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Effect of spraying acetyl salicylic acid to reduce the damaging effects of salt water stress on orange plants (Citrus sinensis L.)

تأثير الرش بالـ Acetyl salicylic acid في تقليل الضرر الحاصل بواسطة الري بالمياه المالحة لشتلات البرتقال (Citrus sinensis L.)

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Abstract

This experiment was conducted on a private fruit nursery at Babylon Governorate the period from the first of March 2008 until 15- July- 2008 to study effect of spraying with Acetyl salicylic acid with two concentrations (1000,2000 mg/liter) and the quality of irrigation water also the interactions.Orange nurslings with 1 year age were chosen at same size and the height, factorial experiment in (R.C.B.D) design included about 9 treatments with three replicates.Results showed that the Acetyl salicylic acid with (1000, 2000 mg/L) increased the plant tolerance of salt water stress significantly in the average of leaf area , the length of vegetative shoots, the fresh and dry weight to the shoots and the amount of total chlorophyll in the leaves.Also the results showed that decreasing in the all of study parameters whenever increased the salinity of irrigation water.

الخلاصة:

أجريت التجربة في أحد المشاتل الخاصة بإكثار نباتات الفاكهة في محافظة بابل للفترة من 1/3/ 2008 ولغاية 15/ 7/ 2008 ،لدراسة تأثير الرش بالـ Acetyl salicylic acid بتركيزين (1000، 2000 ملغم / لتر) ونوعية مياه الري على شتلات البرتقال المزروعة حديثاً وقد استخدم نوعين من مياه الآبار بالإضافة للمياه العادية التي اعتبرت كمعاملة مقارنة.استخدمت شتلات برتقال بعمر سنة متجانسة بالحجم والارتفاع، صممت التجربة كونها عامليه وفق تصميم القطاعات العشوائية الكاملة وتضمنت تسعة معاملات كل معاملة كررت ثلاث.أظهرت النتائج أن Acetyl salicylic acid أدى إلى زيادة تحمل الشتلات المعرضة للشد الملحي معنوياً نتيجة إضافة مياه الري المالحة في معدل طول الأفرع الخضرية، المساحة الورقية، ومعدل الوزن الرطب والجاف للمجموع الخضري و كمية الكلوروفيل الكلية بالأوراق ، ولوحظ أيضا نقصان واضحة بالمعدلات المذكورة عند الري باستخدام المياه المالحة (مياه الآبار) وتزداد معدلات النقص كلما ازدادت ملوحة مياه الري وأظهر التداخل إن هنالك فروقاً معنوية قد حصلت في جميع الموشرات المدروسة.

Introductions

One of the most widely favored of the world's fruits, the orange, sweet orange (**Citrus sinensis** L.), the orange tree, reaching 25 ft or (7.5 m), with great age, up to 50 ft (15 m), evergreen, the orange has become the most commonly grown tree fruit in the world. It is an important crop in the Far East, the Union of South Africa, Australia, throughout the Mediterranean area, and subtropical areas of South America and the Caribbean. The United States leads in world production (**1**),the Iraq have a suitable climate for orange and other citrus trees but according to the neighboring countries, the fact of citrus culture decline, one of the citrus culture problems is the salinity stress whether in the soil or in the irrigation water.

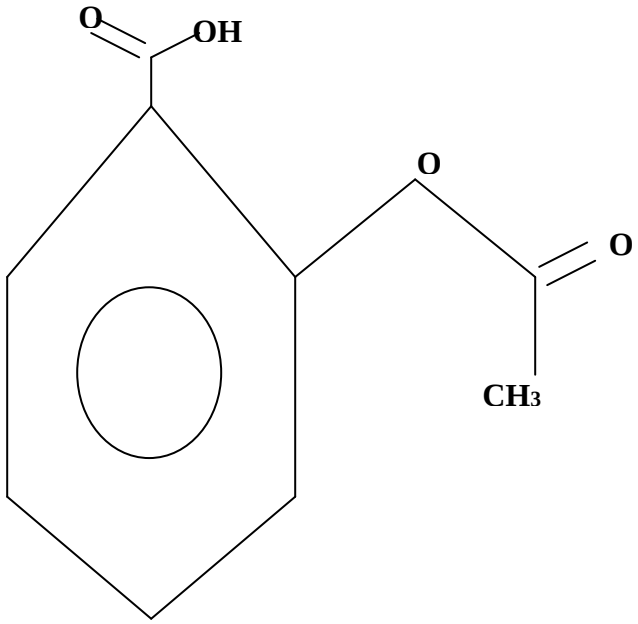
Secondary salinization from irrigation water is a growing worldwide problem as more than 6% of agricultural land has become saline (**2**), the citrus plants classified from the sensitive plants to the salinity despite the difference between the species for the range of salinity tolerance (**3**) & (**4**).

The problem of salt water appeared in world on last years and we can see it in Iraq clearly ,because the drought and the decrease of water levels in Tigris and Euphrates rivers year by year , that mean to compensate this decreasing by using other sources like wells water but at same time we have other problem with its because it consist of a amounts of the salt and some of wells water classified as a salt water , (**5**) showed that the irrigation water carried a lot of salts to the soil due to

increase the soil salinity that makes us in front of a real problem to requires following some methods to increase tolerance of plants for the salinity, some researches mentioned that there is a relation ship between the salicylic acid and the tolerance of salt stress in plants,(**6**) & (**7**), Salicylic acid induced a lot of physiological processes in the plant , activate some processes and inhibit the others (**8**), different levels of acetyl salicylic acid appeared to function as anti transparint in leaves of **Phaseolus vulgaris,** and inhibited the opening of stomata in epidermal strips of **Commelina communis** (**9**). **(10)** Used different levels of salicylic acid in maize plants and he reached that, salicylic acid treatment of salt stressed maize could stimulate their salt tolerance via accelerating their photosynthesis performance and carbohydrate metabolism. Salicylic acid increased the protein contents inside the plant cells that make the plant have ability to tolerant the salt stress (**11**),similarly other study mentioned that salicylic acid increased the activity of NR (Nitrate reductase) in the presence of NO3 and also favored protection of the enzyme against Protenase, trypsin (**12**).

There are more suggestions to explain mechanism of salicylic acid for increasing the plant tolerance to salt stress; however the way of signal regulation of plant resistance to unfavorable factors of environment induced by SA are still not clear (**13**).

The objective of this study was to investigate whether salicylic acid could be reduce the damages of orange plants under the salt stress by irrigation with salt water and increase the plant tolerance for this stress.



The chemical structure of Acetyl salicylic acid

Materials and methods

The study was conducted in a private fruit nursery at Babylon Governorate the period from the first of March 2008 until 15- July- 2008.

The land was prepared before planting by plowing, softing & grading , some physical and chemical characters of the soil were measured (table 1), the land was irrigated before the planting , after 3 days the orange seedlings planted on 1 – march -2008. We used 2 qualities from wells water as well as the river water as a control and the chemical characters were analyzed (table 2).

Acetyl Salicylic acid was initially dissolved in few drops of ethanol and the final volume was reached, using distilled water, Tween40 was added to evoke spreading of the applied solutions on the plant-leaf

surface **(10)**. The solutions were sprayed once on the leaves in the early morning, when the plants had their fourth leaf completely expanded every 20 days started on 10 – march -2008.

The treatments and experimental design:

The study was designed as two factors corresponding to the (RCBD) design; the treatment had 3 replicated with 10 plants to every replicate,

First factor was three kinds of water (W) as following:

1.The river water (control) (W1) Ec = 1.2 ds/m
2.The well water 1 (W2) Ec = 4.8 ds/m
3.The well water 2 (W3) Ec = 8.1 ds/m

Second factor was three concentrations from Acetyl Salicylic acid **(A)** as following:

1.0 mg /liter (control) A1
2.1000 mg /liter. A2
3.2000 mg /liter. A3

The results were analyzed by (SPSS 11) and the means compares according to LSD test at 0.05 (**14**).

The studied parameters:

Leave area (cm^2), length of shoots (cm), fresh and dry shoot weight (gm) and the total of chlorophyll in the leaves.

The average of total chlorophyll in the leaves:

The total chlorophyll in the leaves was measured corresponding to Goodwin method (**15**) and used the UV-Visible Spectrophotometer in (Soil laboratory of Agriculture college (Babylon university) to standard of the pigments by photo absorption with two wave long (645) & (663) nanometer, the pigment amount was measured according to equation: **(mgm/ gm)**

Total chlorophyll = 20.2× D (645) + 8.02×D (663) (V /W×1000) ×100

Table (1) some physical and chemical characters of study soil.

Soil separates	%	Texture
Sand	0.65	Sandy Loam
Silt	0.20	
Clay	0.15	
Chemical characters	Units	value
Ec	ds/m	1.8
pH	—	7.21
Soluble ions		
Ca^{++}	meq /L	5.86
Mg^{++}	meq /L	6.45
Na^{+}	meq /L	4.62
K^{+}	meq /L	0.35
$Co3^{=}$	meq /L	—
$HCO3^{-}$	meq /L	0.52
Cl^{-}	meq /L	7.32
$SO4^{=}$	meq /L	9.86

Table (2) the chemical analysis for qualities of study water.

$SO4^{=}$ meq /L	Cl^{-} meq /L	$HCO3^{-}$ meq /L	$Co3^{=}$ meq /L	K^{+} meq /L	Na^{+} meq /L	Mg^{++} meq /L	Ca^{++} meq /L	pH	Ec ds/m
W1 (River water) control									
5.37	4.41	2.60	–	0.25	3.20	3.85	4.91	7.42	1.2
W2 (Well water 1)									
32.60	21.15	4.25	–	0.31	11.30	23.07	14.02	6.85	4.8
W3 (Well water 2)									
45.41	26.76	7.88	–	0.38	23.82	37.18	20.64	6.76	8.1

The Results and Discussion

The results showed a significant differences in average of leaf area , length of shoots ,fresh and dry weight to shoots and the average of total chlorophyll in the leaves ,when we sprayed Acetyl salicylic acid with the (1000, 2000 mg/liter) concentrations compare with untreated plants (see the tables 3,4,5,6 &7) , maybe the salicylic acid improved the chlorophyll content (**16**) ,similarly Salicylic acid was increased the photosynthesis pigments in wheat's plants (**17**) and we can note that clearly in data of table 7 , the increasing in pigments of photosynthesis will be reversed positively, to improved the vegetative characters of plants, and maybe the salicylic acid induced the nitrate reductase activation due to increased nitrate metabolism in the leaves and roots of plant (**18**).

The results showed a clear decreasing in the averages of study parameters, whenever increased the salt stress by irrigation waters and the least means were at the W3 treatment (Well water 2) see the tables, this decreasing maybe by reason of the high ions concentration in the soil solution , that affected on the enzymes activation in side the plant cells via inhibited the active sites of enzymes and due to metabolism disorder by H^{+}-ATPase pumps breakdown or via inhibited the membranes working ,that affected on the permeability of its as well as the salinity affected on the Photosynthesis , Respiration processes and Electron transport chains (**19**).

The interaction between the Acetyl salicylic acid concentrations and kind of irrigation water gave a significant differences in all of the study parameters (tables 3,4,5,6& 7) , there is a clear tolerance to the salt stress, the concentrations of Acetyl salicylic acid gave the plants ability to tolerant the salinity stress especially to the plants was irrigated with well water 2 (W3), compare with the plants untreated with Acetyl salicylic acid , see the Figures (1,2,3,4,5,6,7,8,9) as following in the next pages. The ability of Acetyl salicylic acid for plant tolerance may explain because the Salicylic acid promotes the formation of ROS in the photosynthetic tissues of plants during salt stress and osmotic stress (**20)**, or may the reason of Salicylic acid pre-treatment also provided protection against salinity in plants, probably due to the increased activation of aldose reductase and APx enzymes and to the accumulation of osmolytes, such as sugars, sugar alcohol or proline (**21, 22 & 23**).and may be the plants pre-treated with salicylic acid exhibited less Ca2+ and more K+ accumulation and soluble sugars in roots at the expense of these contents in the plant shoots. The application of exogenous salicylic acid appeared to induce a pre-adaptive response to salt stress, leading to the promotion of protective reactions to the photosynthetic pigments and the maintenance of membrane integrity in barley plants, which was reflected in an improvement in plant growth (**17**).

Table (3) Effect of Acetyl salicylic acid conc., kind of irrigation water & the interaction on average of leaf area (cm^2).

(A) Acetyl salicylic acid concentrations	Kind of irrigation water (W)			The average
	W1	W2	W3	
A1	192.36	135.60	112.30	145.75
A2	223.50	172.63	145.43	180.52
A3	224.26	176.23	155.33	185.61
The average	213.37	161.48	138.02	

LSD (0.05) A = 2.115
LSD (0.05) W = 2.115
LSD (0.05) A ×W = 3.663

Table (4) Effect of Acetyl salicylic acid conc., kind of irrigation water & the interaction on length of vegetative shoots (cm).

(A) Acetyl salicylic acid concentrations	Kind of irrigation water (W)			The average
	W1	W2	W3	
A1	13.27	9.10	5.63	9.33
A2	14.90	9.33	11.20	11.81
A3	13.93	10.53	9.03	11.16
The average	14.03	9.65	8.62	

LSD (0.05) A = 2.379
LSD (0.05) W = 2.379
LSD (0.05) A×W = 4.103

Table (5) Effect of Acetyl salicylic acid conc., kind of irrigation water & the interaction on fresh weight of shoots (gm).

(A) Acetyl salicylic acid concentrations	Kind of irrigation water (W)			The average
	W1	W2	W3	
A1	40.22	29.86	24.15	31.41
A2	45.16	35.34	30.55	37.01
A3	43.93	37.15	31.07	37..38
The average	43.10	34.11	28.59	

LSD (0.05) A = 0.025
LSD (0.05) W = 0.025
LSD (0.05) A×W = 0.137

Table (6) Effect of Acetyl salicylic acid conc., kind of irrigation water & the interaction on dry weight of shoots (gm).

(A) Acetyl salicylic acid concentrations	Kind of irrigation water (W)			The average
	W1	W2	W3	
A1	23.82	15.17	12.11	17.03
A2	24.45	16.54	15.36	18.78
A3	23.38	18.33	15.55	19.08
The average	23.88	16.68	14.34	

LSD (0.05) A = 0.034
LSD (0.05) W= 0.034
LSD (0.05) A×W = 0.059

Table (7) Effect of Acetyl salicylic acid conc., kind of irrigation water & the interaction on total chlorophyll in the leaves (mgm/ gm).

(A) Acetyl salicylic acid concentrations	Kind of irrigation water (W)			The average
	W1	W2	W3	
A1	60.33	41.20	33.90	45.14
A2	67.36	50.04	45.26	54.34
A3	67.66	49.66	46.33	54.55
The average	65.12	47.08	41.83	

LSD (0.05) A = 0.695
LSD (0.05) W= 0.695
LSD (0.05) A×W = 1.205

Figure (2) irrigated with well water 1 (W2)

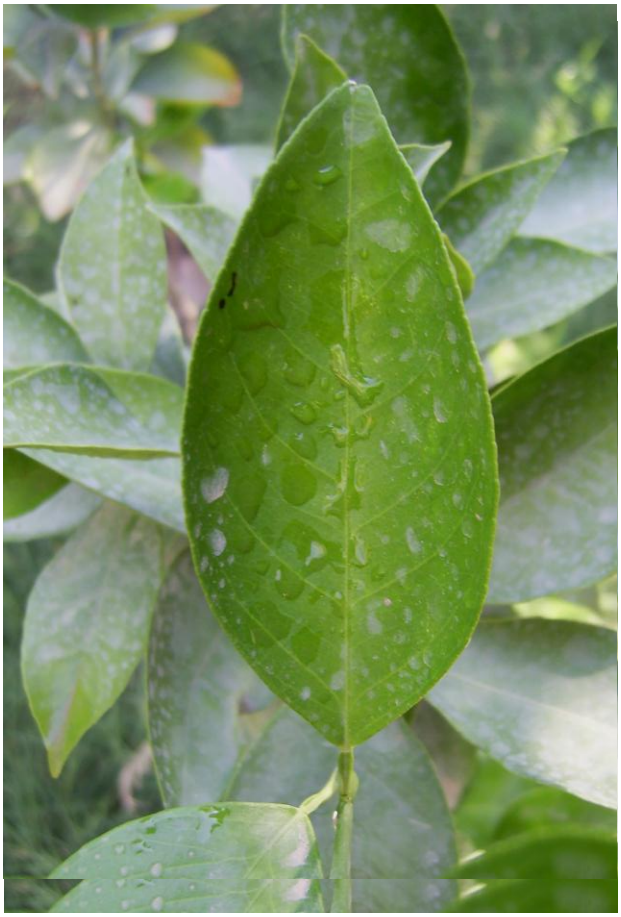


Figure (1) irrigated with river water (control)

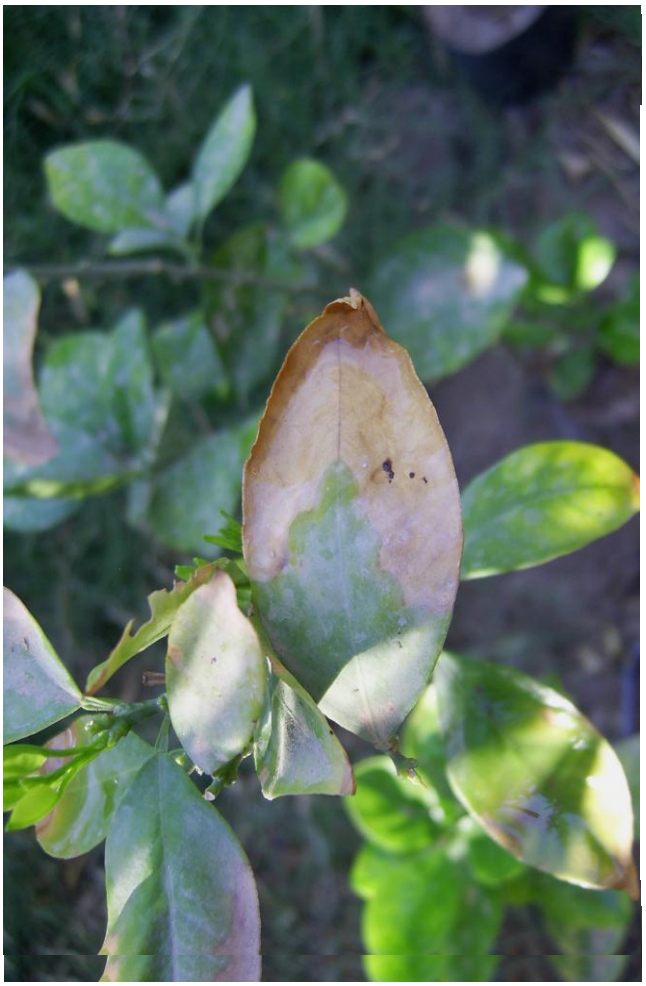


Figure (3) irrigated with well water 2 (W3)

Figure (4) Acetyl salicylic acid 1000 + Well water 1 (W2)

Figure (5) Acetyl salicylic acid 2000 + Well water 1 (W2)

Figure (6) Acetyl salicylic acid 1000 + Well water 2 (W3)

Figure (7) Acetyl salicylic acid 2000 + Well water 2 (W3)

Figure (9) Acetyl salicylic acid 2000 + river water (W1) (W1)

Figure (8) Acetyl salicylic acid 1000 + river water (W1)

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