Effect of Gibberllic Acid, Sulphuric Acid, Thiourea and Potassium Nitrate on Seed Germination and Seedling Growth of C. Sempervirens L. Pyramidalis and C. Sempervirens L. Horizontalis

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ABSTRACT

As a result of presence of dormancy phenomena in the seeds of the two species of cupressus trees, C. sempervirens L. Pyramidalis and C. sempervirens L. Horizontalis which grown well in green mountain area. Because of the importance of these trees that subjected to extinction in it's natural habitat, like many other trees grown in the green mountain. This study has been carried out to investigate the efficiency of using different concentration from the growth regulator "gibberellic acid", concentrated sulphuric acid and some germination promotion chemicals like thiourea and potassium nitrate, on breaking of their seed dormancy and promotion of seedling growth.

The results showed that the highest percentage of seed germination and seedling development were obtained by treating C. sempervirens L. Horizontalis seeds with concentrated sulphuric acid for 10 min followed by treatment with 100 ppm gibberellic acid for 24 hours. While in case of C. sempervirens L. Pyramidalis seeds, treating with concentrated sulphuric acid for 10 min followed with 50 ppm of gibberellic acid or with potassium nitrate (0.2 %) for 24 hours showed the highest percentage of seed germination and maximum development of seedling. However the concentration 400 ppm of thiourea and gibberellic acid inhibited germination and development of both variety seedlings significantly in compared

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with other treatments including untreated seeds. Finally results showed that there were no significant differences between treatments in calculated mean germination time for the seeds of all species used.

Key Words: Seed dormancy; Cupressus seeds; Horizontalis Variety, Pyramidalis Variety.

INTRODUCTION

Seeds of many non cultivated plants are dormant when first shed and will not sprout even when exposed to adequate moisture, temperature and oxygen (Salisbury & Ross, 1978). This phenomenon is mainly due to the hard seed coat, impermeable to water and gases exchange, to internal physiological seed factors or may be related to both factors together.

In Libya, the green mountain (El-Jable El-Akhdar) is considered to be the one of the most important topographical area because of many wild trees species that could be used as frost trees grown there. Cupressus trees one of the most famous frost trees grown in this area, they cover a large scale of El-Jable El-Akhdar and also they have been classified as one of the plant that originated in El-Jable El-Akhdar (Sherif & El-Taife, 1986).

As a result the area is suffering from mismanagement including over grazing (more than 571.75 domestic animals), trampling, fire, over cutting and up rooting of plant (El-Barasi & Buhwarish, 2005) and also, seeds of Cupressus *sempervirens* L. var horizontalis and var. pyramidalis having dormancy phenomena, the normal vegetation of these varieties (like other plants there) are going to decline time by time (El-bakkosh, 2001).

Many studies have been focused different applications to break seed dormancy in many plant sp. For example, they mentioned that sulphuric acid (H_2SO_4)

from 10 to 98% is generally used to soft the hared seed coat (Hadad, 1995). Plant hormones especially gibberellic acid (GA_3) (Abdalla & Mckelvie, 1980), 0.5 – 3% of thiourea (Ibrahim & Haikel, 1991) and 0.1 – 2% of potassium nitrate (KNO_3) (ISTA, 1993).

The present study is preformed to find the best treatments to break dormancy in the seeds of both cupressus variety which grown in El-Jable El-Akhdar and the effect of these treatments in there vegetation growth was also included.

EXPERIMENTAL

Seed Collection:

The seeds of two cupressus varieties were collected randomly from different parts from El-Jable El-Akhdar (El-beida – WadyEl-Koof – Susa – Ras El-Helal). The seeds were dipping in tap water to eliminate weak, embity and infected seeds. A random selected sample of 100 seeds was chosen for seed viability test by application teterazolium test as in (ISTA, 1993).

Gibberellic Acid And Thiourea Treatments:

The seeds of the two varieties had been treated with concentrated (conc) H_2SO_4 for 10 min first, then soaked in different conc (50, 100, 200 and 400 ppm) of growth regulator GA_3 for 24 h or soaked in thiourea solution $CS(NH_2)_2$ for same conc and time as GA_3. For control treatment, the seeds treated with conc H_2SO_4 as before, followed by soaking in tap water for 24 h.

Potassium Nitrate Treatment:

The seeds of two varieties which have been also treated before with conc H_2SO_4 for 10 min put in plates contain filter papers wet with 0.2% KNO_3 and incubated

for 24 h. The filter papers were wet with water instead of KNO_3 in control treatment.

All treated seeds externally sterilized using 10% of sodium hypochlorite for 5 min followed by washing with distilled water. The seeds of each treatment put in 5 plates (20 seed / plate) and all plates put in growth chamber 30º C/12 h light and 20º C/12 darkness.

Numbers of germinated seeds (emergence of radical for 2 mm) have been accounted for 5 weeks. At the end of the period, the percentage of seed germination (% SG), mean germination time (MGT) using the method of (Rawal, Samant & Dhar, 1998). The growth and development of seedling was following up by measuring mean shoot length (MSL) and mean root length (MRL). A complete randomized design (CRD) was used and the data was statically analyzed by using one – way analysis of variance. The significantly between treatments have been detected by using Tukey's test.

RESULS AND DISCUSSION

The resulted data, revealed a big variation in % SG and growth and development of the seedlings between all treatments Fig 1 & 2. As shown in table 1 and 2, the highest % SG were detected in application of 50 or 100 ppm GA_3 among other treatments in both cuppressus variety (35 and 40.1 % in horizontalis, 39.3 and 33.1 % in pyramidalis variety respectively). The data also shown the positive effect of low conc of GA_3 in increasing MSL and MRL in both varieties, by using Tukey' s test it was found that the highest MSL and MRL were achieved in 50 ppm GA_3 in pyramidalis variety (18.1 and 24.1 mm respectively) table 4. However, 100 ppm GA_3 achieved highest MSL and MRL, while treating with 50 ppm GA_3 or 0.2% KNO_3 comes next with non significant difference between them in horizontalis variety table 3.

Table 1. The effect of gibberellic acid, thiourea and potassium nitrate on the % of seed

germination and mean germination time of *C. sempervirens* var horizontalis.

Treatments	Mean ± Standard Error (S.E)	
	Mean Germination Time (MGT)	% Seed Germination (%SG)
Control	7.300 ± 0.484 a	25.67 ± 2.45 *** c
50 ppm Gibberellic Acid (GA_3)	7.300 ± 0.476 a	35.00 ± 3.84 ac
100 ppm (GA_3)	7.300 ± 0.484 a	40.17 ± 4.49 a
200 ppm (GA_3)	7.300 ± 0.504 a	30.33 ± 3.23 ac
400 ppm (GA_3)	7.300 ± 0.552 a	21.00 ± 2.52 c
50 ppm Thiourea ($CS(NH_2)_2$)	7.100 ± 0.495 a	32.50 ± 3.02 ac
100 ppm ($CS(NH_2)_2$)	7.100 ± 0.490 a	31.01 ± 3.16 ac
200 ppm ($CS(NH_2)_2$)	7.100 ± 0.472 a	30.17 ± 2.84 ac
400 ppm ($CS(NH_2)_2$)	7.100 ± 0.547 a	20.83 ± 2.50 c
0.2 % KNO_3	7.300 ± 0.515 a	31.00 ± 3.41 ac

*** Mean there is a significant at 0.001

Means followed by the same letter, within columns aren't significantly different at 0.05

Level of significance according to Tukey's test.

The positive effect of GA_3 is mainly due to its physiological role in stimulating the conversion of complex compounds and elements in the seeds to sucrose, mobile amino acids or amides and or decreasing the inhibition effect of ABA plant hormone (Devlin 1975). Enhanced cell elongation so the radical can push through the endosperm, the hard seed coat that restricts growth

(Salisbury and Ross, 1987). It over comes both kind of seed dormancy in many plant sp as acting as a substitute for low temperature, long day or red light (Abu-Zeid, 1990).

Table 2. The effect of gibberellic acid, thiourea and potassium nitrate on the % of seed

germination and mean germination time of *C. sempervirens* var pyramidalis

Treatments	Mean ± Standard Error (S.E)	
	Mean Germination Time (MGT)	% Seed Germination (%SG)
Control	7.30 ± 0.63 a	22.83 ± 2.80 *** bce
50 ppm Gibberellic Acid (GA_3)	7.30 ± 0.47 a	39.33 ± 2.27 a
100 ppm (GA_3)	7.30 ± 0.48 a	33.17 ± 3.89 ab
200 ppm (GA_3)	7.30 ± 0.49 a	22.67 ± 2.78 bce
400 ppm (GA_3)	7.30 ± 0.65 a	10.83 ± 1.66 cd
50 ppm Thiourea ($CS(NH_2)_2$)	7.10 ± 0.55 a	23.33 ± 2.89 bce
100 ppm ($CS(NH_2)_2$)	7.10 ± 0.51 a	23.83 ± 3.01 bce
200 ppm ($CS(NH_2)_2$)	7.10 ± 0.56 a	17.83 ± 2.31 cde
400 ppm ($CS(NH_2)_2$)	7.10 ± 0.90 a	8.17 ± 1.4 d
0.2 % KNO_3	7.10 ± 0.54 a	30.17 ± 3.32 ae

*** Mean there is a > p

significant at 0.001

Means followed by the same letter, within columns aren't significantly different

at 0.05

Level of significance according to Tukey's test.

Table 3. The effect of gibberellic acid, thiourea and potassium nitrate on the shoot and root length of *C. sempervirens* var horizontalis.

Treatments	Mean ± Standard Error (S.E)	
	Shoot length / mm SL	Root length / mm RL
Control	3.41± 0.57 ce	4.29 ± 0.66 *** cd
50 ppm Gibberellic Acid (GA_3)	10.72 ± 1.14 a	11.87 ± 1.18 b
100 ppm (GA_3)	17.70 ± 1.90 b	23.01 ± 2.40 a
200 ppm (GA_3)	5.72 ± 0.82 c	7.58 ± 0.98 bc
400 ppm (GA_3)	1.71 ± 0.36 de	2.86 ± 0.47 d
50 ppm Thiourea ($CS(NH_2)_2$)	4.67 ± 0.73 ce	5.56 ± 0.76 cd
100 ppm ($CS(NH_2)_2$)	5.40 ± 0.75 cd	6.85 ± 0.88 cd
200 ppm ($CS(NH_2)_2$)	2.15 ± 0.34 ce	3.60 ± 0.44 cd
400 ppm ($CS(NH_2)_2$)	1.02 ± 0.23 e	2.19 ± 0.36 d
0.2 % KNO_3	3.06 ± 0.52 cde	4.90 ± 0.69 cd

*** Mean there is a > p

significant at 0.001

Means followed by the same letter, within columns aren't significantly different at 0.05

Level of significance according to Tukey's test.

Conversely, the data shown a significant decrease in % SG, MSL and MRL as the conc of thiourea or GA_3 increased, to end up with max decline in the above parameters with high conc (400 ppm) of thiourea or GA_3 with no significant difference with control treatment in both cupressus varity. Waali, 1990 mentioned that thiourea and GA_3 considered as induced growing compounds at certain level and when their conc increased they may be give an adverse effect, so they could be act as growth inhibitors and restrict the growing of radical.

This result came in close agreement with the results of (Nasroun & Al-Mana, 1992; Laura, Alvarenga & Arrigoni 1998; Rawal, Samant & Dhar, 1998).

Statistical analysis however, revealed that there is no significant difference in MGT between all treatments in both cupressus varity. These might be due to the close response – although the variation in %SG- of all treatment in inducing sprouting.

Table 4. The effect of gibberellic acid, thiourea and potassium nitrate on the shoot and root length of C. sempervirens var pyramidalis .

Treatments	Mean ± Standard Error (S.E)	
	Shoot length / mm SL	Root length / mm RL
Control	4.99 ± 0.71 c	5.72 ± 0.80 *** c
50 ppm Gibberellic Acid (GA_3)	18.18 ± 2.10 a	24.10 ± 2.87 a
100 ppm (GA_3)	12.03 ± 1.49 b	13.11 ± 1.57 b
200 ppm (GA_3)	4.44 ± 0.68 c	5.39 ± 0.76 c
400 ppm (GA_3)	1.85 ± 0.36 c	2.23 ± 0.39 c
50 ppm Thiourea ($CS(NH_2)_2$)	3.87 ± 1.17 c	3.45 ± 0.46 c
100 ppm ($CS(NH_2)_2$)	2.75 ± 0.42 c	3.76 ± 0.49 c
200 ppm ($CS(NH_2)_2$)	1.91 ± 0.34 c	2.71 ± 0.43 c
400 ppm ($CS(NH_2)_2$)	0.55 ± 0.17 c	1.15 ± 0.25 c
0.2 % KNO_3	11.42 ± 1.57 b	15.42 ± 2.17 b

*** Mean there is a p

significant at 0.001>

Means followed by the same letter, within columns aren't significantly different at 0.05

Level of significance according to Tukey's test.

CONCLUSION

This study demonstrated that the plant growth regulator GA_3 in a certain degree had a positive effect in breaking seed dormancy in the two cupressus variety studied. The decline in %SG in control treatment in both varieties was mainly due to physiological seed dormancy and partially due to the hard seed coat. The most effective treatment in breaking dormancy and increasing the percentage of seed germination, shoot and root system length was soaking pyramidalis seeds sp in 50 ppm GA_3 and 100 ppm GA_3 in horizontalis variety, after all had being treated with conc H_2SO_4 for 10 min, which is popular use for minimizing hardiness of the seed coat.

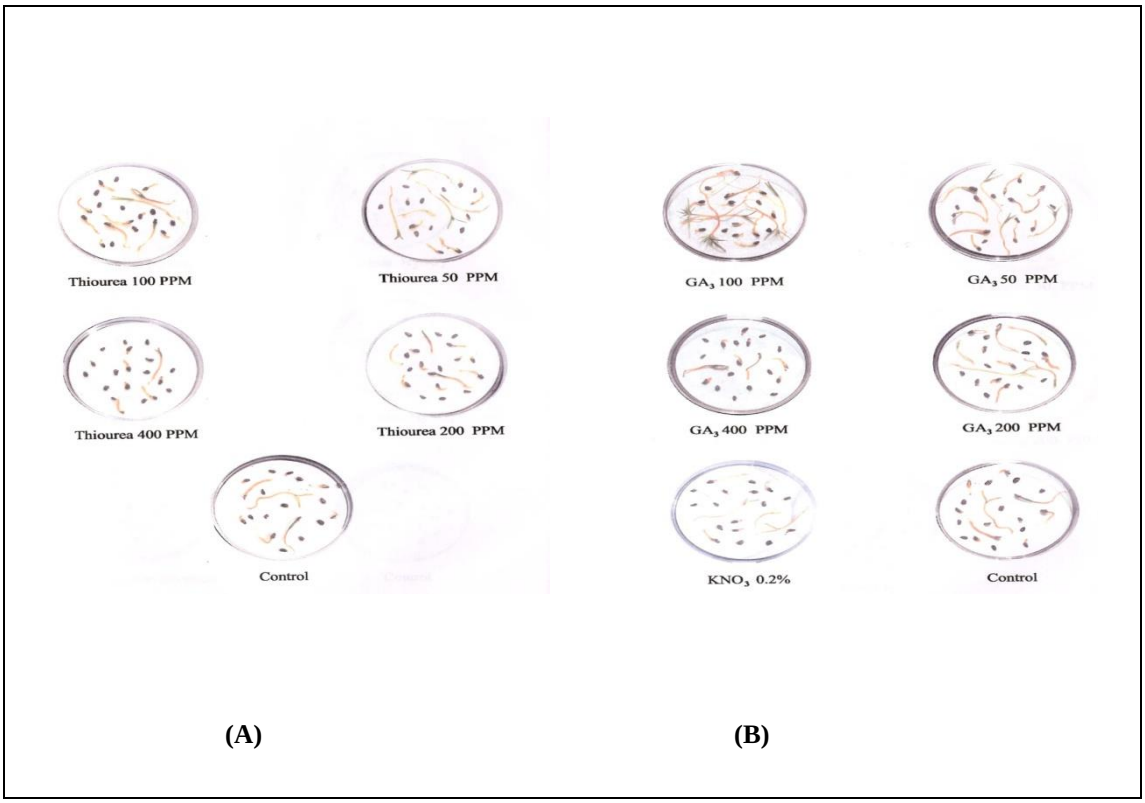

Fig. 1. Effect of (A) CS $(NH_2)_2$ (B) GA_3 and KNO_3 on seed germination of *C . sempervirens* L. Horizontalis.

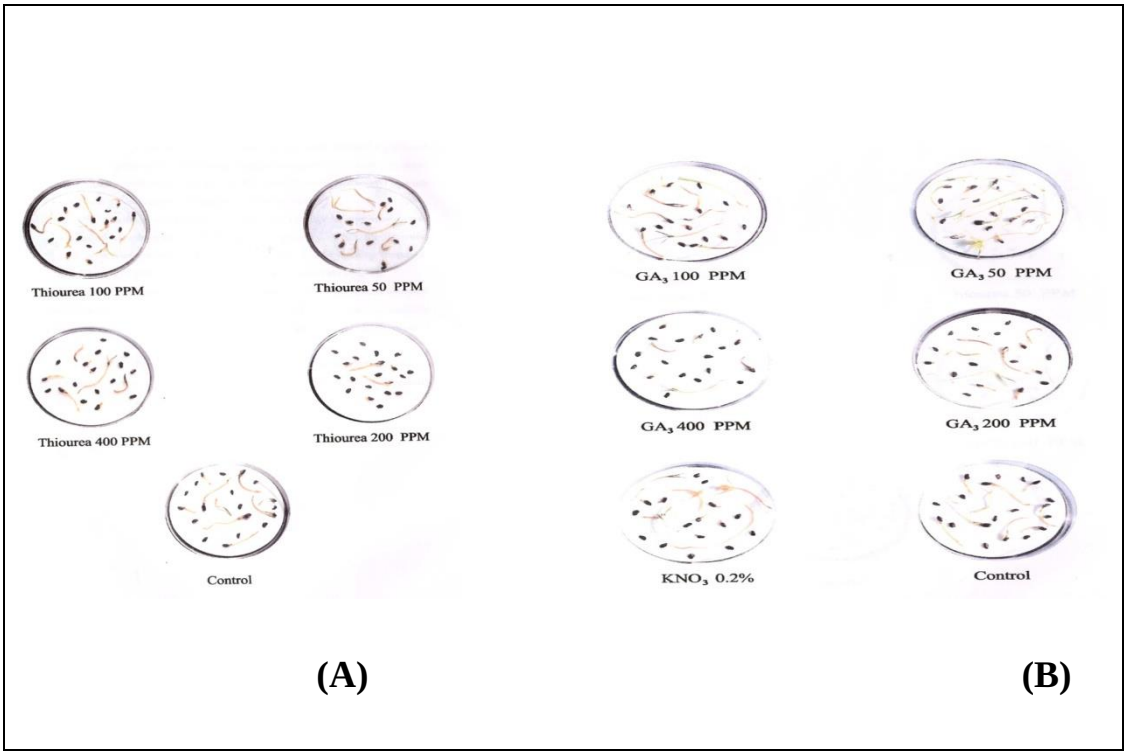

Fig. 2. Effect of (A) CS $(NH_2)_2$ (B) GA_3 and KNO_3 on seed germination of *C . sempervirens* L. Pyramidalis.

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بعض الدراسات البيئية والفسيولوجية لبذور أهم أشجار الغابات النامية في منطقة الجبل الأخضر

ب- تأثير المعاملات بحمض الجبر يليك ، حمض الكبريتيك، الثيوريا و نترات البوتاسيوم علي استنبات و نمو بذور أشجار السرو الأفقي *Cupressus sempervirens* L. Var horizontalis (Mill) و السرو العمودى

Cupressus sempervirens L. Var. pyramidalis

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الملخـــص

نتيجة لوجود ظاهرة السكون في بذور أشجار السرو الأفقي والسرو العمودي مع أهمية هذه الأشجار بمنطقة الجبل الأخضر وتزايد تعرضها لخطر الانقراض جاءت هذه الدراسة لتحقيق التالي:

دراسة تأثير استخدام تراكيز مختلفة من منظم النمو حمض الجبريليك ، حمض الكبريتيك المركز وبعض منشطات الإنبات كالثيوريا, و نترات البوتاسيوم علي الإسراع في إنبات البذور وكذلك مدي تأثيرهم علي نمو وتطور البادرات. أوضحت النتائج تفوق معاملة بذور أشجار السرو الأفقي بحمض الجبريليك تركيز 100 جزء في المليون لمدة 24 ساعة والتي سبق معاملاتها بحمض الكبريتيك المركز لمدة 10 دقائق معنوياً في زيادة النسبة المئوية للإنبات و تطور البادرات. كما أظهرت النتائج تفوق المعاملتان، 50 جزء فى المليون حمض الجبريليك و 0.2 % نترات البوتاسيوم و المعاملتان مسبقاً أيضا بحمض الكبريتيك المركز لمدة 10 دقائق معنوياً في حالة بذور أشجار السرو العمودي علي النسبة المئوية للإنبات وتطور البادرات. في حين سجلت معاملتا بذور أشجار السرو بنوعيه بحمض الجبريليك تركيز 400 جزء في المليون أو الثيوريا 400 جزء فى المليون أدنى نسبة مئوية للإنبات وأقل تطور للبادرات وبفارق معنوي مقارنةً بجميع المعاملات. لم يلاحظ وجود فروق معنوية بين المعاملات المختلفة لبذور السرو بنوعيه في متوسط زمن الإنبات.

مفتاح الكلمات: سكون البذور؛ بذور السرو؛ السرو الأفقى؛ السرو العمودى.

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