content of *Withania somnifera*. The content of chlorophyll b increased by the plant age; however, the highest chlorophyll b content was recorded at the fruiting stage under both control and all salinity treatments (Table 3). Chlorophyll b content under 50 mM NaCl increased by 34, 5 and 9 % at vegetative, flowering and fruiting stages, respectively compared with the control. However, higher NaCl treatments resulted in a slight increase in chlorophyll b content.

Treatment with $CaCl_2$ remarkably increased chlorophyll b content at all growth stages. The most pronounced increase was due to 20 mM $CaCl_2$ treatment at vegetative stage (39%) and to 5 mM at flowering (26%) and at fruiting (35%) stage. The combinations of $CaCl_2$ and NaCl treatments, except 50 mM NaCl decreased chlorophyll b content at the vegetative stage. Most combinations at vegetative and flowering stages increased chlorophyll b over the control value, the effective combinations were those of 10 mM $CaCl_2$ with 50 mM NaCl at the vegetative stage, 5 mM $CaCl_2$ with 150 mM NaCl at the flowering stage and 20 mM $CaCl_2$ with 50 mM NaCl at the fruiting stage.

Treatment with NaCl at low concentration (50 mM) increased carotenoids content at all growth stages compared with the control, with increases by 18.0, 2.5 and 1.0 % at vegetative, flowering and fruiting stages, respectively. Higher NaCl concentrations inhibited carotenoids synthesis. The decrease in carotenoids under salt stress leads to degradation of β-carotene and formation of zeaxanthins, which are apparently involved in protection against photo inhibition (Sharma and Hall, 1991). All $CaCl_2$ concentrations applied singly stimulated the synthesis of carotenoids at all growth stages, except 20 mM at the fruiting stage. The most observed increase in carotenoids content was due to 10 mM $CaCl_2$ treatment at the vegetative stage (20%), to 15 mM at the flowering stage (17%) and to 5 mM at the fruiting stage (34%). The combinations of NaCl and $CaCl_2$ at low concentrations mostly reduced carotenoids content, while with higher NaCl concentration, these combinations increased carotenoids content over the control value in most cases, especially at the flowering stage. The maximum content of carotenoids (1.17 mg/g f.wt) was recorded by 5 mM $CaCl_2$ with 100mMNaCl at the fruiting stage (30 % of the control).

In general, the total of photosynthetic pigments (Chloropyll a, b and carotenoids) in *C. intybus* leaves increased by 50 mM NaCl, and the higher concentrations slightly decreased it (Fig. 1). Also, all $CaCl_2$ concentrations on the opposite, appreciably increased the total of the photosynthetic pigments, the most observed increase was due to 5 mM treatment. Combing $CaCl_2$ and NaCl with their different concentrations was effective as the former ameliorated the inhibitory effect of the later especially at concentrations of 50 and 100 mM NaCl. The highest pigments content was due to the combinations of 20, 10 and 5mM $CaCl_2$ with 50, 100 and 150 mM NaCl, respectively.

Secondary metabolites (Alkaloid, Saponin and Phenolic compounds):

The pharmaceutical compounds identified and isolated from the plant belong mainly to alkaloids, saponins and phenolic group of compounds. The present results indicated that alkaloids were found in greater content in the plant shoot in comparison with saponin and phenolic compounds which could be need by such little content in plants. These secondary metabolites in *C. intybus* shoot varied at the different growth stages evaluating the need for knowing specific

period for obtaining the best yield of each. The studied treatments of sodium and calcium chlorides have various effects on the secondary metabolites of the plant on applying as single or in combination with each other.

The represented results in Table (4) showed highly significant ($P<0.01$) variations in the contents of total alkaloids, saponin and phenolic compounds due to the imposed stresses by the different concentrations of NaCl and $CaCl_2$ singly or in combination at all growth stages. The alkaloids content generally increased progressively from vegetative to flowering stage then it decreased again with the fruiting stage under all treatments. Applied NaCl treatments accelerated a progressive increase in the alkaloids content. The highest alkaloid content was acquired under the highest NaCl concentration (150 mM) where there were increases by 39, 70 and 29 % in comparison with the control at vegetative, flowering and fruiting stages, respectively. This notable alkaloids content at the flowering stage assigned it as the best stage for obtaining *C. intybus* important alkaloids under both normal and saline conditions.

The used concentrations of $CaCl_2$ up to 15 mM increased the total alkaloids content compared to the control, and above that the alkaloids content was slightly decreased but not to lower values than that of the control. The maximum increases in the alkaloids content were due to treatment by 15mM $CaCl_2$ and were by 37, 50 and 54 % of the control value at the vegetative, flowering and fruiting stages, respectively. Combinations of $CaCl_2$ with the lowest NaCl concentration (50 mM) induced higher accumulation of the total alkaloids content compared with either control or single application of NaCl or $CaCl_2$. The most pronounced accumulation of alkaloids was due to the combination of 10 mM $CaCl_2$ with 50 mM NaCl at all growth stages. The combinations of higher NaCl concentrations with $CaCl_2$ had mostly an inhibitory effect on the total alkaloids accumulation as compared with their single application or control. These results are in accordance with those obtained by Brachet and Cosson (1986), Elhaak and Wegmann (1997), William *et al*. (1998) and Elhaak and Migahid (1999). Accumulation of alkaloids was considered as an adaptation to the imposed salinity stress because they have an osmoregulatory role (Elhaak and Wegmann 1997). In other view, Rahman *et al*. (2013) considered the alkaloids accumulation as a disturbance in amino acids metabolism in the salinized callus of *Datura*. William *et al*. (1998) reported that the increase in the alkaloids content as the influence of NaCl is a combination of an osmotic effect and a specific ion effect. He added that the increase of alkaloids in response to salinity may be due to its role in the plant protection against the salt stress effects.

The saponin content of *C. intybus* shoot (Table 4) was decreased with the plant age, where it decreased to its half from vegetative to fruiting stage under the control treatment. The used NaCl concentrations had a progressive stimulatory effect on the production of saponin, where increasing NaCl concentration increased the saponin content in comparison with the control. The highest saponin content was achived by 150 mM NaCl, which increased the saponin content by 47, 10 and 15 % at vegetative, flowering and fruiting stages, respectively. Also, under this concentration the saponin content during the vegetative stage was more than double its content during both flowering and fruiting stages. This result agreed with that of Odjegba and Alokolaro (2013) who observed that drought and salinity treatments enhanced saponin

production in *Acalypha wilkesiana*. Also, De Costa *et al*. (2013) that saponin content in *Quillaja brasiliensis* leaves increased significantly when exposed to salinity. El-Sayed *et al*. (2008) also reported that saponin content in *Trubulus* increased when subjected to water stress. This increase could be related to its protective role against oxidative stress (Lin *et al*., 2009).

The application of $CaCl_2$ also resulted in an increase in the saponin content at vegetative and fruiting stages with a most pronounced increase due to 15 mM $CaCl_2$ at the former stage. On the opposite, during flowering stage $CaCl_2$ concentrations inhibited the metabolism of saponin and its content was decreased by 43% due to 20 mM $CaCl_2$ treatment.

Application of $CaCl_2$ with salinity stress inhibited the stimulating effect by NaCl on the saponin content at all growth stages. The greatest decrease was due to the combination of 20 mM $CaCl_2$ with 100 mM NaCl at vegetative and fruiting stages and the combination of 15 mM $CaCl_2$ with 150 mM NaCl at flowering stage.

Total phenolic compounds content in the shoot system of *Cichorium intybus* decreased gradually from vegetative to fruiting stage under the control or the other NaCl and $CaCl_2$ treatments (Table 4). NaCl at 50 and 100 mM had inhibited the metabolism of phenolic compounds at all growth stages, the marked decrease in the phenolic compounds content was due to 100 mM NaCl and was by 11, 14 and 12 % of the control at the vegetative, flowering and fruiting stages, respectively. Application of 150 mM NaCl on the opposite stimulated increases of phenolic compounds content by 10, 36 and 13 % at the vegetative, flowering and fruiting stages respectively. Accumulation of the phenolic compounds in plants by NaCl stress was reported by many authors as Dhingra and Varghese (1985), Ayaz *et al*. (2000) and Ali and Abbas (2003). Radi *et al*. (2013) reported that salinity stress affect the phenolic compounds content by the induced disturbance of the metabolic processes leading to an increase in phenolic compounds.

Also, single treatments of $CaCl_2$ generally increased total phenolic compounds as compared with the control at all growth stages. The marked increase in phenolic compounds content was at 15 mM $CaCl_2$ that increased the total phenolic compounds by 39, 28 and 52 % at the vegetative, flowering and fruiting stages respectively. Combination of all $CaCl_2$ with NaCl concentrations increased the plant shoot phenolic compounds content in comparison with NaCl alone or in most cases the control content. The most effective combination was that of 150 mM NaCl with 10 mM $CaCl_2$, where the total phenolic compounds increased by 86, 70 and 68 % of the control at the vegetative, flowering and fruiting stages respectively.

The mean of the alkaloids, saponin and phenolic compounds in *C. intybus* at the whole growth season (mean of the three growth stages) (Fig. 2) showed that alkaloids increased progressively in response to the different NaCl treatments until the highest value under the highest NaCl concentration (150 mM). Also, the increase in $CaCl_2$ concentration increased alkaloids until the maximum value with 15mM after which alkaloids decreased to lower value than those of the other treatments except the control. Combination of $CaCl_2$ with NaCl resulted in remarkable increases in alkaloids under the different salinity levels. The maximum values were produced by combining 10mM $CaCl_2$ with all NaCl treatments.

Table.1 Variations in shoot system dry weight (g/plant) in *C. intybus* under the effect of NaCl, $CaCl_2$ and their combination treatments at the different growth stages

CaCl2 (mM)	NaCl (mM)			
	0	50	100	150
	Seedling (leaves)			
0	0.067 ± 0.018	0.050 ± 0.007	0.045 ± 0.014	0.039 ± 0.005
5	0.042 ± 0.004	0.047 ± 0.007	0.041 ± 0.005	0.033 ± 0.005
10	0.046 ± 0.001	0.041 ± 0.008	0.037 ± 0.002	0.033 ± 0.002
15	0.058 ± 0.007	0.039 ± 0.006	0.043 ± 0.002	0.035 ± 0.002
20	0.050 ± 0.002	0.043 ± 0.000	0.036 ± 0.002	0.031 ± 0.004
	Vegetative (leaves)			
0	0.64 ± 0.06	0.59 ± 0.01	0.53 ± 0.06	0.37 ± 0.03
5	0.36 ± 0.03	0.55 ± 0.06	0.50 ± 0.08	0.34 ± 0.00
10	0.47 ± 0.05	0.52 ± 0.06	0.37 ± 0.04	0.36 ± 0.07
15	0.41 ± 0.02	0.39 ± 0.04	0.30 ± 0.05	0.37 ± 0.03
20	0.43 ± 0.03	0.42 ± 0.08	0.33 ± 0.07	0.25 ± 0.03
	Flowering (shoot)			
0	2.51 ± 0.35	2.37 ± 0.92	2.23 ± 1.2	1.83 ± 0.07
5	1.56 ± 0.54	2.23 ± 0.51	1.70 ± 0.67	1.69 ± 1.19
10	2.00 ± 0.79	1.90 ± 0.72	1.96 ± 0.52	1.19 ± 0.49
15	1.92 ± 1.50	1.76 ± 0.72	1.42 ± 0.46	1.30 ± 0.19
20	1.85 ± 0.50	1.20 ± 0.42	1.69 ± 0.68	1.44 ± 0.43
	Fruiting (shoot)			
0	2.81 ± 0.80	2.70 ± 0.41	2.63 ± 0.62	2.22 ± 0.23
5	1.96 ± 0.22	2.54 ± 0.62	2.34 ± .056	2.02 ± 0.48
10	2.48 ± 0.48	2.34 ± 0.35	2.47 ± 0.62	1.73 ± 0.42
15	2.42 ± 0.28	2.34 ± 0.41	1.98 ± 0.32	1.81 ± 0.29
20	2.34 ± 0.17	2.01 ± 0.41	2.20 ± 0.37	1.91 ± 0.62

Statistical analysis:

Stage	Treatment	F test	P value	LSD at 0.05
Seedling	NaCl	5.179	**	0.014
	$CaCl_2$	1.137	Ns	0.014
	$NaClxCaCl_2$	1.676	Ns	
Vegetative	NaCl	29.72	**	0.036
	$CaCl_2$	28.61	**	0.040
	$NaClxCaCl_2$	17.5	**	
Flowering	NaCl	7.342	**	0.499
	$CaCl_2$	1.521	Ns	0.499
	$NaClxCaCl_2$	1.759	Ns	
Fruiting	NaCl	32.75	**	0.937
	$CaCl_2$	6.425	Ns	0.937
	$NaClxCaCl_2$	18.48	Ns	

** = Highly significant (P<0.01) ns = not significant (P>0.05).

Table.2 Variations in shoot system water content of *C. intybus* (%) under the effect of NaCl, $CaCl_2$ and their combination treatments at the different growth stages

$CaCl_2$ (mM)	NaCl (mM)			
	0	50	100	150
	Seedling (leaves)			
0	1010 ± 62	972 ± 7.0	852 ± 66	818 ± 74
5	1129 ± 64	1034 ± 86	935 ± 78	840 ± 74
10	1099 ± 54	1086 ± 126	890 ± 54	893 ± 34
15	1080 ± 55	987 ± 36	852 ± 51	848 ± 47
20	1047 ± 15	994 ± 17	880 ± 55	824 ± 39
	Vegetative (leaves)			
0	1345 ± 63	1200 ± 47	1116 ± 160	992 ± 180
5	1290 ± 128	1216 ± 43	1160 ± 115	1075 ± 122
10	1255 ± 34	1218 ± 73	1123 ± 210	1090 ± 49
15	1223 ± 48	1190 ± 97	1099 ± 22	1120 ± 87
20	1190 ± 42	1289 ± 94	1141 ± 63	1081 ± 119
	Flowering (shoot)			
0	572 ± 47	546 ± 37	531 ± 25	516 ± 28
5	531 ± 27	537 ± 59	647 ± 9	543 ± 7
10	496 ± 60	604 ± 14	633 ± 54	508 ± 14
15	498 ± 12	604 ± 39	637 ± 63	532 ± 30
20	426 ± 25	638 ± 36	575 ± 34	514 ± 28
	Fruiting (shoot)			
0	549 ± 58	531 ± 10	516 ± 25	509 ± 70
5	516 ± 21	555 ± 15	550 ± 15	517 ± 29
10	487 ± 28	571 ± 22	578 ± 18	485 ± 8
15	459 ± 14	538 ± 22	589 ± 60	464 ± 6
20	433 ± 33	502 ± 58	507 ± 26	458 ± 30
	Mean			
NaCl alone	869	812	754	709
NaCl+$CaCl_2$	822	848	800	738

Statistical analysis

Stage	Treatment	F	P	LSD at0.05
Seedling	NaCl	107.0	**	54.5
	$CaCl_2$	9.5	**	54.5
	NaCl x $CaCl_2$	34.0	**	
Vegetative	NaCl	37.1	**	70.5
	$CaCl_2$	5.6	**	70.5
	NaCl x $CaCl_2$	26.9	**	
Flowering	NaCl	85.5	**	25.9
	$CaCl_2$	24.8	**	25.9
	NaCl x $CaCl_2$	31.1	**	
Fruiting	NaCl	33.7	**	24.2
	$CaCl_2$	15.6	**	24.2
	NaCl x $CaCl_2$	47.6	**	

** = Highly significant ($P<0.01$).

Table.3 The content of chlorophyll a, b and carotenoids (mg/g f. wt.) in *C. intybus* under the different concentrations of NaCl and $CaCl_2$ at the different growth stages

$CaCl_2$ (mM)	Chlorophyll a				Chlorophyll b				Carotenoids			
	0	50	100	150	0	50	100	150	0	50	100	150
	Vegetative											
0	1.1	1.38	1.06	0.81	0.41	0.55	0.42	0.3	0.55	0.7	0.54	0.41
5	1.18	1.22	1.1	0.85	0.48	0.46	0.47	0.4	0.57	0.6	0.56	0.43
10	1.29	1.41	1.13	0.94	0.49	0.55	0.43	0.34	0.66	0.65	0.57	0.49
15	1.15	1.19	1.15	1.25	0.46	0.51	0.46	0.51	0.57	0.54	0.61	0.68
20	1.37	1.3	1.14	1	0.57	0.55	0.47	0.42	0.65	0.63	0.58	0.52
	Flowering											
0	1.32	1.51	1.5	1.12	0.61	0.63	0.64	0.43	0.42	0.43	0.41	0.39
5	1.69	1.53	1.24	1.67	0.77	0.67	0.52	0.69	0.46	0.44	0.37	0.5
10	1.47	1.29	1.34	1.49	0.58	0.54	0.57	0.58	0.43	0.41	0.38	0.45
15	1.56	1.5	1.12	1.55	0.66	0.64	0.46	0.64	0.49	0.49	0.4	0.45
20	1.57	1.48	1.6	1.43	0.66	0.64	0.66	0.58	0.47	0.47	0.49	0.46
	Fruiting											
0	2.2	1.93	1.28	1.77	0.79	0.86	0.62	0.76	0.96	0.97	0.67	0.91
5	2.2	2.08	2.14	3.06	1.07	0.67	0.93	0.67	1.29	1.07	1.17	0.77
10	2.3	1.97	3.02	1.95	1	0.87	0.73	0.84	1.19	1.05	0.81	0.99
15	2.29	2.1	2.01	1.91	1.01	0.89	0.92	0.82	1.21	1.09	1.05	0.94
20	1.98	2.04	1.73	2.05	0.69	0.96	0.82	0.87	0.86	1.01	0.88	1.01

Statistical analysis

Stage		Chlorophyll a			Chlorophyll b			Carotenoids		
	Treatment	F	P	LSD	F	P	LSD	F	P	LSD
Vegetative	NaCl	182.4	**	0.05	76.88	**	0.03	1	ns	0.05
	$CaCl_2$	60.08	**	0.05	33.14	**	0.03	1	ns	0.05
	Interaction	28.11	**		15.57	**		1	ns	
Flowering	NaCl	6.68	**	0.08	6.99	**	0.05	1.72	ns	0.04
	$CaCl_2$	6.57	**	0.08	4.64	*	0.05	3.08	ns	0.04
	Interaction	8.38	**		4.79	**		1.53	ns	
Fruiting	NaCl	24.08	**	0.22	17.55	**	0.10	28.76	**	0.10
	$CaCl_2$	30.73	**	0.22	10.47	**	0.10	28.71	**	0.10
	Interaction	10.39	**		16.42	**		11.03	**	

** = Highly significant (P<0.01). * = Significant (P<0.05). ns = not significant

Table.4 The content of alkaloids, saponins and phenolic compounds (mg/g d. wt.) in *C.intybus* under the different concentrations of NaCl and CaCl2 at the different growth stages

	Alkaloids				Saponins				Phenolic compounds			
NaCl	0	50	100	150	0	50	100	150	0	50	100	150
$CaCl_2$	Vegetative stage											
0	24.72	27.55	29.89	34.40	11.60	15.50	15.80	17.10	0.79	0.71	0.70	0.87
5	29.40	32.13	33.33	31.70	13.70	14.40	14.80	15.20	0.85	0.74	1.04	1.22
10	31.24	34.31	38.36	32.50	13.00	14.80	14.50	16.60	0.83	0.84	1.07	1.47
15	33.96	34.13	25.00	23.06	14.00	15.20	14.80	14.50	1.10	0.80	0.83	1.11
20	29.46	32.00	22.60	17.93	12.00	14.80	12.20	14.20	1.06	0.84	1.12	0.96
	Flowering stage											
0	35.49	36.69	45.59	36.41	7.90	8.20	8.40	8.70	0.50	0.48	0.43	0.68
5	40.85	43.25	47.39	49.13	6.90	7.32	6.15	6.21	0.57	0.57	0.47	0.76
10	48.44	52.64	58.33	54.30	6.52	6.21	5.84	5.28	0.60	0.56	0.49	0.85
15	53.43	52.00	53.46	57.00	5.22	6.08	6.21	4.47	0.61	0.52	0.55	0.73
20	38.32	39.10	33.00	31.06	5.40	6.08	6.95	6.52	0.55	0.53	0.52	0.69
	Fruiting stage											
0	30.14	33.48	35.40	38.89	6.20	6.60	6.80	7.10	0.40	0.37	0.35	0.45
5	38.01	34.39	30.56	26.53	8.01	6.30	6.20	6.62	0.60	0.44	0.41	0.51
10	41.06	37.74	33.68	25.47	9.37	6.15	5.22	7.00	0.60	0.40	0.49	0.60
15	46.26	34.80	29.86	27.19	9.62	5.27	6.10	6.90	0.62	0.39	0.54	0.63
20	32.30	34.30	22.98	17.30	8.40	6.20	5.09	7.00	0.57	0.39	0.47	0.50

Statistical analysis:

Stage	Treatment	Alkaloids			Saponins			Phenolics		
		F	P	LSD	F	P	LSD	F	P	LSD
Vegtative	NaCl	6.08	*	0.57	193.00	*	0.50	50.10	*	0.07
	$CaCl_2$	375.00	*	0.64	35.30	*	0.56	19.80	*	0.07
	Interaction	117.00	*		27.30	*		10.20	*	
Flowering	NaCl	82.36	*	1.02	31.40	*	0.53	12.10	*	0.05
	$CaCl_2$	1070.00	*	1.02	15.80	*	0.53	14.70	*	0.05
	Interaction	218.20	*		22.50	*		29.90	*	
Fruiting	NaCl	16.95	*	3.08	552.00	*	0.26	170.00	*	0.02
	$CaCl_2$	15.60	*	3.45	180.00	*	0.29	124.00	*	0.02
	Interaction	4.23	*		117.00	*		72.10	*	

** = Highly significant (P<0.01) LSD at 0.05 level

Fig.1 The relationship between shoot weight and NaCl concentration (mM) with or without $CaCl_2$ treatments

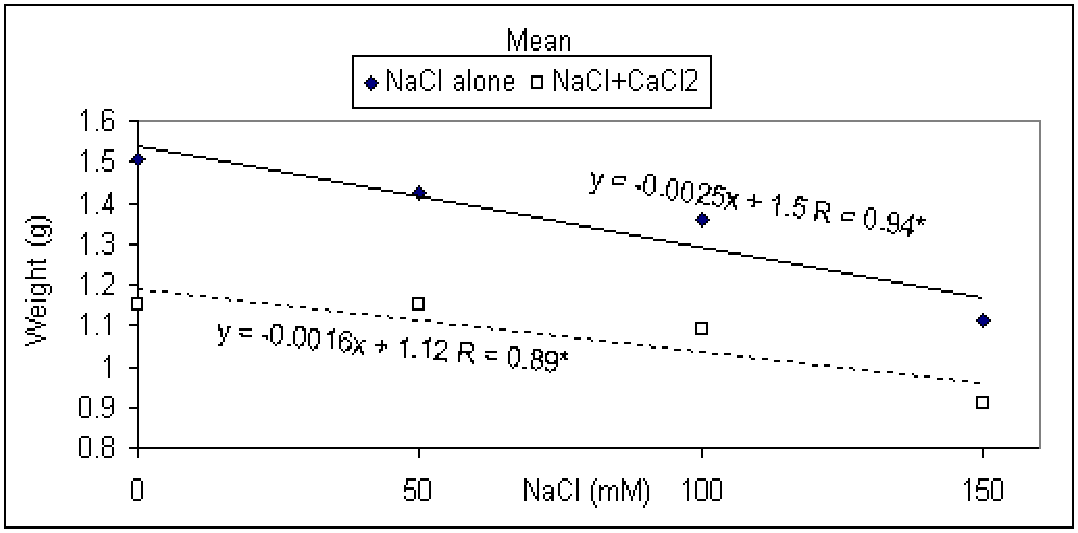

Fig.2 The total of the studied secondry metabolites in *Cichorium intybus* under different concentrations of sodium and calcium chlorides

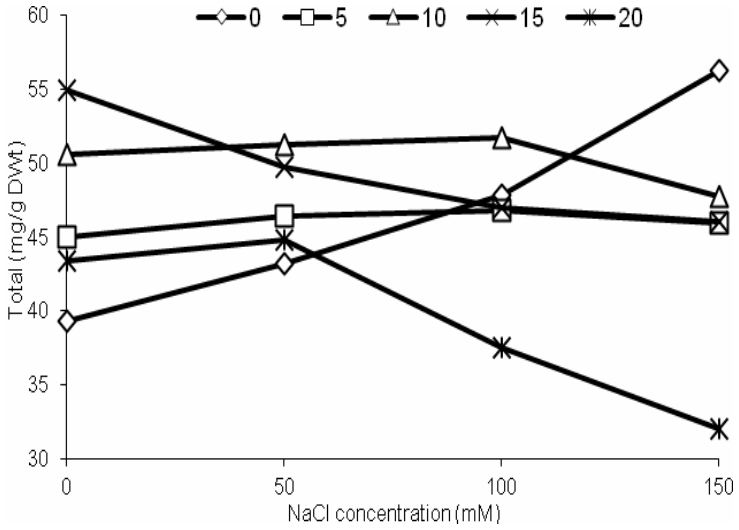

Table.5 The regression equations of the linear relationships between NaCl and $CaCl_2$ and alkaloids, phenolic compounds and saponins

Treatment	Equation	R_2 value
NaCl	Alkaloid=0.095 NaClmM+28.8	0.97*
NaCl+$CaCl_2$	Alkaloid=-0.037 NaClmM+39.0	0.95*
NaCl	Phenolic compounds= not significant	0.68
NaCl+$CaCl_2$	Phenolic compounds= not significant	0.47
NaCl	Saponin=0.015 NaClmM+8.8	0.94*
NaCl+$CaCl_2$	Saponin= not significant	0.50

The mean of saponin increased progressively by increasing NaCl concentration. The maximum saponin was greater by 25% than the control under the highest NaCl concentration (150mM). $CaCl_2$ treatments, except 5mM, increased saponin in comparison with the control. The combined application of NaCl with $CaCl_2$ decreased saponin content to lower values than those produced by their single application and in some cases lower than the control. The mean of phenolic compounds increased only after 100mM NaCl salinity treatment but it decreased lower than the control value by other lower treatments. All $CaCl_2$ treatments increased the phenolic compounds under all salinity treatments. The maximum of phenolic compounds was achieved by the combination of 150mM NaCl and 10mM $CaCl_2$ which increased it by 45% in comparison with the control value.

Relationship between NaCl and alkaloids, saponin and phenolic compounds:

Table (5) represented the linear regression equations governing the relationships between the used concentrations of NaCl, applied singly or in combination with the study $CaCl_2$ treatments, and the content of each alkaloids, saponin and phenolic compounds of the shoot system of *C. intybus*. These relationships could be used for calculating the previous compounds content in the plant shoot under any prevailing salinity stress conditions by NaCl concentration alone or with the used concentrations of $CaCl_2$. These relationships indicated significant positive correlation denoting to NaCl concentration increase will led to significant increase in the plant shoot alkaloids and saponin content. Positive correlations were observed between the NaCl levels from 50-200mM and solasodine alkaloid accumulation by Abdel Gawwad and Jasmin (2011). Significant decrease in the alkaloids occurred when $CaCl_2$ was added combined with NaCl referring to the suppressive effect of $CaCl_2$ to NaCl effect. The relationships showed also that the used salinity range was not correlated with the synthesized amounts of phenolic compounds in the plant. This is confirmed by the start of enhanced metabolism of phenolic compounds at the highest salinity level (150 mM).

The medical capacity of the plant

The total amount of the secondary metabolites alkaloids, saponin and

phenolic compounds in *C. intybus* shoot, represents the medical capacity of the plant, increased paralleling the plant growth towards the maximum value at the flowering stage which followed by a slight decrease in their content at the fruiting stage. Application of all combinations of $CaCl_2$ and NaCl increased the total of these secondary metabolites to greater content than that of the control, except the combination of 20mM $CaCl_2$ with both 100 and 150 mM NaCl. It was also clear that 50mM NaCl increased the total sum of secondary metabolites in the plant, and in combination with $CaCl_2$ the increase was higher than that occurred by NaCl individually. The total of these secondary metabolites had a greater value with the single application of 15 mM $CaCl_2$.

The medical capacity of *Cichorium intybus* as represented by the total amount of the secondary metabolites alkaloids, saponin and phenolic compounds acquired its highest capacity at the vegetative and flowering stages. Application of $CaCl_2$ was important with salinity by NaCl as it increased the total of these secondary metabolites especially when applied with low salinity levels. There was a greater value of total secondary metabolites with the single application of 15 mM$CaCl_2$. This value was slightly lower than that caused by the 150mM NaCl treatment but 15 mM $CaCl_2$ is preferable than the 150 mM NaCl in the view of salinity stress.

The study concluded that the measured secondary metabolites in *C. intybus* were induced by slight salinity levels which suggested cultivation or collection of the plant from the slightly saline fields. $CaCl_2$ proved to be important to increase the content of alkaloids and phenolic compounds in the shoot of the plant. This importance increases under salinity stress conditions as $CaCl_2$ not only counteracted the salinity stress but also enhanced the metabolism of these secondary metabolites. The vegetative stage was the best growth stage for the best crop of phenolic compounds followed by the flowering stage which was the best for alkaloids and with slightly less value for phenolic compounds. Also, alkaloids were the major components of the plant shoot.

C. intybus photosynthetic pigments (chlorophyll a, b and carotenoids) and secondary metabolites alkaloids, phenolic compounds and saponin in its edible shoot implies for the plant importance. The promising content of these secondary metabolites in the plant shoot and root evaluates its economic utilization as a complementary food as well as for their free radical scavenging activity (Karori *et al*., 2006). The plant grows naturally accompanied with *Trifolium* and other plants in the field. The plant collection from the field could not a real source of the plant in addition its cultivation in the field instead of any crop plant is not economic enough. This leads to the need of its cultivation in new areas where soil is still saline or in changed soil into saline due to wrong cultivation practices. However, studies to alleviate the effects of salinity on the plant growth and metabolism are important.

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