

Effect of some growth regulators on seed germination of some plants grown under different levels of salinity

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Summary

This investigation was conducted at the College of Science , Basrah University during 2010 to investigate the effect of salinity levels and growth regulators (Ethephon and Kinetin) on germination of *Coriandrum sativum* , *Lactuca sativa* and *Raphanus sativus* seeds .The plants were grown under salinity levels (NaCl) of 0.0 , 2.5 , 5 and 10 ds/m . The seeds were soaked with concentration of ethephon (50, 75 and 100) ppm and Kinetin (1, 5 and 10) ppm while the control treatment was distilled water.

The results showed that seed germination was significantly decreased as the salinity level increased up to 10 ds/m level. The results revealed that increasing salinity levels not only reduced seed germination but also decreased seed vigor traits which measured by (mean germination time and seed germination index). Fresh and dry weight of seedling was significantly decrease as salinity level increased up to 10 ds/m comparing with control treatment.

Soaking seed in ethephon or kinetin improved seed germination, seed and seedlings vigor under salinity levels comparing with the distilled water . The maximum increase in seed germination was obtained by soaking seeds with ethephon of 100 ppm and Kinetin 10 ppm .The results showed that treatment with ethephon at 100 ppm and Kinetin 10 ppm caused significant increase in fresh and dry weigh of seedling. Ethephon 75 ppm and Kinetin 10 ppm improved the seeds germination under salinization up to 10 ds/m . Ethephon at 100 ppm and 10 ds/m salinity showed a significant increase in seed germination 80% in *Raphanus sativus*. Kinetin at 10 ppm and 5 ds/m salinity was 90 % in *Coriandrum sativum* .

Introduction

Salinity is a worldwide problem in arid and semi arid regions that substantially reduces the yield of major crops by more than 50% (Bray *et al.*, 2000). The UNEP (United Nations Environment Program) estimates that 20% of the agricultural and 50% of the cropland in the world is salt stressed (Flowers

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Sahar Abdul Abbas Al-Saadi , A'lla N. H. Al-Waheeb and Yeo, 1995).The soils in Iraq are affected with salinity specially the central and southern area of Iraq , be moderate to high salinity (Dieleman *et al.* , 1977; Szaboges,1989).

Many social and economic problems are caused by salinity that affects the growth, plant density, productivity and distribution of plants or delayed germination, high rate of seedling mortality, stunted growth and reduced yield are some of the most common effects of salted soils. Salinity is one of the most serious factors limiting crops production, especially the sensitive ones (Zadeh and Naeini, 2007). A high salt concentration in the root affects the growth and yield of many important crops (Taffouo *et al.*, 2006).Salinity reduces the ability of plants to take up water and minerals leading to growth reduction as well as metabolic change similar to those caused by the water stress (Munns, 2002). Salinity may reduce crop yield by upsetting water and nutritional balance of the plant (Khan *et al.*, 2007) Water availability and nutrient uptake by plant roots are limited because of high osmotic potential and toxicity of Na and Cl ions (Bybordi *et al.*, 2010)

Salt stress affects all growth stages of a plant, seed germination and seedling growth stages are known to be more sensitive for most plant species (Cuartero *et al.*, 2006). Kabar (1990) studied the germination of 6 species of each *Gramineae* (monocots) and dicots on saline media after pretreatment of the dry seeds with kinetin and GA3 observed that in dicots investigated, kinetin was found to be more effective in alleviation salt stress on germination, In addition, the shoot of control seedlings was more sensitive to salt than the radicle. While Bybordi (2010) showed that different salinity stress levels had significant effect on germination percentage, germination speed, shoot and root length. In the pot experiment there was a significant effect on plant height, leaf area, and dry matter.

The evidence for hormone involvement comes from correlation of hormone concentration with specific development stages, effects of applied hormones and the relationship of hormones to metabolic activities. Das Gupta *et al.* (1994) recorded that foliar application of plant growth regulators like IAA and GA helped the plant to restore retardation in water content in Mungbean plants subjected to water stress. Al-Samaraea(2000)reported that using lower concentrations of ethephon increasing the dry weight of *Rosa hybrid L.* Chakrabarti and Mukherji (2002) noticed that GA3 used to overcome the adverse effects in Mungbean plants. Cha-um *et al.*, (2009) indicated that growth characters including shoot length, fresh weight and dry weight of salt-stressed rice seedlings were inhibited, depending on NaCl concentrations. While using ethephon concentrations were 0, 1, 10 and 20 ppm , significant increase with all fertilization treatments for all studied characters in *Coriandrum sativum* such as : herb weight, number of umbels /plant and number of bisexual flowers /umbel

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and significant decrease for number of male flowers /umbel, sex ratio (bisexual /male flowers) compared to the control (El- Mekawey *et al.*, 2010).

The objectives of this study were to evaluate the effects of plant growth regulators (kinetin and ethephon) treatments on three species differing in salt tolerance under saline conditions.

Materials and Methods

This experiment was conducted in Laboratory of Biology, College of Science, University of Basrah, to study the effect of salinity and growth regulators (kinetin and ethephon (2-chlorethylphosphonic acid, C₂H₆ClO₃P) on seed germination and seedling on some plants. In this experiment used follows concentrations of NaCl: 0 (control), 2.5, 5 and 10 ds/m. The second factor growth regulators ethephon (0.0, 50, 75, and 100) ppm and kinetin (0.0, 1, 5, and 10) ppm.

Germination experiment

The three species *Coriandrum sativum* , *Lactuca sativa* and *Raphanus sativus* were collected from Iraq. The same size seeds were surface-sterilized for 5 min in sodium hypochlorite solution (10%) and then they were 3-5 times rinsed with distilled water. After sterilization, 10 seeds were transferred into 9 cm sterile Petri dishes on filter paper and then were wetted with 7 ml distilled water (control) or saline water solution at 0 (control), 2.5, 5 and 10 ds/m NaCl and regulator growth (Kinetin and ethephon). To prevent infection and evaporation of solution, all of the plates were closed. The Petri dishes were labeled and incubated in a germinator at 25°C. Computation of germinated seeds was done daily until the end of the 15 days. After that, five germinated seeds were removed and their morphological traits were assayed.

Pot experiment

The seeds were surface-sterilized for 5 min in sodium hypochlorite solution (10%) and then were rinsed with distilled water for 3 to 5 times. they were soaked in distilled water (control) or in aqueous solutions of growth regulators in predetermined concentrations. Then 10 seeds were sowed in a plastic pot (35 cm height and 30 cm diameter) contained clay: sand: silt: organic matter (1: 1: 1: 1) at depth of 1 cm. For each seed treatment (control, Kinetin and ethephon), the different concentration of NaCl solution was added to each pot gradually. Pots were transferred to glasshouse under conditions of 25°C and natural light. Computation of germinated seeds was done daily until the end of the 15 days. After that, five germinated seeds were removed and their morphological traits were assayed. The germination percentage, germination energy and mean germination time were calculated.

1- Germination percentage (%) = $n / N \times 100$ (ISTA rules, 1999).

Where n: number of germinated seed on the day and N: number of seeds.

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2- Speed germination index (SGI): It was calculated as described in the Association of Official Seed Analysis (AOSA, 1983) by following formula:

$$SGI = \frac{\text{No. of germinated seed}}{\text{Days of first count}} + \dots + \frac{\text{No. of germinated seed}}{\text{Days of final count}}$$

The seeds were considered germinated when the seedling was at least 2 mm long.

3- Mean germination time (MGT) was used to determine the speed of germination: It was calculated based on the following equation of Ellis and Roberts (1981).

$$MGT = \frac{\sum Dn}{\sum n}$$

Where (n) is the number of seeds, which were germinated on day, (D) is number of days counted from the beginning of germination.

At the final count, ten normal seedlings from each replicate were randomly taken to measure seedling fresh and weight . Seedling dry weight (DSW) can be measured by putting the seedling in oven at 75 °C as long as 48 hours.

Statistical analyses:

Significant variation in the estimated variable in relation to the different treatments was determined using analysis of variance (ANOVA) of completely randomized design and the complementary test least significant difference (LSD), using SPSS version 11 (2001, SPSS Inc., Chicago).

Results and discussion

1-Effect the salinity on germination

The germination percentage of *Coriandrum sativum* , *Lactuca sativa* and *Raphanus sativus* was significantly depressed by NaCl salinity specially in higher salinity concentration (10 ds /m NaCl) which inhibitory germination of all seeds. The results in fig (1) showed that a gradual decrease in germination percentage with increasing salinity levels. Maximum average value for germination percentage 100 % in *Coriandrum sativum* and *Raphanus sativus* in control treatment, while the minimum average (percentage) in *Coriandrum sativum* in 10 ds /m concentration was 2% (Fig 1) . The effect of salinity causes osmotic stress (Nandawall *et al.*, 2000) or specific ion effects , which delay , reduces or completely inhibit seed germination (Hanselin and Eggen ,2005).while El-Saht (1994) mentioned that the effect of salinity on plant growth might be due to the decrease in the rate of water uptake and/ or the toxic effect of accumulated sodium and chloride ions .

Table (1) represent the interaction between the different levels of salinity

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and the tested growth substances showed varied responses with the different species , depending on the doses used . All interactions between the different levels of salinity and growth regulator (ethephon or kinetin) increased significantly the germination percentage of all seeds treatment, and favored significantly in the moderate and high levels of NaCl, only the higher doses of ethephon and Kinetin increased significantly the germination percentage of all seeds tested. Ethephon 100 ppm and 10 ds/m salinity showed a significant increase in seed germination of *Raphanus sativus* (80%), while kinetin at 10 ppm and 5 ds/m salinity was 90% in *Coriandrum sativum*, this results are in agreement with Babu and Kumar(1979), who stated that leguminous plants are more sensitive to salinity , particularly at the germination stage. Furthermore Anwar *et al.*, (2001) reported reduced germination under saline conditions in some other medicinal plants. As data on the effect of interaction between salinity and kinetin or ethephon indicate , at least there are some favorable effect of Kinetin and ethephon on the germination percentage of *Coriandrum sativum* , *Lactuca sativa* and *Raphanus sativus* seeds under all tested levels of salinity .In this regard Soliman (1998) recorded similar enhancive effects of ccc on germination of berseem seeds under saline condition .Both kinetin and ethephon substantially accelerated the germination of the germination of species inhibited by salinity .In this regard the addition of kinetin might improve promoters balance in leaves tissue resulting anabolic products (Mostafa *et al.*, 1984).

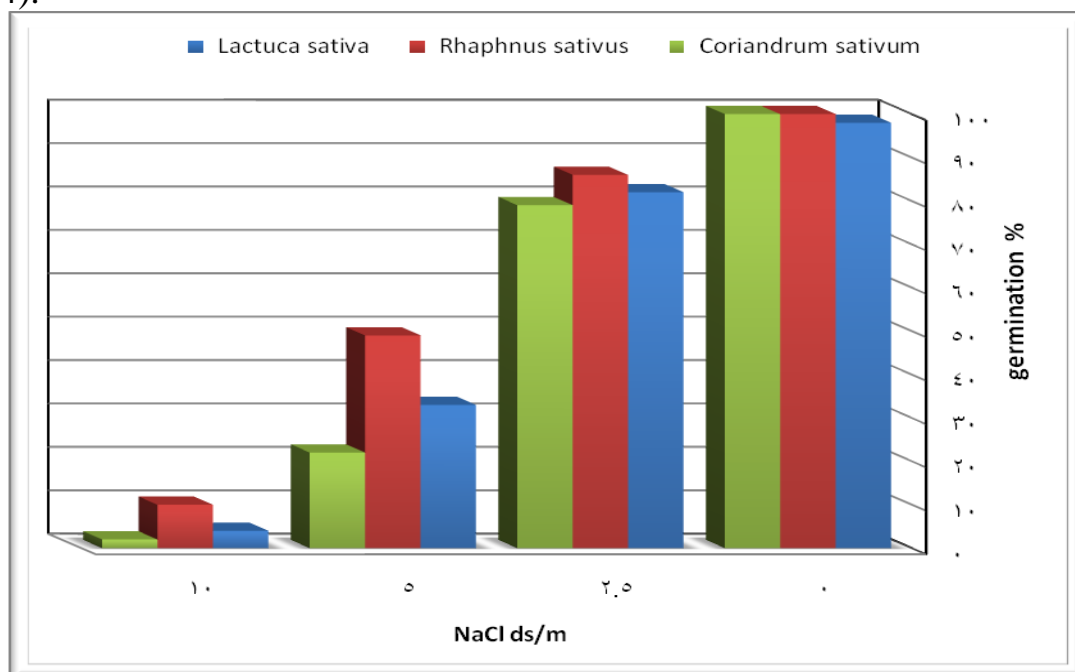


Fig (1) Effect of salinity on germination percentage of *Coriandrum sativum*, *Raphanus sativus* and *Lactuca sativa* seeds.

Table (1) Effect of the interaction between salinity and growth substances on

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germination percentage of the different species.

NaCl (ds/m)	Growth substances (ppm)	<i>Coriandrum sativum</i>	<i>Lactuca sativa</i>	<i>Raphanus sativus</i>
0	Kin 1 ppm	90	98	93
	Kin. 5 ppm	90	95	96
	Kin. 10 ppm	98	95	96
	Ethephon 50 ppm	90	90	91
	Ethephon 75 ppm	93	95	93
	Ethephon 100 ppm	95	100	95
2.5	Kin 1 ppm	90	86	88
	Kin. 5 ppm	91	89	93
	Kin. 10 ppm	94	92	96
	Ethephon 50 ppm	96	92	89
	Ethephon 75 ppm	96	94	94
	Ethephon 100 ppm	99	99	95
5	Kin 1 ppm	62	50	40
	Kin. 5 ppm	69	64	58
	Kin. 10 ppm	90	64	75
	Ethephon 50 ppm	54	54	35
	Ethephon 75 ppm	63	57	51
	Ethephon 100 ppm	74	88	83
10	Kin 1 ppm	45	46	42
	Kin. 5 ppm	55	55	60
	Kin. 10 ppm	69	68	77
	Ethephon 50 ppm	41	65	52
	Ethephon 75 ppm	55	62	54
	Ethephon 100 ppm	71	72	80
L.S.D.(0.05)		3.06	3.89	2.83

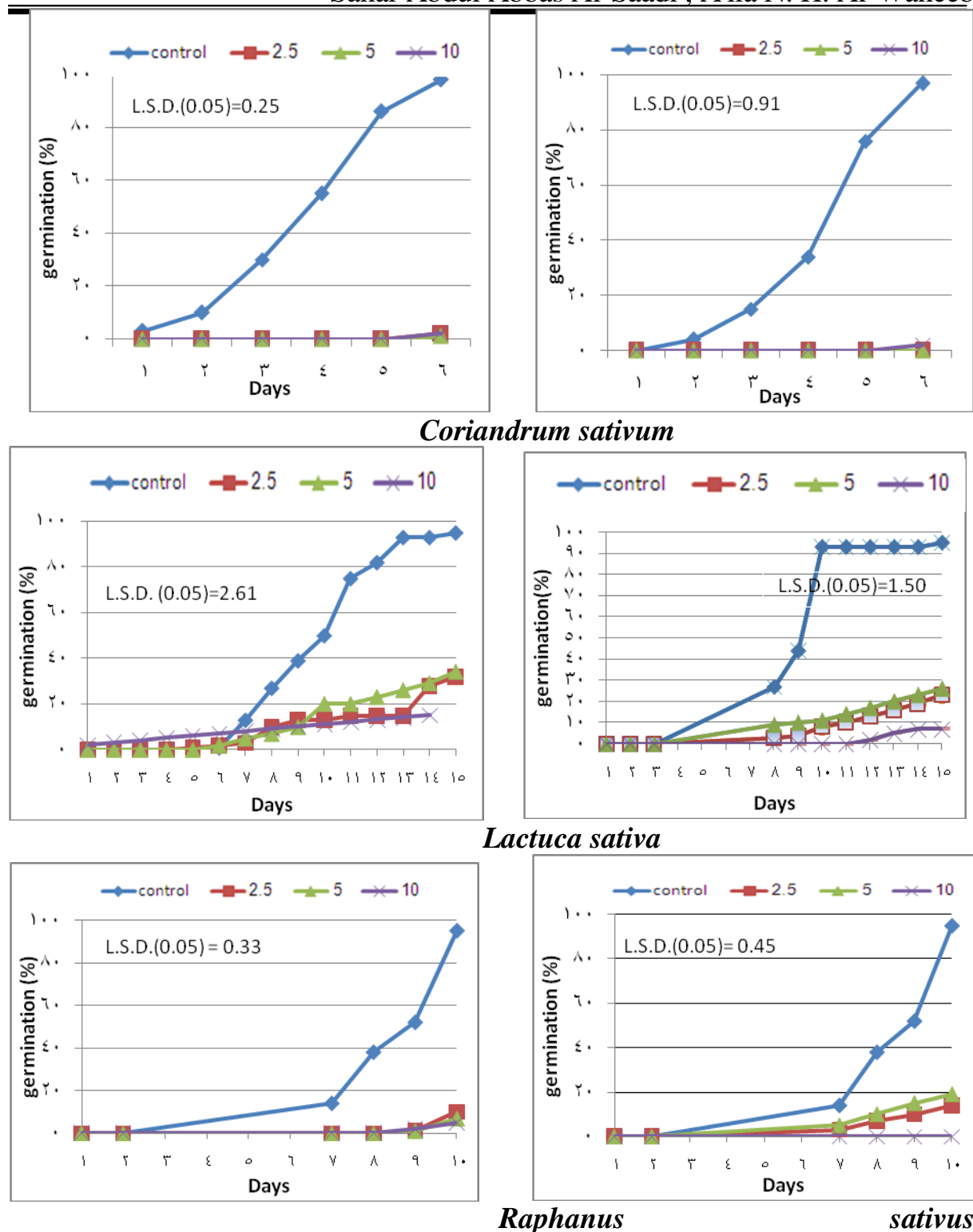
Fig (2) represents the effect of salinity on germination percentage in incubator and pots on *Coriandrum sativum*, *Lactuca sativa* and *Raphanus sativus* seeds. The data show a significant decrease in germination percentage by using the higher concentration of salinity .The 2.5 ds /m slightly increased germination percentage compared with 10 ds/m. while the germination percentage reach to 100% in each *Lactuca sativa* and *Raphanus sativus* ,while record 98% in *Coriandrum sativum* in control treatment. Also noticed that the seeds were not germination in the concentration 5 and 10 ds/m in *Coriandrum sativum* seeds (Fig 2). In *Raphanus sativus* the seeds grown after 2 days in control treatment, while the seeds which treated with salinity grown after 7 and 9 days in incubator and pots respectively (Fig 2). *Coriandrum sativum* in control treatment the seeds grown after one day, while the seeds which treated with salinity grown after 5 days was (2%) .

Incubator

pots

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Fig(2) Effect of salinity on seed germination percentage of the *Coriandrum sativum* , *Lactuca sativa* and *Raphanus sativus* in incubator and pots.

2- Effect of growth regulation on germination percentage:

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Data in (Fig. 3) showed effect of the ethephon on germination percentage of the *Coriandrum sativum*, *Lactuca sativa* and *Raphanus sativus* seeds in incubator and pots. The data show a significant increase in germination percentage was obtained by using ethephon at 100 ppm which recorded maximum in all crops tested. The highest concentration of ethephon (100 ppm) showed germination percentage (100 %) in *Lactuca sativa* and *Raphanus sativus*, while 96 and 94 in *Coriandrum sativum* in incubator and pots respectively (Fig 3). These results are in agreement with those obtained by (El-Mekawey *et al.*, 2010). This increase might be due to ethephon is known to affect some processes in plant; stimulates the release of dormancy, shoot and root growth and differentiation, may has a role in adventitious root formation, induction of femaleness in dioecious flowers, stimulates flower opening and fruit ripening. (Raven *et al.* 1992).

Fig (4) shows that using 1, 5 and 10 ppm of kinetin resulted in a significant increase in the germination percentage, The maximum values in germination percentage is 100% which obtained in *Lactuca sativa* and *Raphanus sativus* ,but the lowest value founded in *Coriandrum sativum*. Similar results were obtained by Mohanty and Sahoo (2006).

In both the cases ethephon or kinetin, the germination percentage increases when the concentration increased, the application of plant growth regulator could increased the seed germination and other physiological activity by the reason of tolerance to the toxic particles which was found in consistent with the finding of (Haroun *et al.* ,1991; Hoque and Haque ,2002). The pre-soaking with different treatments evident that soaked seed could improve in germination and seedling establishment and this observation was found equivalent the observation of (Ahmad *et al.*, 1998). While Harris *et al.*, (1999) mentioned that the soaking period of 24 hrs increased the total uptake of water which help the maximum imbibitions rate, this in turn aid to the quick biochemical changes and time period was found suitable for seed germination.

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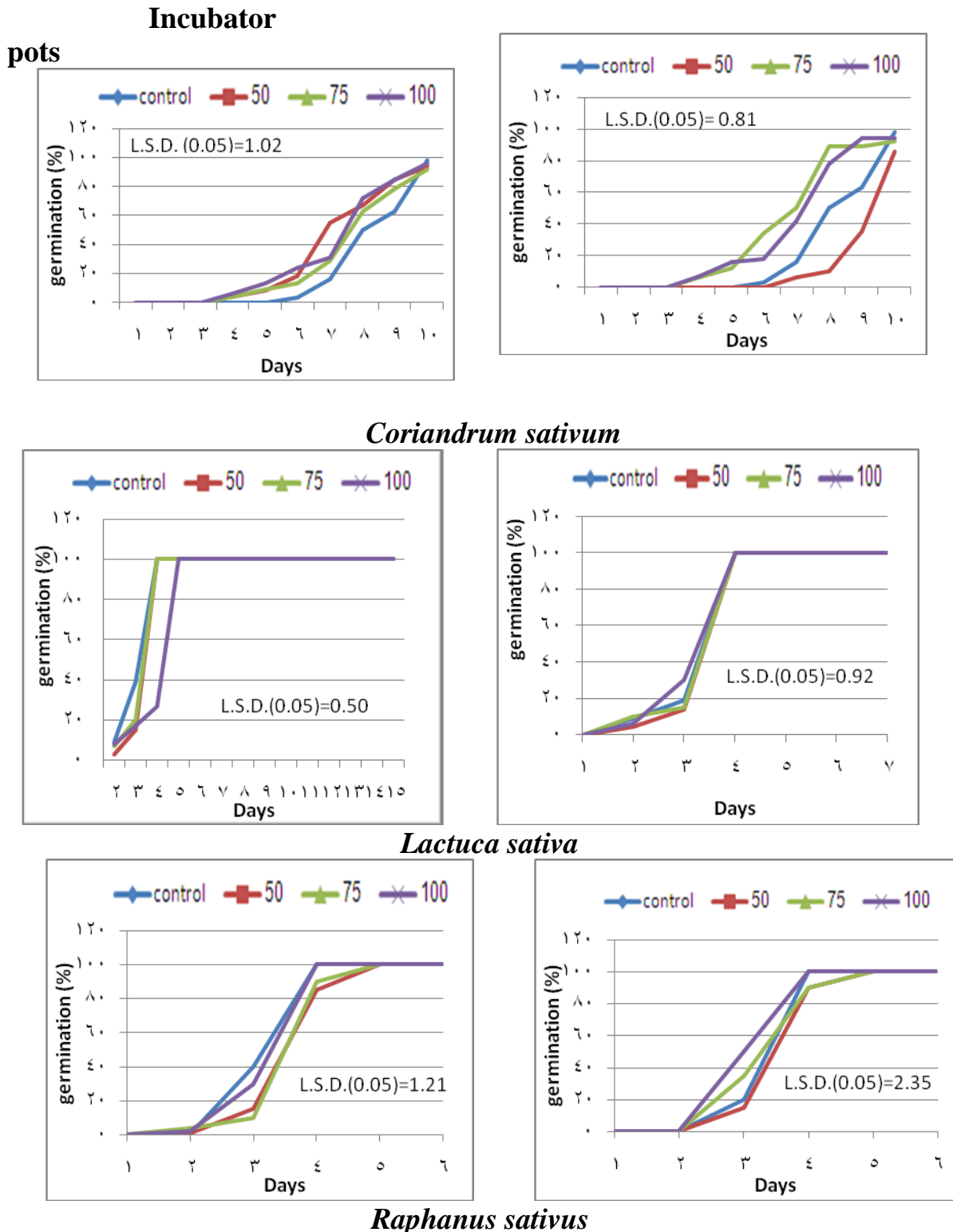
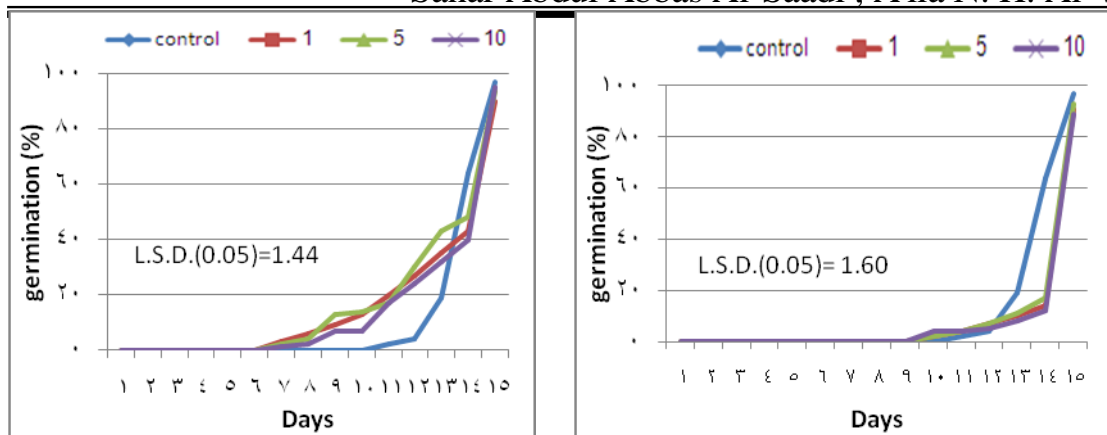


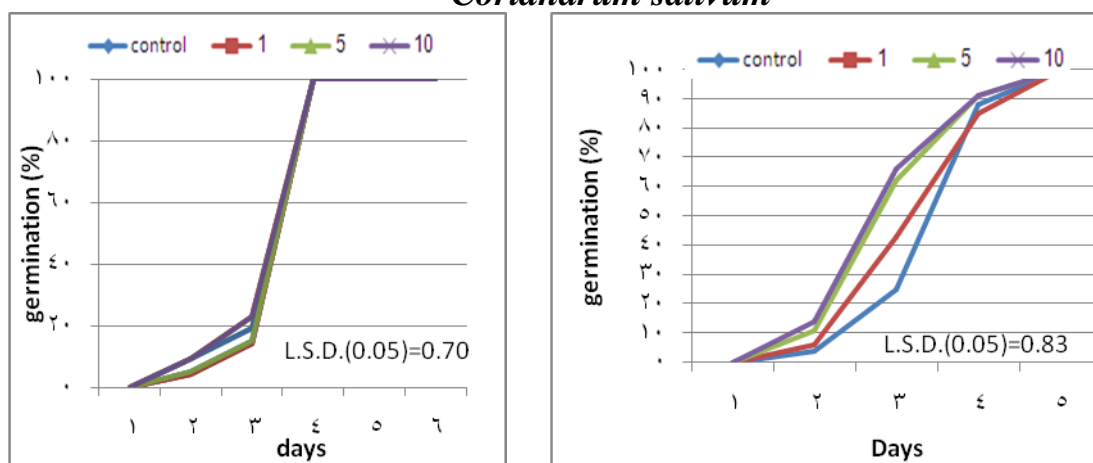
Fig (3) Effect of ethephon on seed germination percentage of the *Coriandrum sativum* , *Lactuca sativa* and *Raphanus sativus* in incubator and pots.

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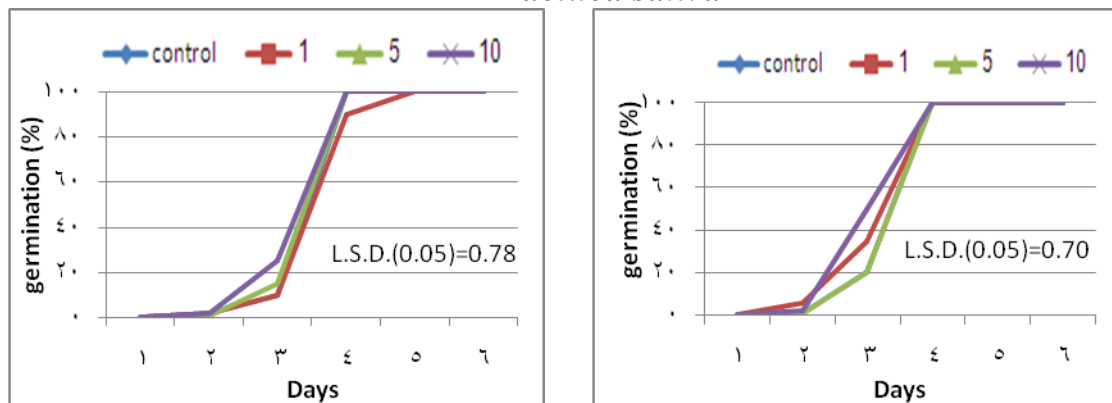
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Coriandrum sativum



Lactuca sativa



Raphanus sativus

Fig(4) Effect of kinetin on seed germination percentage of the *Coriandrum sativum* , *Lactuca sativa* and *Raphanus sativus* in incubator and pots.

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3- Growth characters:

1- Fresh weight:

Data presented in (table 2) show a reduction in fresh weight of the three studied plant as salinity level increased in the soil and Petri dish. The magnitude of fresh weight reduction under saline conditions varied with the different species. Under NaCl concentration of 2.5 , 5 and 10 ds/m , fresh weight was depressed by 1.97, 1.50 and 1.31 gm for *Lactuca sativa* 1.50 , 1.40 and 1.39 gm for *Raphanus sativus* and 1.1, 1 and 1 gm for *Coriandrum sativum*.

Under Kinetin concentration of 1, 5 and 10 ppm, fresh weight was 2.10, 2.10 and 2.30 gm for *Lactuca sativa* 2.75, 2.70 and 3.20 gm for *Raphanus sativus* and 2.32, 2.37 and 2.53 gm for *Coriandrum sativum*, respectively. While under the ethephon concentration of 50, 75 and 100 ppm, fresh weight was 2.10, 2.35 and 2.40 gm for *Lactuca sativa* 2.10, 2.30 and 3.15 gm for *Raphanus sativus* and 2.60, 2.60 and 2.83 gm for *Coriandrum sativum* respectively. These results are in agreement with those obtained by (Mostafa *et al.*, 1984) .This increase might be due to an active role in cell division played by kinetin leading to more growth as well as in the initiation and development of the aerial portions of the plant as mentioned by Mostafa *et al.* (1984).

2 - Dry weight:

The results in table (2) show that salinity at control (0%) significantly increases fresh and dry weight. The reduction in dry weight as a result of salinity proved significant in all tested species. Dry weight of *Lactuca sativa* seedling was reduced by 0.73, 0.62 and 0.51 gm when grown under 2.5, 5, and 10 ds/m NaCl The reduction in dry weight of *Raphanus sativus* plants subjected to the same levels of salinity was 0.75, 0.65 and 0.45 gm in *Raphanus sativus* , and 0.23, 0.22 and 0.20 gm in *Coriandrum sativum* .

Regarding the effect of using growth regulators causes which favored an increase in dry weight of the three species (table 2). The maximum increase in seed germination was obtained by soaking seeds with ethephon of 100 ppm and Kinetin 10 ppm .The results showed that treatment with ethephon at 100 ppm and Kinetin 10 ppm caused significant increase in fresh and dry weigh of seedling.

This results agree with (Jenning *et al.*, 1976); Yasseen *et al.*, 1988) which reported that the effect osmotic salt and Ion acuminate (Na⁺) in plant tissue result to reduction growth process for fresh and dry weight. The mechanism of inhibition of germination and seedling growth by NaCl, maybe related to radicle emergence due to insufficient water absorption, or may be ascribed to toxic effects on the embryo.

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Table (2) Effect salinity levels and growth regulators(ethephon and kinetin) on fresh and dry weight on some plants.

Treatment		<i>Lactuca sativa</i>		<i>Raphanus sativus</i>		<i>Coriandrum sativum</i>	
		Fresh weight (gm)	Dry weight (gm)	Fresh weight (gm)	Dry weight (gm)	Fresh weight (gm)	Dry weight (gm)
control		2.50	0.92	3.20	2.05	2.25	0.51
Salinity	2.5	1.97	0.73	1.50	0.75	1.1	0.23
	5	1.50	0.62	1.40	0.65	1	0.22
	10	1.31	0.51	1.39	0.45	1	0.20
L.S.D.(0.05)		1.23	0.70	2.83	0.08	0.15	1.50
Kinetin	Kin 1 ppm	2.10	0.87	2.75	0.4 9	2.32	0.61
	Kin. 5 ppm	2.10	0.81	2.70	0.62	2.37	0.41
	Kin. 10 ppm	2.30	0.80	3.20	0.81	2.53	0.83
L.S.D.(0.05)		1.44	0.21	1.85	0.70	0.60	0.70
Ethephone	Eth. 50 ppm	2.10	0.90	2.10	0.30	2.60	0.21
	Eth. 75 ppm	2.35	0.91	2.30	0.32	2.60	0.5 4
	Eth. 100 ppm	2.40	0.96	3.15	0.95	2.83	0.67
L.S.D.(0.05)		0.64	2	0.01	2.05	0.54	0.41

Kord and Khalil (1995) reported that the major effect of salinity on germination may be attributed to a reduce hormone delivery throughout the seedling which inhibit the growth.

Table (2) represented the effect of kinetin and ethephon on fresh and dry weight .The data show a significant increase in the fresh and dry weight of seedling .This might be due to the increase in leaf anabolism.The increase and decrease in seedling weight might depend upon the physiological effect of the balance between the growth regulators within the plant which effect directly or indirectly the anabolism beside the katabolism (Mostafa *et al.*, 1984).

Data presented in table (2) show that ethephon at 100 ppm significantly increased the fresh weight of seedling, similar results were obtained by (Gupta and Modam ,1967). Ethephon might enhance the anabolism pathway than the katabolism wich resulted in more reserved stuff (Mostafa *et al.*, 1984).

3- Speed germination

The results in table (3) show that effect of salinity levels and growth regulators (ethephon and kinetin) on seed mean germination time and speed germination index on the *Coriandrum sativum*, *Lactuca sativa* and *Raphanus sativus* . The data show with the increase in salinity levels from the control (zero) to 10 ds/m, the mean germination time decrease in all plant studied. The reduction of germination percentage was signification at 0 -10 ds/m , also germination speed decrease when salinity levels was raised.The best mean

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germination time in ethephon treatment in concentration 100 ppm for all plants studies , Then Kinetin treatment (table 3).

The result confirmed the report of that high salinity levels leads to ion imbalance, osmotic regulation disorders and decrease in water absorption by seeds (Bybordi, 2010). Also data cleared that, speed germination index significantly affected. this was agree with germination percentage also salinity can affect germination of seeds by creating osmotic potentials which prevent water uptake (Poljakoff -Mayber *et al.*, 1994). These results also agreed with (Dubey and Rani, 1989; Khanam *et al.*, 2007 and Cha-um *et al.*, 2009). Kord and Khalil (1995) reported that the major effect of salinity on germination may be attributed to a reduce hormone delivery throughout the seedling which inhibit the growth. Also the same effects for increasing salinity levels was observed on the other seed vigor traits such speed germination index and mean germination time. It can be concluded that the growth regulators effect large and important traits studied in this research, which caused the increase in speed of germination for plants extend and differently by the differences in plant species, and on that basis can we recommend the development of research at the level of the morphological characters and using salt- affected soils.

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تأثير بعض منظمات النمو على أنبات بذور بعض النباتات النامية تحت مستويات ملحية مختلفة

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الخلاصة

أجري هذا البحث في كلية العلوم - قسم علوم الحياة - جامعة البصرة خلال موسم 2010 لدراسة تأثير مستويات من الملوحة كلوريد الصوديوم (0، 2.5، 5، 10) ديسي سيمنز. م⁻¹ ودراسة تأثير منظمي الايثفون بتركيز (50، 75، 100) جزء في المليون والكينتين (1، 5، 10) جزء في المليون على أنبات بذور نباتات الخس *Lactuca sativa* والفجل *Raphanus sativus* والكزبرة *Coriandrum sativum* مع دراسة تأثير التداخل بين الملوحة ومنظمات النمو .

تبين من البحث انخفاض النسبة المئوية للإنبات بارتفاع مستويات الملوحة من (0.0-10) ديسي سيمنز. م⁻¹، كما لوحظ أن بذور النوعين الفجل والكزبرة لا تثبت نهائياً عند التركيز 10 ديسي سيمنز. م⁻¹ بينما كانت نسبة الإنبات في معاملة المقارنة 100 % . كما لوحظ انخفاض النسبة المئوية للإنبات بارتفاع مستويات الملوحة من (0.0-5) ديسي سيمنز. م⁻¹. أدت زيادة مستويات الملوحة إلى انخفاض صفات قوه البذور والتي تم قياسها بواسطة (متوسط زمن الإنبات ودليل سرعة الإنبات) وقوة البادرات والتي تم قياسها بواسطة (الوزن الطري والجاف للبادرة) مقارنة بمعاملة السيطرة . فقد وجد أن زيادة مستويات الملوحة حتى تركيز 10 ديسي سيمنز. م⁻¹ أدى إلى نقص معنوي في الوزن الطري والجاف للبادرات كلما ازدادت تراكيز الملوحة. وقد ظهر من النتائج أن استخدام منظمي النمو الايثفون والكينتين أدى إلى تسريع الإنبات والحصول على أعلى نسبة للإنبات مقارنة مع المعاملة بالتراكيز الملحية وأن التركيزين 100 جزء في المليون من منظم الايثفون و 10 جزء في المليون من منظم الكينتين قد تفوقتا معنوياً عن باقي المعاملات وأعطيتا أعلى نسبة للإنبات ، أما بالنسبة للتداخل بين الملوحة ومنظمات النمو فقد كان لها دور في تشجيع الإنبات في البذور بشكل أكثر تحت الظروف الملحية وكانت لمنظمات النمو تأثير محفز للبذور على الإنبات وذلك بتقليل تأثير الملوحة على أنبات البذور وقد تفوقت المعاملة (75 جزء في المليون + 10 ديسي سيمنز. م⁻¹) لمنظم الايثفون عن باقي المعاملات وكانت النسبة المئوية للإنبات 80% في الفجل *Raphanus sativus* . أما بالنسبة لمنظم الكينتين فقد تفوقت المعاملة (10 جزء في المليون + 5 ديسي سيمنز. م⁻¹) معنوياً وكانت 90% في الكزبرة *Coriandrum sativum* .