Effect of salt stress, application of salicylic acid and proline on seedlings growth of sweet pepper (*Capsicum annum L.*).

Ali H. Jasim **Basheer A.** **Wassan M.**
Coll.of agric. Univ. of al-qasim **Coll.of sci. Univ. of babylon**

Abstract :

Factorial experiment was conducted to study the effect of salt stress on seedlings growth of sweet pepper (*Cpsicum annuum* L.) planting individually in pots (5kg) and its interactions with exogenous application of salicylic acid and proline. Sodium chloride (NaCl) was added (in water irrigation in two concentrations (1.3 and 5 dsm/cm). Three concentrations of salicylic acid (SA) $(0,5*10^{-5},10^{-4}$ M) and four concentrations of proline (0, 1, 5, 10 mM) were sprayed exogenously on seedlings. The results showed that salt stress was negatively affect wet weight, leaves number; leaves surface area and shoot length coincided with free proline accumulation both in shoots and roots.Exogenous application of SA resulted in maintaining almost growth parameters of the plant. Wherease, supplied prolin caused decrease in almost growth parameters of the plant. The interaction of SA 10^{-4} M+ 5 mM proline was the best in maintaining almost growth parameters of stress the plants.

تأثير الاجهاد الملحي ، رش حامض الساليسلك والبرولين في نمو شتلات الفلفل الحلو(*Cpsicum annuum* L.)

علي حسين جاسم **بشيرعبد الحمزة العلواني** **وسن مضر ابو التمن**

الخلاصة :

اجريت تجربة عاملية لدراسة تاثير الاجهاد الملحي في نمو شتلات الفلفل الحلو المفردة في اصص (5 كغم تربة) وتداخلاتها مع رش النباتات بحامض الساليسيلك والبرولين. استعمل كلوريد الصوديوم مع ماء الري بتركيز 1.3 و 5 ديسيسمنز/ سم . واستخدمت ثلاث مستويات من حامض الساليسلك (0 , $5*10^{-5}$, 10^{-4} مول) واربع مستويات من البرولين (0 , 1 , 5 , 10 ملي مول) للرش على الشتلات . اظهرت النتائج ان الاجهاد الملحي اثر سلبا في الوزن الرطب ، عدد الاوراق ، المساحة السطحية للاوراق وطول الساق مع تجمع للبرولين الحر في كلا المجموعين الجذري والخضري للنبات . الاضافة الخارجية لحامض الساليسيلك ادت الى المحافظة على اغلب مؤشرات النمو للنبات . بينما الاضافة الخارجية للبرولين سببت بشكل عام تقليل في مؤشرات النمو للنبات . التداخل بين الساليسلك 10^{-4} مول + البرولين 5 ملي مول كانت الافضل في المحافظة تقريبا على مؤشرات النمو للنبات المعرضة للاجهاد.

البحث مستل من أطروحة دكتوراه للباحث الثالث

ISSN 2072-3875

Introduction :

Soil salinity is the most common problem in the arid and semi arid regions where the rains are rarely, and in irrigated and un-irrigated regions (Pessarakli, 1999), so it's significantly affect the physiological processes of the plant. It causes deficiency in surface leaf area, dry weight, chlorophyll content, stomata conductance and photosynthesis average (Shah, 2007).

Sweet pepper (*Capsicum annum* L.) is the most widely vegetable grown in all over the world, it is susceptible and cannot survive under high soil salinity levels. It causes deficiency in chlorophyll content, proline accumulation, increase antioxidant enzyme activity such as catalase both in roots and leaves (Chookhampaeng, 2011). In addition, it causes a deficiency in soot and root length, dry weight and surface leaf area (Ziaf *et al*, 2009).

To alleviate this problem, many studies were conducted with the aim of alleviating or decreasing the inhibitory effect of salt stress on plant growth and increase plant tolerance.(Noreen and Ashraf , 2008 ;Houimli *et al*, 2008).

Salicylic acid is one of phenol derivatives which previously isolated from willow bark(John Buchner,1928).Salicylic acid is classified under the group of plant hormones and is assigned divers regulatory roles in plant growth and increase plant tolerance to salt stress (Hayat and Ahmed, 2007; Amin *et al*, 2009 ; Shahba *et al*, 2010). It acts on increasing the activity of antioxidant enzymes such as catalase, super oxide dismutase, and peroxidase (Arfan, 2009; Erdal *etal,* 2011; Chookhampaeng, 2011). Furthermore, increase in growth due to exogenously application of SA may be attributed to the changes in photosynthesis (Noreen and Ashraf, 2008).

Proline is one of nonessential amino acids found in plants, and one of the most important dominant compound produced in response to salt stress (Marin *et al*, 2010). Its main role is probably to protect plant cells against negative effect of salt by maintaining the osmotic balance, stabilizing sub cellular structure such as proteins and membranes in addition to scavenging ROS (Ashraf, and Foolad, 2007). Exogenous application of proline considered as an important agent to maintain osmotic potential of the plant cell (Ali, et al, 2007) and it considered as an antioxidant agent through its role in increasing the ability of plant to tolerate salt stress (Okuma et al, 2004).

The aim of this research is to alleviate harmful effect of salt stress on sweet pepper by individually and simultaneous application of S.A and proline.

Materials and methods:-

This experiment was conducted under saran canopy at the Department of Biology, collage of science Babylon University. Sweet pepper (*Capsicum annuum* L.) seedlings of 45 days old were obtained from gbela/babylon .The original seeds were irrigated with water of (1.3 dsm/cm). The seedlings were planted in plastic pots containing 5 kg of soil

ISSN 2072-3875

(six pots for each treatment).Each one supplied with 0.5 gm of NPK and granular fungicide. Seedlings were irrigated with tap water (1.3 dsm/cm) for ten days twice a day before salinity treatment, followed by irrigation (half of seedlings) with salted water (5 dsm/cm) every day until seedlings were reaching 75 days old.

Plants were sprayed twice with different concentrations of S.A($0,5*10^{-5},10^{-4}$) M and proline (0,1,5,10) mM. The first treatment occurred when the plants were 60 days old and the second treatment after a week of the first one. The interaction between S.A and proline was applied by spraying seedlings with proline in the concentrations mentioned above after two days of S.A application. Shoot length, node length, fresh weight and leaves number were measured. Leaf surface area was measured by planimiter.

Proline colorimetric determination preceded according to (Bates *et al*., 1973, Marín *et al*., 2010) based on proline's reaction with ninhydrin ratio of 1:1:1 solution of proline, ninhydrin acid and glacial acetic acid was incubated at 100ºC for 1 hour. The reaction was arrested in an iced bath and the chromophore was extracted with 1 ml toluene and its absorbance at 520 nm was determined spectrophotometerically .0.1 gm of shoot and root tissues was suspended with 1 ml of 3% sulfosalicylic acid and after centrifugation (10 min at 12,000 rpm) was mixed in a 1:1:1 ratio with ninhydrin acid and glacial acetic acid. The reaction and determination of proline were carried out similarly to that described above The concentration of proline in tissues were determined depending on standard curve of pure proline.

This factorial experiment included three factors (24 treatments) . Each pot was treated as one replicate and all the treatments were repeated three times. The data were analyzed statistically with SPSS-17 statistical software. Means were statistically compared by L.S.D test at $p<5\%$ level.

Results and discussion

Table (1) explains the effect of salt, S.A, proline and the interaction between them on the wet weight of sweet pepper plants. It showed that plants which exposed to salt stress resulted in a decrease of wet weight. It was related to decrease of leaves number ,leaf surface area and shoot length (Table 1,2,3) in contrast to un- stressed plants, or, it may be related to decrease in water availability in pepper plants exposed to salt treatment.(Houimli *et al*,2008) suggested that the decline in plant growth was related to the decrease in water availability. The same result was concluded by (Hirpara *et al*.2005) in *Butea monosperma*. Or due to reduce the uptake of essential elements such as K and N (Mgbeze and Omodamwen, 2011) or may be affect on photosynthetic rate. These conclusions were compatible with (Desingh and Kanagaraj, 2007). They conclude that photosynthetic rate and activities of RuBP carboxylase and sucrose phosphate synthase decreased with increasing salinity level;(Bethke and Drew,1992) showing that high salinity levels had up to 85% inhibited photosynthesis. In addition, spraying of stressed plants with S.A in both tested concentrations maintains wet weight of plants. These

ISSN 2072-3875

results were compatible with other researchers such as (Canakci,2011; Erdal *et al*, 2011) who demonstrated that low SA concentration has an inhibitory effect on salinity than high concentrations or it could be related to the ability of SA to decrease membrane deterioration and inhibited Na^+ accumulation and increase K^+, Ca^+ and Mg^+ content of stressed plants(Ben Ahmed *et al*,2009).Alternatively, it may be related to the role of SA to mitigate the damage of leaves and roots of pepper plant through its effect on sugar accumulation (Amin *et al*,2009),or it could be related to the effect of SA on diminution transpiration rate in maize (Noreen and Ashraf,2008) and in sunflower (Tuna *et al*,*2007*).

The negative effect was seen when the plants sprayed with proline in all concentrations, it coincided with the decrease in leaves numbers and leaf surface area (Table 2, 3consiquently, wet weight was obviously decreased. Proline effect was negative in un- stressed plants but it maintains the wet weight in stressed plants. These results leads to thought that the central effect of proline emerge under stressed conditions. This results were agreed with (Karima *et al*,2005;Ali *et al*,2007;Gerdakaneh *et al*,2010;Nawaz *et al*,2010).It may be related to the role of proline as osmoprotectant compound (Ali *et al*,2008) by its maintenance of water equilibrium (Gerdakaneh *et al*,2010),or act as free radical scavenger (Yan *et al*,2011).

However,(Table 1) showed that the interaction between S.A 10^{-4} M + proline 5, 10 mM was the best in maintaining plant wet weight. These results may be attributed to the inhibitory effect of S.Aon the deleterious effect of proline on plant growth through its effect in increasing water content of the leaves and ultimately decreasing proline content (Table 6).Moreover, it is compatible with the finding of (Shahba *et al*,2010). In addition, the same table elucidates the triple interaction between salt, S.A and proline. It explained that there were no significant effects of S.A treatment on stressed and un-stressed plants.

Spraying un-stressed plants with S.A and proline caused decrease in wet weight, meanwhile this treatment lead to increase plant tolerance in stressful plants, especially the treatment with S.A 10^{-4} M + proline 5mM when compared to stressful plants but without supplying SA .This concentration of proline may had a synergistic effect with SA in improving plant wet weight. This may be depend on proline content of the cells (Table 6) and the activity of antioxidant enzymes (Tuna *et al*,2007;Bin Ahmed *et al*,2009) or preventing membrane damage(Yan *et al*,2011).

Table (2) demonstrated that irrigation of plants with salted water caused a significant decrease in leaves number. This results was compatible with the findings of (Hirpara *et al*,2005; Ziaf *et al*,2009).On the other hand the plants which treated with S.A and proline showed a significant decrease in leaves number ,it may be referred to the ability of these compounds to enhance the acclimation of plants to stress by reducing leaves number and then reducing water loss by transpiration(Tari *et al*,2002). The interaction between salt and SA showed no significant effect of un- stressed plants, whereas, there were a decrease in leaves number in stressful plants.

ISSN 2072-3875

Table(1):The effect of salt , S.A , proline and its interactions on wet weight (gm)

Salt concentration Dsm/cm	S.A concentration M	Proline concentration mM				Mean of salt* S.A
		0	1	5	10	
1.3	0	16.835	12.303	9.953	5.510	11.1504
	10^{-4}	15.653	10.557	11.700	11.827	12.4342
	$5*10^{-5}$	18.568	6.690	9.437	10.600	11.3237
5	0	6.990	7.110	7.227	9.413	7.6850
	10^{-4}	4.790	7.803	10.500	8.750	7.9608
	$5*10^{-5}$	8.980	4.017	4.800	9.700	6.8742
Mean of proline		11.969	8.080	8.936	9.300	
L.S.D $_{0.05}$	salt * SA * proline =2.514 salt * SA =1.26 proline=1.03					Mean of salt
Salt * Proline	1.3	17.019	9.850	10.363	9.312	11.636
	5	6.920	6.310	7.509	9.288	7.507
L.S.D $_{0.05}$	salt * proline =1.452 salt =0.73					Mean of S.A
S.A * Proline	0	11.913	9.707	8.590	7.462	9.418
	10^{-4}	10.222	9.180	11.100	10.288	10.198
	$5*10^{-5}$	13.774	5.353	7.118	10.150	9.099
L.S.D $_{0.05}$	SA * proline = 1.78 SA=0.889					

Spraying un-stressed pepper plants with proline resulted in disappearance of significances when compared with the control except 5mM concentration that caused significant decrease in leaves number of the plant. Whereas, all concentrations of proline caused decrease of leaves number in stressed plants. This table also clarify the interaction between S.A and proline, almost these interactions showed a negative effect on leaves number, except the combinations between S.A, 10^{-4}M + proline ,5mM and S.A ,$5*10^{-5}$M + proline,10 mM. These combinations may be enhance endogenous hormones level which was responsible on leaves formation which affected negatively during salt stress. Hamayun *et al*, 2010 suggested that the reduction in growth under salt stress conditions was associated by reduced production of GAs and he suggested that SA had an important role in protecting the plant during salt stress. Alternatively, its effect on auxin availability in the plant which agreed with(Iglesias *et al*,2011) who concluded that the modulation of growth program as part of adaptive plant strategy against both biotic and abiotic stress involves down regulation of auxin-mediated signaling.

The interaction between salt, S.A and proline was revealed that spraying of un-stressed plants with S.A and proline resulted in no significance in their effect, whereas,

ISSN 2072-3875

stressed plants showed decrease in leaves number, except the combination of S.A 10^{-4}M +proline 5mM .

Table (3) demonstrated that there was a decrease in leaves surface area in plants which irrigated with salted water; about 32.8% compared with un-stressed plants. These results were compatibility with the findings of (Hirpara *et al*, 2005; Ziaf *et al*, 2009).

The same table showed that the differences between S.A treated plants and the control were absent, this was compatible with (Hussein *et al*, 2007)who demonstrated that there were not significances between leaves surface area sprayed with SA at 200 ppm. Whereas, a decrease in leaves surface area was found in plant treated with proline compared to control plants. The interaction between salt and S.A elucidate no significance between the means of un-stressed plants. The same result was seen in stressed plants, but the treatment with S.A could not alleviate harmful effect of salinity. This observations were similar to (Mahmood *et al*,2010) who find that the exogenous supply of SA showed almost non significant effect on sorghum plants that grown under salt stress and to (Farahbakhsh and Siid,2011) who mentioned that salinity and SA interaction had no significant effect on leaf area.

Table (2): The effect of salt, S.A , proline and its interactions on leaves number.

Salt concentration Dsm/cm	S.A concentration M	Proline concentration mM				Mean of salt* S.A
		0	1	5	10	
1.3	0	14.500	13.333	12.333	13.000	13.1818
	10^{-4}	13.667	11.000	12.667	12.000	12.3333
	$5*10^{-5}$	14.667	12.667	11.000	14.000	12.3333
5	0	13.333	11.667	10.000	7.667	10.6667
	10^{-4}	7.000	9.667	11.000	9.000	9.1667
	$5*10^{-5}$	10.000	5.667	9.000	8.667	8.3333
Mean of praline		12.310	10.667	10.667	10.722	
L.S.D $0_{.05}$	salt * SA * proline =3.66 salt * SA =1.83 proline=1.494					Mean of salt
Salt * Proline	1.3	14.278	12.333	12.000	13.000	12.903
	5	10.111	9.000	10.000	8.444	9.389
L.S.D $_{0.05}$	salt * proline =2.113 salt =1.05					Mean of S.A
S.A * Proline	0	13.611	12.500	11.167	10.333	12.093
	10^{-4}	10.333	10.333	11.833	10.500	10.750
	$5*10^{-5}$	12.333	9.167	10.000	11.333	10.708
L.S.D $_{0.05}$	SA * proline =2.59 SA=1.294					

ISSN 2072-3875

Treatment of un-stressed plants with proline caused a reduction in leaf surface area compared to control. It may be related to the excessive accumulation of supplied proline in the leaves (Table 6) which then led to the damage of the plant cells. These finding was similar to these of (Mattoli *et al* ,2009) who mentioned that osmotic stress caused by environmental factors may seriously damaged the plant cells and it is likely to be counteracted by proline accumulation, while the significance was absent between stressed plants which sprayed with S.A.These forgoing results suggested that proline activity protrudes under stress conditions (Nawaz *et al*,2010).The interaction between S.A and proline elucidates that the combination between S.A10^{-4} or $5*10^{-5}$ M + proline 10 mM were the best.

Table (3): The effect of salt, S.A, proline and its interactions on leaves surface area (cm^2)

Salt concentration Dsm/cm	S.A concentration M	Proline concentration mM				Mean of salt* S.A
		0	1	5	10	
1.3	0	24.515	17.320	23.4867	20.1667	21.3715
	10^{-4}	23.940	19.197	22.0267	25.6767	22.7100
	$5*10^{-5}$	29.080	16.987	19.2733	19.4667	21.2017
5	0	18.760	17.170	15.5800	10.9800	15.6225
	10^{-4}	13.673	14.343	12.9100	12.7767	13.4258
	$5*10^{-5}$	13.880	15.353	10.3233	19.7167	14.8183
Mean of proline		20.641	16.728	17.267	18.131	
L.S.D $_{0.05}$	Salt*S.A*Proline= 4.67 Proline= 1.91 Salt*S.A=2.33					Mean of salt
Salt * Proline	1.3	25.844	17.834	21.596	21.770	21.761
	5	15.438	15.622	12.938	14.491	14.622
L.S.D $_{0.05}$	Salt= 1.35 Salt*Proline= 2.695					Mean of S.A
S.A * Proline	0	21.636	17.245	19.533	15.573	18.497
	10^{-4}	18.807	16.770	17.468	19.227	18.068
	$5*10^{-5}$	21.480	16.170	14.798	19.592	18.010
L.S.D $_{0.05}$	S.A*Proline= 3.3 S.A= 1.65					

The triple interaction between the above parameters demonstrated that un-stressed plants sprayed with S.A or proline showed no significant effect on leaves surface area except proline 1mM that caused inhibition. However, S.A concentrations 10^{-4} and $5*10^{-5}$ M caused decrease in leaves surface area of stressed plants because of the high level of proline produced by plant tissues (Table 6) may be toxic, whereas, proline maintains leaves area in its normal range of stressed plants.

Table (4) illustrated the effect of salinity on node length. It showed no significant effects on node length. Whereas, the general effect of SA was clarify a significant

ISSN 2072-3875

decrease in node length in the plants treated with S.A. Salinity, the treatment with proline had no significant effect on node length.

The same table showed that spraying of un- stressed and stressed plants with S.A at concentrations $5*10^{-5}$ and 10^{-4} caused a decrease in node length that is the concentrations between the first and the second case it was coincined with the increase of intracellular proline of roots cells(Table 7),while proline treatment had no significant effect on node length both in stressed and un- stressed plants.

Spraying the plants with S.A and proline simultaneuosly caused a decrease in node length, but the interactions between S.A 10^{-4} ,$5*10^{-5}$M + proline 10 mM were the best in maintaining node length compared with the control. The triple interaction between salt, S.A, and proline showed no significant effect both in stressed and un-stressed plants, except the concentrations S.A 5* 10^{-5}M + proline 0,5 mM of un-stressed plants caused decrease of node length.

Table (4): The effect of salt, S.A,proline and its interactions on node length(cm)

Salt concentration Dsm/cm	S.A concentration M	Proline concentration mM				Mean of salt* S.A
		0	1	5	10	
1.3	0	3.200	2.433	4.067	3.067	3.1917
	10^{-4}	3.167	2.500	2.667	3.767	3.0250
	$5*10^{-5}$	1.667	2.167	1.833	2.867	2.1333
5	0	3.433	2.983	2.367	2.900	2.9208
	10^{-4}	2.333	2.167	2.100	2.333	2.2333
	$5*10^{-5}$	2.067	2.167	2.767	2.667	2.5236
Mean of praline		2.644	2.403	2.633	2.933	
L.S.D $_{0.05}$	salt * SA * proline =1.146 salt * SA =0.56 proline=0.465					Mean of salt
Salt * Proline	1.3	2.678	2.367	2.856	3.233	2.783
	5	2.611	2.439	2.411	2.633	2.524
L.S.D $_{0.05}$	salt * proline =0.657 salt =0.33					Mean of S.A
S.A * Proline	0	3.317	2.708	3.217	2.983	3.056
	10^{-4}	2.750	2.333	2.383	3.050	2.629
	$5*10^{-5}$	1.867	2.167	2.300	2.767	2.275
L.S.D $_{0.05}$	SA * proline =0.81 SA=0.403					

Salinity also affect shoot length as indicated in (Table5).It caused decrease about 29.740% in shoot length. This result was compatible with the finding of(Deivanai et al,2011).Such decrease may be related to the alterations of photosynthetic activity and water relation characteristics which accompanied by reduction of shoot elongation (Hirpara *et al*,2005; Ziaf *et al* ,2009; Ben Ahmed *et al* ,2010;Ben Ahmed *et al*,2011).

ISSN 2072-3875

Commonly S.A treatment at the concentration $5*10^{-5}$M caused a decrease in shoot length, while proline treatment had no effect on this parameter which was coincided with the decrease in node length (Table 4). S.A has an important role in maintaining shoot length during stress, in spite of its negative effect in un-stressed plants. In contrast proline at 5 and 10 mM had effect negatively on stressed plant, These results were similar to the finding of (Nawaz *et al*,2010).The interaction s between S.A and proline illustrated that all combinations maintains shoot length as it was the case in control plants.

The triple interaction between salt, SA and proline demonstrated that individually proline at 1,5,10 mM ,and S.A in $5*10^{-5}$M caused an increase in shoot length in un-stressed plants. It was compatible with (Karima *et al*,2005).It could be related to the stimulatory effect of low concentrations of SA on shoot length (Canackei *et al*,2011), or, because increasing the intracellular pool of free proline both in shoots and roots (Table 6,7) which plays as osmoprotectant during physiological responses (Nounjana *et al*,2012) ,whereas, proline in 5,10 mM, S.A10^{-4} M+ proline 10 mM and S.A $5*10^{-5}$M+ proline 1,5 mM caused decrease in shoot length in stressed plants.

Table (5):The effect of salt, S.A, proline and its interactions on shoot length (cm).

Salt concentration Dsm/cm	S.A concentration M	Proline concentration mM				Mean of salt* S.A
		0	1	5	10	
1.3	0	20.667	29.333	26.000	30.000	26.5000
	10^{-4}	24.000	22.000	23.000	24.000	23.2500
	$5*10^{-5}$	27.333	22.333	23.667	21.333	23.6667
5	0	21.333	18.000	16.667	14.000	17.5000
	10^{-4}	19.000	17.667	19.667	15.000	17.8333
	$5*10^{-5}$	17.000	14.667	13.333	20.000	17.1944
Mean of praline		21.556	20.667	20.389	20.722	
L.S.D 0.05	salt*SA* proline=4.38 salt * SA =2.19 proline=1.787					Mean of salt
Salt * Proline	1.3	24.000	24.556	24.222	25.111	24.472
	5	19.111	16.778	16.556	16.333	17.194
L.S.D $_{0.05}$	salt * proline = 2.53 salt =1.264					Mean of S.A
S.A * Proline	0	21.000	23.667	21.333	22.000	22.000
	10^{-4}	21.500	19.833	21.333	19.500	20.542
	$5*10^{-5}$	22.167	18.500	18.500	20.667	19.958
L.S.D $_{0.05}$	SA * proline =3.095 SA=1.55					

ISSN 2072-3875

Table (6):- The effect of salt, S.A, proline and their interaction on proline content in leaves µm/gm F.W

Salt concentration Dsm/cm	S.A concentration M	Proline concentration mM				Mean of salt* S.A
		0	1	5	10	
1.3	0	.5791	1.1719	1.7086	2.6943	1.5385
	10^{-4}	.5654	1.4143	1.5306	1.5251	1.2589
	$5*10^{-5}$	1.4211	1.4485	1.3965	.9652	1.3078
5	0	1.8003	1.7839	1.9126	1.2335	1.6826
	10^{-4}	3.5993	1.8962	1.3759	1.2924	2.0409
	$5*10^{-5}$	3.0777	3.8485	2.5602	2.0249	2.8778
Mean of proline		1.840	1.927	1.747	1.623	
L.S.D	salt * SA * proline =0.484 salt * SA =0.242 proline=0.198					Mean of salt
Salt * Proline	1.3	.855	1.345	1.545	1.728	1.368
	5	2.826	2.510	1.950	1.517	2.200
L.S.D $_{0.05}$	salt * proline =0.28 salt =0.099					Mean of S.A
S.A * Proline	0	1.190	1.478	1.811	1.964	1.611
	10^{-4}	2.082	1.655	1.453	1.409	1.650
	$5*10^{-5}$	2.249	2.648	1.978	1.495	2.093
L.S.D $_{0.05}$	SA * proline =0.342 SA=0.171					

Table (6) clarifies a significant increase in proline content in the leaves of pepper plants during salinity. These results were agreed with the finding of (Nounjan *et al*,2012).Spraying plants with SA at $5*10^{-5}$ caused an increase in proline content, due to its role in plant tolerance in stressed plants. This result was compatible with (Hayat *et al*,2007).The same result was found with proline treatment in un-stressed plants due to increase of intracellular pool of free proline in the shoot (Karima *et al*,2005),while its content decreased in stressed plants refer to its recovery after salt treatment(Nounjana *et al*,2012).

Although the individual treatment of plants with SA 10^{-4} or proline caused an increase in proline content, the interaction between these compounds cause a decrease in proline content, whereas SA $5*10^{-5}$ in combination with proline caused an increase in proline content, due to the synergistic effect of these compounds in plant to protrude an additional role in protection the plant during stress conditions (Hayat *et al*,2007;Ben Ahmad *et al*,2011). Proline content in the triple interaction was increased significantly in stressed and un-stressed plants, but this increment was higher in stressed plants than un-stressed plants.

ISSN 2072-3875

Table(7):- The effect of salt, S.A, proline and its interactions on proline content in roots µm/gm F.W

Salt concentration Dsm/cm	S.A concentration M	Proline concentration mM				Mean of salt* S.A
		0	1	5	10	
1.3	0	0.8310	1.8127	1.5457	2.3616	1.6378
	10^{-4}	1.5785	2.3466	1.0295	1.7237	1.6696
	$5*10^{-5}$	1.3773	2.9914	1.4088	2.0509	1.9571
5	0	2.6136	2.1029	1.1856	1.3855	1.8219
	10^{-4}	3.0763	1.5429	1.4772	1.2034	1.8250
	$5*10^{-5}$	0.7256	3.9621	2.0509	2.1933	2.2330
Mean of proline		1.700	2.460	1.450	1.820	
$L.S.D_{0.05}$	salt * SA * proline =0.389 salt * SA =0.195 proline=0.159					Mean of salt
Salt * Proline	1.3	1.262	2.384	1.328	2.045	1.755
	5	2.138	2.536	1.571	1.594	1.960
$L.S.D_{0.05}$	salt * proline =0.23 salt =0.11					Mean of S.A
S.A * Proline	0	1.722	1.958	1.366	1.874	1.730
	10^{-4}	2.327	1.945	1.253	1.464	1.747
	$5*10^{-5}$	1.051	3.477	1.730	2.122	2.095
$L.S.D_{0.05}$	SA * proline =0.275 SA=0.137					

Table (7) illustrated proline content in root tissue. It showed that salinity treatment increased proline content. This observation was similar to (Marin *et al* ,2010) .Low concentration of SA caused an increase in proline content which increase plant tolerance to salt stress(Sahba *et al*,2010). Almost interactions caused an increase in proline content due to its effect on increase plant tolerance to salt stress.

References:-

Ali Q., Muhammad A. and Habib-Ur-Rehman A., 2007. Exogenously applied proline at different growth stages enhances growth of two maize cultivars grown under water deficit conditions. *Pak. J. Bot*., 39(4): 1133-1144.

Ali Q.; Ashraf M.; Shahbaz M. and Humera H., 2008. Ameliorating effect of foliar applied prolin on nutrient uptake in water stress maize (*Zea mays* L.) plants. *Pak. J. Bot*., 40(1): 211-219.

ISSN 2072-3875

Amin B; Mahleghah G. ; Mahmood H.R.M.; And Hossein M.,2009. Evaluation of interaction effect of drought stress with ascorbate and salicylic acid on some of physiological and biochemical parameters in okra (*Hibiscus esculentus* L.). *Research Journal Of Biological Sciences.*,4(4): 380-387.

Arfan M.; Athar H.R. and Ashraf M., 2007. Does exogenous application of salicylic acid through the rooting medium modulate growth and photosynthetic capacity in two differently adapted spring wheat cultivars under salt stress, *J. Plant Physiol*., 164:685-694.

Ashraf M. And Foolad M.R., 2007. Roles of glycine betaine and proline in improving plant abiotic stress resistance. *Env. Exp. Bot*., 59: 206-216.

Bates, L.S.; R.P, Waldren And I.D. Tears, 1973. Rapid determination of free proline for water-stress studies. *Plant and Cell*., 39: 205-207.

Ben Ahmed C.; Ben Rouina B.; Sensoy S.; Boukhriss M.,And Ben Abdullah F.,2010. Exogenous proline effects on photosynthetic performance and antioxidant defense system of young olive tree. *J. Agric. Food Chem*. 58:4216–4222.

Ben Ahmed C.H; Magdich S.; Ben Rouina B.; Sensoy S.; Boukhris M.And Ben Abdullah F., 2011. Exogenous proline effects on water relations and ions contents in leaves and roots of young olive. *Amino Acids* 40:565–573.

Ben Ahmed; Hela; Abidi; Farouk; Manaa; Arafet; Mimouni; Zid H. And Ezzeddine, 2009. Salicylic acid induced changes on some physiological parameters in tomato grown under salinity. *The Proceedings of the International Plant Nutrition Colloquium Xvi.*

Bethke P.C. & Drew M.C.,1992 . Stomatal and nonstomatal components to inhibition of photosynthesis in leaves of *Capsicum annuum* during progressive exposure to NaCl salinity. *Plant Physiology*.,99: 219–226.

Canakci S., 2011. Effect of salicylic acid on growth, biochemical constituents in pepper (Capsicum annuum L.) seedlings. *Pakistan Journal of Biological Sciences*., 14(4):300-304.

Chookhampaeng S.,2011. The effect of salt stress on growth, chlorophyll content proline content and antioxidative enzymes of pepper (*Capsicum annuum* L.) *seedling. European Journal Of Scientific Research*.,4(1): 103-109.

Deivanai S, R. Xavier, V. Vinod, K. Timalata and O.F. Lim, 2011. Role of exogenous proline in ameliorating salt stress at early stage in two rice cultivars. *Journal of Stress Physiology & Biochemistry,* 7(4): 157-174.

Desingh R. And Kanagaraj G., 2007. Influence of salinity stress on photosynthesis and antioxidative systems in two cotton varieties. *Gen. Appl.* Plant Physiology. *33 (3-4): 221-234.*

Erdal S,.; Aydın M.; Genisel M.; Taspınar M.S.; Dumlupinar R.; Kaya O. and Gorcek Z., 2011. Effects of salicylic acid on wheat salt sensitivity. *African Journal Of Biotechnology*., 10(30):5713-5718.

ISSN 2072-3875

Farahbakhsh H. And Saiid M.S.,2011. Effects of foliar application of salicylic acid on vegetative growth of maize under saline conditions. *African Journal Of Plant Science*., 5(10): 575-578.

Gerdakaneh M.; Mozafari A.; Khalighi A. And Sioseh-Mardah A., 2010. The effects of exogenous proline and osmotic stress on morpho-biochemical parameters of strawberry callus. *African Journal Of Biotechnology* ., 9(25). 3775-3779.

Hamayun M.; Khan S.A.; Khan A.L.; Shinwari Z.K.; H. J.; Sohn E.E.Y.; Kang S.; Kim Y.; Khan M. A. And Lee I., 2010. Effect of salt stress on growth attributes and endogenous growth hormones of soybean cultivar hwangkeumkong. *Pak. J. Bot*., 42(5): 3103-3112.

Hayat S. And Ahmad A.,2007. Salicylic Acid: A Plant Hormone, Published By Springer, Xv, P. 401.

Hirpara K.D.; Ramoliya P. J.; Pate A.D. & Pandey A.N., 2005. Effect of Stalinization of soil on growth and macro- and micro-nutrient accumulation in seedlings of *Butea monosperma* (Fabaceae). *Anales De Biologia.*, 27: 3-14.

Houimli S.I.M.; Mounir D. and B.A. Salim , 2008. Induction of salt tolerance in pepper (*Capsicum annuum*) by 24-epibrassinolide. *Eur. Asian Journal of Bioscines*.,2:83-90.

Hussein M.M.; Balbaa K. And Gaballah M.S.,2007. Salicylic acid and Salinity effects on growth of maize plants. *Research Journal of Agriculture And Biological Sciences*, 3(4): 321-328.

Iglesias M. J.; Terrile M.C. And Casalongué C.A.,2011. Auxin and salicylic acid signalings counteract during the adaptive response to stress. *Plant Signaling & Behavior*., 6(3): 452-454.

Karima M.; Gamal El-Din; And Abd El-Wahed M.S.A.,2005. Effect of some amino acids on growth and essential oil content of chamomile plant. International *Journal Of Agriculture Biology* ., 7:(3).

Mahmood T.; Iqbal N.; Raza H.; Qasim M. And Ashraf M. Y.,2010. Growth modulation and ion partitioning in salt stressed sorghum (*Sorghum bicolor* L.) by exogenous supply of salicylic acid. *Pak. J. Bot*., 42(5): 3047-3054.

Marin J.A.; P. Andreu; A. Carrasco; A. Arbeloa,2010. Determination of proline concentration, an abiotic stress marker, in root exudates of excised root cultures of fruit tree rootstocks under salt stress. *Revue Des Régions Arides – Numéro Spécial – 24*.

Mattioli Ro.;Berto; Costantino P. And Trovato M.,2009. Proline accumulation in plants. *Plant Signaling & Behavior*. 4, 11: 1016-8.

Mgbeze G.C. And Omodamwen J.O., 2011. Nutrient uptake in pepper (*Capsicum annuum* L.) grown under salt stress. *Journal of Agriculture and Biological Sciences*.,(4) Pp.099-107.

ISSN 2072-3875

Nawaz K.; Talat A.;Iqra, Hussain K. And Abdul Majeed ,2010. Induction of salt tolerance in two cultivars of sorghum (*Sorghum bicolor* L.) by exogenous application of proline at seedling stage. *World Applied Sciences Journal* 10 (1): 93-99.

Noreen S. And Muhammad A.,2008. Alleviation of adverse effects of salt stress on sunflower (*Helianthus Annuus* L.) by exogenous application of salicylic acid: growth and photosynthesis. *Pak. J. Bot*., 40(4): 1657-1663.

Nounjana N.; Nghiab, P.T. and Theerakulpisuta P., 2012. Exogenous proline and trehalose promote recovery of rice seedlings from salt-stress and differentially modulate antioxidant enzymes and expression of related genes. *Journal of Plant Physiology*. 169 :596– 604.

Okuma E.;Murakami Y.;Shimoishi Y.;Tada M.;And Murata Y.,2004. Effect of exogenous application of proline and betain on the growth of tobacco cultured cells under saline conditions. *Soil. Sci.Plant Nutr*.,50(8):1301-1305.

Pessarakli M.,1999.Handbook Of Plant And Crop Stress.2nd Ed.Marcel Dekker,Inc.

Sahu S., Das P., Ray M., Sabat S.C..2010. Osmolyte modulated enhanced rice leaf catalase activity under salt-stress. *Advances In Bioscience And Biotechnology*.,1: 39-46.

Shahba; Zahra; Amin B.; Mehdi Y., 2010. The salicylic acid effect on the tomato (*Lycopersicum esculentum* Mill.) germination, growth and photosynthetic pigment under salinity stress (NaCl). *Journal Of Stress Physiology & Biochemistry* ., 6(3) :4-16.

Tari I.;Csiszar J., Szalai G.; Horvath F.; Pécsvaradi A.;Gabriella.,2002. Acclimation of tomato plants to salinity stress after a salicylic acid pre-treatment. *Acta Biologica Szegediensis*. 46(3-4):55-56.

Tuna L.; Kaya C.; Dikilitas M.; Yokas I.; Burun B. And Altunlu H.,2007. Comparative effects of various salicylic acid derivatives on key growth parameters and some enzyme activities in salinity stressed maize (*Zea mays* L.) plants. *Pak. J. Bot*., 39(3): 787-798.

Yan Z.; Guo S.; Shu S.; Sun J. And Tezuka T.,2011. Effects of proline on photosynthesis, root reactive oxygen species (ROS) metabolism In two melon Cultivars (*Cucumis Melo* L.) under NaCl stress. African Journal Of Biotechnology Vol. 10(80), Pp. 18381-18390.

Ziaf K.; Amjad .; Pervez M. A.; Iqbal Q.; Rajwana I. A. And Ayyub M.,2009. Evaluation of different growth and physiological traits as indices of salt tolerance in hot pepper (*Capsicum annuum* L.). *Pak. J. Bot*., 41(4): 1797-1809.

ISSN 2072-3875