In Vitro Preservation of *Ecballium elaterium* L. Plants in Sinai

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Abstract: *Ecballium elaterium* seeds were surface sterilized under a running tap- water with a few drops of a liquid soap then transferred to a solution of 20% clorox (containing 5.25% sodium hypochlorite for 5-20 min.). Finally the seeds were washed three times with a distillated water to remove all traces of chlorine. Seeds were germinated on a solidified MS medium supplemented with 3% sucrose, 0.5 mg l^{-1} NAA, 0.5 mg l^{-1} 2,4-D and 0.8% agar. The present work aimed to clarify to which extent plantlets and callus of *Ecballium elaterium* tolerate preservation period for 1-6 months, suitable temperature storage (4 and 22°C) and capacity to tolerate both sucrose (30, 45 and 60 g/l) and mannitol (0.0, 10.0 and 15.0 g/l) concentrations in the medium without subculture, besides study effect of photoperiod conditions (dark and light) on vegetative traits for plantlets and callus. The results showed that culturing on MS medium with sucrose at 60 g/l for 3 months at 4°C in dark was the best condition for plantlet and callus preservation. While, the best callus fresh weight resulted from preservation was obtained on MS medium with 15g/l mannitol at 22°C in light for four months.

Keywords: *Ecballium elaterium,* plantlet, callus, temperature, preservation, mannitol, sucrose, MS, 2,4-D, kin, BAP.

INTRODUCTION

Ecballium elaterium L. A. Rich (Fam. *Cucurbitaceae*), a powerful hydragogue cathartic known as squirting cucumber. Perennial somewhat fleshy monoecious. Stems 30-100 cm. long, rough hairy, prostrate, branched from base, tendrils absent. Leaves 6-10 cm. long petiole, triangular to heart-shaped, rough on the upper surface, density white hairy on the lower surface. Flowers greenish-yellow; female flowers in the leaf axils, usually solitary; male flowers in racemes. Fruit a large juicy berry, 3-5cm anote-oblong, nodding, detaching it self explosively at maturity scattering seeds and juice. Flowering in March and September or all year (Tackholm, 1974). In folk medicine the roots are used as analgesic and in treatment of hemorrhoids, fruits in sinusitis Jaundice, nocturia, lumbago and otalgia (Toker *et al.,* 2003). The *Ecballium elaterium* L. plant is one of the in danger plants due to that the growth of plant depends upon the available rain fail in Sinai in addition the intensive grazing which may cause the lost of this plant.

It has been proved, particularly in the recent time that the purpose of germplasm preservation is to ensure the availability of useful germplasm at any time. Attempts in the *in vitro* storage of plant material have focused on imposing some sort of stress on the cultures in order to achieve reduced growth. One mean of growth limitation has been attained by reducing temperatures to near freezing. Braun (1988) found on medicinal plants, that the germplasm remains viable and can be grown into plants in case of immediate demand when culture stored in the refrigerator at 4°C for up to 18 months. Engelmann (2000) reported that *in vitro* conservation and cryopreservation offer the only safe and cost-effective option for long term conservation of genetic resources of recalcitrant species. Drew (2000) found that the medium-term conservation strategies have been developed for numerous species but their routine use is still restricted with limited application in tropical species. Amoroso (2006) suggested using *in vitro* culture as an *ex-situ* conservation and propagation strategy for some endemic, endangered, forests of some ornamental plant for economically important plants. Sharma, *et al.* (2006) studied that rate of germplasm preservation through excised root culture and found that it may promote establishment of *in vitro* gene banks of elite genotypes of neem requiring diverse agroclimates.

The objective of this work was to study the effect of osmotic inhibitors, preservation temperature and photoperiod conditions on reducing vegetative growth of the callus and plantlet of *Ecballium elaterium.*

MATERIALS AND METHODS

This work was carried out in the Plant Tissue Culture Laboratory at El-Sheik Zuwyed Research Station, Desert Research Center (DRC), North Sinai Governorate, Egypt during the period from 2004 to 2006.

Ecballium elaterium L. Fam. cucurbitaceae seeds were collected from El-Sheik Zuwyed region, North Sinai. The seeds were surface sterilized under a running tap water with a few drops of a liquid soap, then transferred to a solution of 20 % clorox (containing 5.25 % sodium hypochlorite for 5-20 min. Finally, they were washed three times with a distillated water to remove all traces of chlorine. The seeds were germinated on a solidified MS medium supplemented with 3% sucrose, 0.5 mg l^{-1} NAA, 0.5 mg l^{-1} 2,4-D and 0.8 % agar.

The callus was maintained on MS medium supplemented with 2 mg l^{-1} 2, 4-D and 0.1 mg l^{-1} kin. plantlet and callus tissues of 0.5 – 1.0g were transferred to MS medium supplemented with 0.05 mg l^{-1} NAA, 1mgl^{-1} BAP, different concentrations of mannitol (0.0, 10 and 15g l^{-1}) and Different concentrations of sucrose (30, 45 and 60 g l^{-1}) for growth studies under osmotic stress. The different storage conditions treatments for callus and plantlet preservation are shown in (Table 1).

Samples were taken for measurements after one month of cultivation (shoot length (cm), shoot number, leaf number, and callus fresh weight (g)) until the end of experiments.

The obtained data were subjected to statistical analysis according to Snedecor and Cochran (1990).

Table (1): Different storage conditions {(4 and 22 °C), (dark, light)} and storage treatments (mannitol; (0.0, 10 and15 g l^{-1}), sucrose (30, 45 and 60 g l^{-1}) for preservation media for *Ecballium elaterium* scallus and plantlets.

Treatments			Concentration ($g l^{-1}$)
Temp.	light	Sugar kind	
4°C	24hr.D.	Mannitol	0
			10
			15
		Sucrose	30
			45
			60
	16hr.L.+ 8 hr. D.	Mannitol	0
			10
			15
		Sucrose	30
			45
			60
22°C	24hr.D.	Mannitol	0
			10
			15
		Sucrose	30
			45
			60
	16hr.L.+ 8 hr. D.	Mannitol	0
			10
			15
		Sucrose	30
			45
			60

Temp. = Temperature preservation, hr = hours, D = dark, L= light, g= grams, l = liter.

RESULTS AND DISCUSSION

Effect of temperature

Data in Table (2) indicate that preservation at 22°C gave higher values for shoot length (cm), number of shoot/ explant, number of leaves/ shootlet and callus fresh weight (g) than those with preservation at 4°C. The highest values were 3.76cm for shoot length, 2.88 for leaf number/ shootlet, 1.09 for shoot number/ explant and 2.78g for callus fresh weight with 22°C treatment. However, the lowest values for vegetative traits in this respect were 3.64cm, 0.49, 1.50 and 1.12g, respectively, under preservation at 4°C. These results are in agreement with Staritsky, *et al.* (1986) who found on some tropical crops, that increasing storage temperature (9, 13, 17 and 25°C) gave normal growth criteria including the green shoots, number of newly doubling shoots, number of dying leaves and number of transplanted plantlets formed in each tube as comparing to storage the plantlet, at low temperature (4°C). Also, Bertrand-Desbrunais (1991) and Kubota and Kozai (1994) reported that growth reduction of callus fresh weight was achieved with temperature reduction. In addition, Miedema (1982) reported that at low temperature (1-10°C) the shoot cultures growth *in vitro* was reduced for many tropical plants.

Effect of photoperiod

Results in Table (3) indicate that storage under light gave an increase in number of leaves/ shootlet, number of shoots/ explant and callus fresh weight, while storage at dark decreased these values. In this respect, the differences were significant. Concerning shoot length, the increase was significant under storage at dark and gave the maximum value 3.32cm. while, storage under light conditions resulted the minimum shoot length (2.16 cm.). These results are in harmony with Staritsky, *et al.* (1986) who found that in the dark the culture became etiolated and most of those in the light browned and died and shoot incubated in the light browned green color and did not suffer etiolation. Also, Armitage, *et al.* (1990) found that *Oxypetalum caeruleum* subjected to photoperiod reduction produced the opposite effect on decreasing with the liner increase in stem length, node number and liner decrease in stem diameter and number of stem.

Effect of preservation period

Table (4) show that long storage period (4, 5 and 6 months) generally decreased shoot length, shoot number/ explant and leaf number/ shootlet as compared to storage for 2 and 3 months. Shoot length, leaf number/ shootlet and callus fresh weights were increased at short storage period (1-3 months) with highly significant different over long storage period (4-6 months) treatments .

These results are in line with those obtained by Tyagi and Prakash (2004) who found that a significant difference was observed for the conservation period of *Simmondsia chinensis* (Link) jojoba cultures of all the genotypes on MS+10 µm BA which supported the shoot cultures of jojoba especially maximum conservation period for (16 months).

Also, Malik, *et al.* (2005) reported that *in vitro* conservation of *Garcinia indica* could be achieved by reducing the growth rates using different strategies under the longest subculture duration of 10-11 months on reduced BAP concentration of 0.5 µM and keeping sucrose concentration at 3%.

Effect of sugar concentration

Data in Table (5) show that storage at sugar concentration of 60g/l sucrose gave the maximum shoot length, leaf number/ shootlet and shoot number/ explant while, 15g/l mannitol gave the highest value of callus fresh weight. Similar results were reported by Hunter, (1986) who found that increasing concentration of mannitol generally resulted in an increased number of axillary buds. Also, Sairam, *et al.* (2003) concluded that the effect of mannitol was comparable with other sugars tested at day seven and earlier, a marked decrease in the growth of callus was observed after 21 days of culture. Continuous incubation on mannitol for 28 days resulted

Effect of the interaction between temperature and photoperiod on plantlet vegetative characters and callus fresh weight:

Data in Table (6) indicate that the interaction between temperature and photoperiod (light or dark) caused an increase in leaf number/ shootlet, shoot number/ explant and callus fresh weight when conservation was in light compared to storage at dark. However, under light treatment, leaf number/ shootlet, shoot length, shoot number/ explant and callus fresh weight were highly significant increased. Similar results were obtained by Mullin and Schlegel (1976) on strawberry (*Fragaria* × *ananassa*) and Cantos, *et al.* (1998) on *Atropa baetica.*

Effect of temperature and storage period interaction on vegetative characteristics and callus fresh weight:

Data in Table (7) indicate that plantlet and callus stored for 4 months at 22°C showed vigorous growth for shoot length, leaf number/ shootlet, shoot number/ explant and callus fresh weight as compared with storage at 4°C which gave lower values in this respect, after 4 months of storage shoot length, leaf number/ shootlet, shoots number/ explant of plantlet and callus fresh weight did not increase regardless of the storage treatment causing stem distortion and leaves yellowing. These results are in harmony with Kubota and Kozai (1994) on *Brassica oleracea* plant and Yabe and Ogawa (1995) on *Japanese butterbur.*

Table (2): Effect of temperature on some plantlets vegetative characters and callus fresh weight for *Ecballium elaterium.*

Temp.	Plantlets vegetative characters			Callus fresh weight (g)
	Shoot length (cm)	Leaf number / shootlet	Shoot number / explant	
22 °C	3.76	1.09	2.88	2.78
4 °C	3.64	0.49	1.50	1.12
L.S.D $_{p<0.05}$	0.49	0.25	0.38	0.33

Table (3): Effect of photoperiod on plantlets vegetative characteristics and callus fresh weight for *Ecballium elaterium.*

Photoperiod	Plantlets vegetative characters			Callus fresh weight (g)
	Shoot length (cm)	Leaf number / shootlet	Shoot number / explant	
Light	2.06	1.66	0.49	2.22
Dark	5.34	2.73	1.13	1.69
L.S.D $_{p<0.05}$	0.49	0.38	0.26	0.33

Table (4): Effect of preservation period (months) on plantlet vegetative characteristics and callus fresh weight for *Ecballium elaterium*

Months	Plantlets vegetative characters			Callus fresh weight (g)
	Shoot length (cm)	Leaf number / shootlets	Shoot number / explant	
1	5.76	3.1	0.95	2.06
2	5.31	2.66	0.97	2.12
3	5.51	2.91	1	2.62
4	3.62	2.09	0.85	2.48
5	1.98	1.77	0.74	2.41
6	1	0.62	0.24	0.00
L.S.D $_{P<0.05}$	0.86	0.65	0.44	0.58

Table (5): Effect of sugar concentration on plantlet vegetative characteristics and callus fresh weight for *Ecballium elaterium:*

Type	Sugar con.	plantlets vegetative characters			Callus fresh weight (g)
		Shoot length (cm)	Leaf number / shootlet	Shoot number / explant	
Mannitol	0.0	2.13	1.65	0.38	1.93
	10	3.15	1.22	0.38	1.79
	15	2.86	1.47	0.46	2.34
Sucrose	30	4.22	2.10	0.68	1.79
	45	4.17	2.36	0.88	1.96
	60	5.67	4.35	1.98	1.89
L.S.D $_{P<0.05}$		0.86	0.65	0.44	0.58

Table (6): Effect of the interaction between temperature and photoperiod on plantlet vegetative characteristics and callus fresh weight.

Temp.	Photoperiod	Plantlets vegetative characters			Callus fresh weight (g)
		Shoot length (cm)	Leaf number / shootlet	Shoot number / explant	
22°C	Light	4.11	3.30	0.90	3.18
	Dark	3.41	2.47	1.28	2.38
4°C	Light	0.0	0.0	0.0	1.26
	Dark	7.26	2.99	0.98	0.99
L.S.D $_{P<0.05}$		0.70	0.53	0.36	0.47

Effect of temperature and sugar concentration on vegetative characteristics and callus fresh weight:

Data in Table (8) show that shoot length, leaf number/ shootlet and shoot number/ explant were decreased at the high concentration of mannitol (15g/l) at 4°C and 22°C. However, leaf number in high mannitol concentration gave the maximum value. While, storage at sucrose concentration 60g/l gave the maximum shoot length, leaf number/ shootlet, shoot number/ explant and callus fresh weight at 22°C.

Effect of photoperiod and storage period on vegetative characteristics and callus fresh weight:

Data in Table (9) reveal that the interaction between storage periods until three months at dark conservation had significant increase in shoot length leaf number/ shootlet and shoot number/ explant. Callus fresh weight was nonsignificantly increased under the same storage period (3 months) under light conservation when compared with conservation at dark. However, data show that leaf number/ shootlet, shoot number/ explant and callus fresh weight were decreased with increasing storage periods to reach minimum after six months at light conditions. While, the maximum value was at light moderate storage period of 3 months. Similar results were obtained by Staritsky, *et al.* (1986) who found that shoots incubated in the dark was remained green in color and did not suffer etiolation, but, in the light many leaves browned and died. A lower light intensity might be less damaged in both the light and dark treatments; the processes of etiolating of shoots in the dark and their browning in the light were greatly accelerated, resulting in exhaustion of the medium and death of the cultures after 20-30 weeks.

Effect of photoperiod and sugar concentration:

Data in Table (10) show that shoot length, leaf number/ shootlet and shoot number/ explant were decreased at the high concentration of mannitol (15g/l) under light. Storage at sucrose concentration of 60g/l gave the maximum value of shoot length, shoot number/ explant and leaf number/ shootlet at dark.

Effect of the interaction between storage period and sugar concentration:

Data in Table (11) show that the interaction between storage period and sugar concentration gave highly significant increase with increasing both preservation period and sucrose level up to two months at 45 g/l for shoot length which reached the maximum values. On the other hand, shoot length and shoot number/ explant were nonsignificantly affected. Leaf number/ shootlet and callus fresh weights were affected with highly significant different. Similar results were obtained by Drew (1992)who recorded that the plants on sucrose for three increased grow and abscessed all leaves after 2 month, after 4 months the plants in sucrose although still dormant had healthy a axillary buds. After 12 months, one third of the plants were achieved on sucrose. Similar results were obtained by Mamiya and Sakamoto (2000) they studied the effects of sugar concentration and strength of basal medium was studied to produce fest asparagus plants form somatic embryos as there was a significant difference among concentrations of sugar but not among kinds of sugar for shoot when the sucrose concentration was 10, 30, or 50g l^{-1} as the fresh weights of shoots were 31.5, 14.9, or 8.6 mg per plant. There was a significant difference due to basal medium strength in shoot growth under culture in half, full, or double strength basal medium as the fresh weights of shoot were 8.9, 31.0 or 60 mg per plant, respectively. The effect of sugar concentration and strength of basal medium were also studied in the post-culture process to produce encapsulatable units, and in their conversion not only the sugar concentration in the conversion medium but also the growth of shoots. These results go in harmony with those obtained by Hunter (1986) who found that at the end of 65 day period it is possible that the medium had become limiting in sucrose. The high CO_2 and low O_2 concentration may have induced physiological changes that result in growth stunting.

Effect of the interaction among temperature, photoperiod and storage period on plantlet vegetative characteristics and callus fresh weight:

Data in Table (12) show that, temperature (4, 22°C), storage period (1, 2, and 3months) and photoperiod (light and dark) had highly significant effect on leaf number/ shootlet. On the other hand, this interaction was significant on shoot length and callus fresh weight. Also, data showed that maximum value for all vegetative traits were obtained from short stage period (3 months)and storage in 4 °C at dark. While, the minimum values of vegetative traits were resulted from long storage period (3months) at 22°C, under dark storage comparing with other treatments. Furthermore, with increasing storage period under dark conditions for mostly decreased vegetative traits. However, the different vegetative traits were increased with increasing storage period under each dark and light. These results go in harmony with those obtained by **Hunter** (1986) who found that at the end of 65 days period it is possible

Table (7): Effect of temperature and storage period interaction on plantlets vegetative characters and callus fresh weight for *Ecballium elaterium*.

Temp.	Month	Plantlets vegetative characters			Callus fresh weight (g)
		Shoot length (cm)	Leaf number/ shoot let	Shoot number/ explant	
22°C	1	5.61	3.92	1.39	2.19
	2	5.61	3.92	1.44	2.56
	3	5.60	4.17	1.50	3.28
	4	2.87	2.42	1.03	4.57
	5	2.87	2.83	1.17	4.07
	6	0.0	0.0	0.0	0.0
4°C	1	5.09	2.28	0.51	1.92
	2	5.01	1.89	0.51	1.68
	3	5.41	1.15	0.51	1.95
	4	1.09	1.12	0.45	0.39
	5	2.37	1.34	0.53	0.76
	6	2	1.23	0.48	0.0
L.S.D $_{P<0.05}$		1.22	0.93	0.62	0.82

Table (8): Effect of temperature and concentration of sugar on plantlet vegetative characteristics and callus fresh weight for *Ecballium elaterium*.

Temp.	Sugar con.	Plantlets vegetative characters			Callus fresh weight (g)
		Shoot length (cm)	Leaf number/ shootlet	Shoot number/ explant	
22°C	0.0	1.37	2.48	0.51	2.57
	10	3.68	1.67	0.51	2.52
	15	3.21	2.33	0.67	3.00
	30	2.64	1.95	0.84	2.86
	45	4.45	2.64	0.97	2.61
	60	7.22	6.20	3.06	3.06
4°C	0.0	2.90	0.81	0.26	1.29
	10	2.62	0.76	0.26	1.06
	15	2.50	0.59	0.26	1.62
	30	5.80	2.26	0.53	0.72
	45	3.88	2.09	0.78	0.86
	60	4.11	2.51	0.89	1.17
L.S.D $_{P<0.05}$		1.22	0.93	0.62	0.82

Table (9): Effect of photoperiod and storage period on plantlet vegetative characteristics and callus fresh weight for *Ecballium elaterium* .

Photoperiod	Month	Plantlets vegetative characters			Callus fresh weight (g)
		Shoot length (cm)	Leaf number/ shootlet	Shoot number/ explant	
Light	1	2.98	2.01	0.51	2.34
	2	2.98	2.01	0.56	2.47
	3	3.02	2.26	0.62	3.42
	4	1.69	2.04	0.45	2.71
	5	1.69	1.62	0.59	2.35
	6	0.00	0.00	0.00	0.00
Dark	1	7.65	4.20	1.39	1.77
	2	8.54	3.81	1.39	1.77
	3	8.00	3.06	1.39	1.81
	4	3.56	2.14	1.12	2.25
	5	2.27	1.9.00	1.03	2.48
	6	2.00	1.23	0.48	0.00
L.S.D $_{P<0.05}$		1.22	0.92	0.92	0.82

Table (10): Effect of photoperiod and sugar concentration on plantlet vegetative characteristics and callus fresh weight for *Ecballium elaterium*.

Photoperiod	Sugar con.	Plantlets vegetative characters			Callus fresh weight (g)
		Shoot length (cm)	Leaf number/ shootlet	Shoot number/ explant	
Light	0.0	0.41	1.23	0.26	2.26
	10	1.93	0.92	0.26	2.72
	15	1.59	1.01	0.26	2.05
	30	1.25	0.98	0.42	1.87
	45	2.99	1.67	0.42	2.28
	60	4.17	4.12	1.12	2.12
Dark	0.0	3.85	2.06	0.51	1.61
	10	4.36	1.51	0.51	1.53
	15	4.12	1.92	0.67	1.96
	30	7.18	3.22	0.94	1.71
	45	5.34	3.06	1.3	1.64
	60	7.17	4.58	2.83	1.65
L.S.D $_{P<0.05}$		1.22	0.93	0.62	0.82

Table (11): Effect of the interaction between storage period and concentration of sucrose on plantlet vegetative characteristics and callus weight on *Ecballium elaterium*.

Month	Sugar con.	Plantlets vegetative characters			Callus fresh weight (g)
		Shoot length (cm)	Leaf number/ shootlet	Shoot number/ explant	
1	0.0	4.29	4.00	0.75	3.37
	10	6.71	3.00	0.75	3.04
	15	5.71	2.92	0.92	2.92
	30	6.09	2.59	0.75	1.00
	45	4.59	2.34	0.86	0.84
	60	6.25	3.75	1.67	1.15
2	0.0	4.21	3.25	0.75	2.77
	10	7.17	2.59	0.75	2.29
	15	5.04	3.34	.92	2.49
	30	4.46	2.17	.75	1.41
	45	4.88	2.50	0.84	2.33
	60	7.03	3.59	1.84	1.44
3	0.0	5.89	2.59	.75	1.61
	10	4.25	1.67	0.75	1.33
	15	6.34	2.50	0.92	3.49
	30	4.63	2.67	0.75	2.68
	45	5.02	2.09	0.84	3.40
	60	6.92	4.42	2.00	3.20
4	0.0	0.00	0.00	0.00	1.83
	10	0.00	0.00	0.00	1.78
	15	0.00	0.00	0.00	2.74
	30	2.38	2.25	0.84	2.80
	45	3.79	2.67	1.00	3.08
	60	5.67	0.00	2.59	2.66
5	0.0	0.00	5.67	0.00	1.99
	10	0.00	0.00	0.00	2.30
	15	0.00	0.000	0.00	2.40
	30	4.46	2.42	0.75	2.82
	45	4.92	2.84	1.17	2.11
	60	6.32	7.25	3.17	2.868
6	0.0	0.00	0.00	0.00	0.00
	10	0.00	0.00	0.00	0.00
	15	0.00	0.00	0.00	0.00
	30	3.30	1.09	0.26	0.00
	45	1.80	1.17	0.59	0.00
	60	0.91	1.42	0.59	0.00
L.S.D $_{P<0.05}$		2.11	1.60	1.08	1.42

Table (12): Effect of the interaction among temperature, photoperiod and storage period on plantlet vegetative characteristics and callus fresh weight of *Ecballium elaterium*.

Temp	Photoperiod	Months	Plantlets vegetative characters			callus fresh weight (g)
			Shoot length (cm)	Leaf number/ shootlet	Shoot number/ explant	
22°C	Light	1	5.94	4. 00	1.00	2.00
		2	5.94	4. 00	1.11	2.71
		3	6.02	4.50	1.22	5.41
		4	3.37	3.23	1.78	4.24
		5	3.37	4.06	1.17	4.69
		6	3.00	3.83	0.89	0.00
	Dark	1	5.28	0.00	0.00	2.37
		2	5.28	3.83	1.17	2.40
		3	5.18	1.62	1.78	2.33
		4	2.37	3.83	1.78	3.74
		5	2.37	1.62	1.17	3.45
		6	0.00	0.00	0.00	0.00
4°C	Light	1	0.00	0.00	0.00	2.67
		2	0.00	0.00	0.00	2.22
		3	0.00	0.00	0.00	2.60
		4	0.00	0.00	0.00	0.00
		5	0.00	0.00	0.00	0.00
		6	0.00	0.00	0.00	0.00
	Dark	1	10.82	4.56	1.00	1.17
		2	10.02	3.78	1.00	1.14
		3	11.81	2.28	1.00	1.30
		4	2.17	2.23	0.89	0.77
		5	4.74	2.67	1.06	1.52
		6	3.99	2.45	0.95	0.00
	L.S.D $_{p<0.05}$		1.72	1.31	0.88	1.56

either that the medium had become limiting in sucrose or that high CO_2 and low O_2 concentration may have induced physiological changes that results in growth stunting.

CONCLUSION

These results showed that the best preservation condition of *Ecballium elaterium* were at temperature of 4°C, in dark and the modified MS medium with sucrose concentration was 60g/l for three months. Shoot length, leaf number/ shootlet and callus fresh weight of *Ecballium elaterium* were preserved on 15g/ l mannitol. Also, modified MS medium at 22°C in light for four months.

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الحفظ المعملي لنبات بلحة بن جحا في سيناء

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عقمت بذور نبات بلحة بن جحا تحت الماء الجاري مع إضافة بضع نقط من الصابون السائل، ثم نقلت إلى محلول ٢٠% كلوراكس (يحتوى على ٥,٣٥% صوديوم هيبوكلوريت) لمدة ٥-٢٠ دقيقة وفى النهاية تم الغسيل ثلاث مرات بالماء المقطر المعقم لإزالة بقايا الكلور، ثم زرعت على بيئة إنبات موراشج وسكوج مضافا إليها ٣% سكروز و ٢/١ مليجرام /لتر نفثالين حمض الخليك و ٢/١ مليجرام ٢,٤ ثنائي كلورفينوكسى حمض الخليك و٨% أجار. وقد خزنت النباتات والكالس على بيئة موراشج وسكوج ٠,٠٥ مليجرام نفثالين حمض الخليك و١ملجرام /لتر بنزيل أدنين بيورين بتركيزات من المانيتول (صفر، ١,٠، ١,٥ %) و بيئات أخري تحتوي علي تركيزات من السكروز (٣، ٤,٥، ٦,٠%) وذلك تحت ظروف من الإضاءة (١٦ساعة) والإظلام التام (٢٤ ساعة) علي درجات حرارة ٤، ٢٢°م لدراسة أفضل ظروف حفظ للكالس والنباتات. وأوضحت النتائج المتحصل عليها أن أفضل ظروف للحفظ لنبات بلحة بن جحا كانت علي درجة حرارة ٤°م في الإظلام التام عند تركيز ٦,٠% سكروز لبيئة معدلة من موراشج وسكوج لمدة ثلاثة أشهر. بينما أفضل وزن طازج للكالس لنبات بلحة بن جحا كان عند الإضاءة ١٦ ساعة في درجة ٢٢°م بتركيز ١,٥ % من المانيتول لبيئة معدلة من موراشج وسكوج لمدة أربعة أشهر.