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In vitro conservation of *Populus alba* and *Melaleuca ercifolia* germplasm

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ABSTRACT

Slow growth storage, for up to 12 months, has been achieved for *Populus alba* and *Melaleuca ercifolia* explants using seven treatments of conservation. Storage explants on MS medium under low temperature at 4ºC, addition of activated charcoal (AC) at 3g/l and supplemented with either osmotic active compound (mannitol at 45.5 mg/l or 9.1 mg/l) or inhibitor substance (cycocel at 25 mg/l or 2.5 mg/l) as well as control media under normal conditions cultures at 25±1ºC were achieved. The survival percentage, shooting behavior and rooting rate evaluated after 4, 8 and 12 months of storage. Micropropagation ability of conserved explants and the change of some endogenous chemical compound were investigated after each three storage periods. The data revealed that, low temperature at 4 ºC was the best treatments to conserve explants of *Populus alba* and *Melaleuca ercifolia* explants with keep the highest survival percentage. While supplementation the conservation media with (AC) gave significant increased in shooting behavior, rooting percentage and micropropagation characters of explants after conservation periods. The pigments, total phenols and total indoles on conserved explants were declined significantly with increasing the periods of storage.

Key words: *In vitro* conservation, *Populus alba storage, Melaleuca ercifolia,* mannitol, low temperature, a ctivated charcoal.

Introduction

In the last ten years, in vitro storage of cultures has been applied with varying degree of success to a wide range of species and culture systems and successful slow growth systems were developed for different species (Engelmann, 1991; Janeiro *et al.,* 1995). Growth reduction is generally obtained by lowering the culture temperature, reducing or suppressing light intensity, applying growth inhibitors or osmotic substance to the culture media (Mkumbo *et al.*, 1998 and Dodds, 1991).

The poplar (*Populus spp.)* is rapidly being adopted as a model for studying different aspects of forest tree genetics for a number of reasons: its relatively small genome size, considerable genetic variation (both natural and resulting from breeding activity), fast juvenile growth, case of *in vitro* clonal propagation and efficient transformation (Taylor 2002).

Also, *Melaleuca ercifolia* Smith is an evergreen tree and an excellent landscape, street trees and sheared hedges. It grows well even in poor alkaline soils and stands high temperature, exposure to wind, and salt air. From leaves the volatile oils, which have antimicrobial and preservative properties, are widely applied for several medicinal and pharmaceutical purposes (Everett, 1981; Brophy *et al.,* 1990 and Aboutable *et al.* 1991).

Thus, the present study aims to find a suitable protocol for conserve each of the two forest tree *Poplus alba* and *Melaleuca ercifolia* for short – term conservation methods under *in vitro* conditions, and investigate the effect of different conservation treatments on the longevity rate, growth behavior and biochemical composition of explants *in vitro.* In addition, investigate the micropropagation potentiality of the conserved explants after the end of each periods of the storage conditions.

Materials and Methods

This study has been carried out in the Tissue Culture and Germplasm Conservation Res. Lab., Hort. Res. Institute (ARC) Egypt, during the period from 2008 to 2011.

Explant source and disinfection:

The explants used were taken from *Populus alba* L. and *Melaleuca ercifolia* Smith shrubs grown at green house of the Tissue Culture and Germplasm Conservation Res. Lab., Hort. Res. Institute (ARC) Giza. The shoot tips explants were washed in soapy water using septol soap, then agitated in disinfectant solution of savlon (3%)

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for 30 min and rinsed with running tap water for one hour. Thereafter, under aseptic 30 sec in 70% ethanol, then 20% (V/V) Clorox for 10 min, further sterilized 15 min in 0.1 mercuric chloride solution (w/v) , emended with a few drops of tween-20 as emission, and gently rinsed in sterile distilled water for three times. After culturing on MS (Murashig and Skoog, 1962) medium, the obtained shootlets of the *in vitro* plantlets were aseptically microcut (2 -5 mm) and used as source of the explants to serve in all of the conservation treatments.

Culture media:

In both conservation and micropropagation experiments, the basal salts of MS medium at full strength enriched with benzylaminopurine (BAP) (0.5 mg/l), indole-3-butyric acid (IBA)0.1 mg/l) and sucrose (25g/l) for *Populus alba* explants while for *Melaleuca ercifolia* the basal salts of MS medium at full strength provide with IBA (0.5 mg/l), Kintien (1 mg/l), casein (100 mg/l) and sucrose (25 g/l). After adjusting the pH to 5.7±0.1 and adding Anchamia agar (7g/l), 25 ml of the prepared medium were dispensed into 200 ml- capacity glass jars before autoclaving at 121ºC and 1.0 k/cm^2 for 20 min.

Incubation conditions:

The cultures of *in vitro* conservation and micropropagation trials were inocubated in growth chamber at 24ºC ± 1 day/ night temperature and 3 k lux light intensity from 110 cm fluorescent lamps (40 watts) for 16 hours photoperiod.

Procedures and experiments:

The study split into two experiments as follows:

First experiment: Effect of different *in vitro* conservation treatments on longevity rates, growth characters and chemical composition of the germplasm explants.

For each one of the two germplasms of studies wood tree species, twenty five explants in five replicates were used. They were submitted to seven conservation treatments for three stage periods (***e.g.*** 4, 8 and 12 months) as follows:

- Low temperature, the *in vitro* cultures were kept in a refrigerator at 4ºC under dark conditions.
- Control treatment, the *in vitro* cultures were stored without using any conservation agents.
- The media of the *in vitro* cultures were provided with 3 g/l activated charcoal (AC).
- Mannitol sugar was used as an osmotic active compound applied to the media 9.1 or 45.5 g/l.
- Cycocel [(chlorocholine chloride – CCC) was used as growth retardant at 2.5 or 25 mg/l of the media.

The above mentioned chemical (AC, mannitol and cycocel) were added by the determination concentrations to the media before adjusting pH. For each one of the five conservation chemical treatment and the control were stored under room temperature (24±1ºC) and light. At the end of each stored period studied (4, 8 and 12 months), the cultures were randomizly divided into three groups. The first was used for determining the longevity rates and growth characters of the explants. The second group was used for chemical analysis. The third group was used in the micropropagation trail. The parameters recorded were longevity rates of the explants, shootlets number, length of shootlets (cm), number of leaves formed per each shootlet and rooting rates of the explants.

Biochemical analysis:

In three replicates, shootlets resulting after each conservation stage were cut into small pices and severed for pigments, indoles, phenols and sugars analysis. For pigments determination, the metabolic extractions were submitted to the procedures of Saric ***et al.*** (1967) to quantitatively determine the endogenous chlorophyll-a, -b and carotenoids. For total indoles determination, the method of Selim ***et al.*** (1978) was applied. For total phenols determination the procedure of Daniel and George (1972) was used. Total soluble sugars contents were determined, using the phenol sulfuric method according to Dubois ***et al.*** (1966).

Second experiment:

Effect of different conservation treatment on the micropropagation characters of the two germplasms of studied wood tree species.

The growing shootlets resulted after the conservation treatments used as a source of microcutting explants (2-5mm). Twenty five microcutting explants in five replicates were aseptically cultured into free conservation agents MS medium. The cultures were incubated conditions (24±1ºC under light) for eight weeks, for each

period, under which the following parameters were recorded; longevity rate, number of shootlets formed, length of shootlet (cm), number of leaves formed per explants and rooting ability; determined as percentage the number of the explants rooted/number of explants cultured.

Statistical analysis:

The data obtained from the first and second experiments were statistically analyzed using completely randomized design (two factors). The data recorded as percentage were transferred into angular values (arcsin/ percentage). The least significant differences (LSD) test was applied for the comparison among means as described by Steel and Torrie (1980).

Results And Discussion

1.1. Shooting behavior of Populus alba conserved explants:

Survival percentage:

From the data in Table (1) it can be noticed that increasing the period of conservation from 4 to 12 months reduced the survival percentage significantly, the percent was declined from 72 to 22%.The highest level of survived (86%) was recorded for explants storage for 4 months under low temperature 4ºC compared to other treatments. Also, using low temperature significantly raised survival percent recorded(52.0 %) for each other two periods 8 and 12 months. In this respect Anabela and Maria (1999) on *Quercus suber* found that cultures were stored *in vitro* on multiplication medium at 5±1ºC without any intervening subculture for two years. Moreover, Ivaylo *et al.* (2009) showed that using slow growth conditions (4ºC, in darkness) was successfully applied to white poplar (*Populus alba* L.) and hybrid aspen (*P. tremula* L. X *P. termuloids* Michx) for 6 months of storage periods. On the other hand, addition of mannitol at (45.5 g/l) more than 8 months led to explants to die compared with other treatments.

Shootlets number per explants:

Data in Table (1) revealed that there was no significant effect on shootlet number per explants with increasing storage periods. While, supplemented the conservation medium with (AC at 3 g/l) and mannitol at (9.1 g/l) recorded the greatest mean number of shootlet/explant (1.8 and 1.7) . However, low temperature and mannitol at (45.5g/l) reduced the number of shoot to (1.1) explant. While, the greatest number of shootlet / explant (2.1) was recorded after 12 months when (AC) at 3g/l and mannitol at 9.1 g/l to conserve media. On the other hand low temperature decreased number of shoot to the lowest number after the first tow conservation period.

Connecting with such results, El-Sayed-Hwida (2005) reported that, number of shoot proliferation of *Sequoia sempervirns* increased when the storage medium was provided with mannitol at (0.25 M). Also, Said (1991) on *Eucalyptus citriodara* , stated that highest shoot number was obtained when MS medium was provided with 1% (AC).

Shootlet length (cm):

The shootlet length of explant was increased significantly when the period of storage was increasing (3.4, 6.7 and 5.7 cm) after 4,8 and 12 months respectively, as shown in Table (1). The shootlet length was varied from 0.3 to 10.9 cm. The longest shootlet was obtained when the conserved medium was supplemented with 3 g/l (AC), however, the shortest one resulted from using low temperature 4ºC.The data indicated that addition of (AC) at 3 g/l to the conservation medium significantly raised the length of shootlet recorded the highest value (16 cm) after 12 months of storage compared to (8.0 cm) for control, however the lowest shootlet (0.1 cm) resulted by using low temperature. These results are in agreement with other workers as Capuana and Gianvini (1997) on *Cupressus sempervirens,* Youssef and Helmy (1998) on *Azadirachta indica* and El-Sayed-Hwida (2005) on *Sequoia sempervirens.*

Number of leaves / shootlet:

Data in Table (1) indicated that second period of storage (8 months) showed significantly increasing leaves number per shootlet recorded (15), while after 4 and 12 months the leaves number was declined to 9.9 and 10.0,respectively. The greatest number of leaves / shootlet (17.0) as shown when medium of storage was supplemented with (AC) 3 g/l, while the lowest number of leaves (2.9) obtained when low temperature was

applied. Regarding to the interaction between conservation treatment and periods, the data showed that largest number of leaves (22) was produced after third and second conservation period when shootlets storage under medium provided with 3 g/l (AC) and 25 mg/l cycocel.

Rooting percentage:

According to the data illustrated in Table (1), there was no significant effect on rooting percentage with increasing storage periods, while, the largest percent of rooting 72 % was recorded for each control and (AC) treatments after 12 and 8 months. On the other hand, using cold treatment and mannitol at (45.5 mg/l) caused significant decrease in rooting recorded the lowest percentage 0.0 after 12 months for each of them. In this regard, Jouira *et al.* (1997) on *Ulmus pumila X U. japonica* and El-Sayed (2000) on *Acacia melanoxylon,* found that addition of (AC) to the conservation media raised the rooting capacity of the explant.

Table 1: Effect of some conservation treatments on the survival percentage and some growth characters of *Poulus alba* explants *in vitro.*

Period months (P.) Treatments (T.)	Survival %				Shootlet No.				Shootlet length (cm)				Leaves No.				Rooting %			
	4	8	12	Mean (T.)	4	8	12	Mean (T.)	4	8	12	Mean (T.)	4	8	12	Mean (T.)	4	8	12	Mean (T.)
Control %	84	40	8	44	2	1	2	1.5	5	6.9	8	6.5	12	16	10	12.7	48	44	72	54.7
√%	75	40	12	42.2													44	43	62	49.9
Cold 4ºC %	86	52	52	63.3	1	1	2	1.1	0	0.5	0.1	0.3	3.8	1.7	3.1	2.9	20	8	0	9.3
√%	74	47	44	55.2													25	8.8	1	11.5
AC. 3 g/l %	80	40	33	51	2	1	2	1.8	6	11	16	10.9	11	18	22	17	68	72	39	59.7
√%	73	38	33	47.7													60	59	36	51.8
M . 45.5 g/l %	52	8	0	20	1	2	0	1.1	0	0.8	0	0.4	4	7.5	0	3.8	0	4	0	1.3
√%	38	12	1	16.7													1	6.3	1	2.8
M . 9.1 g/l %	52	12	8	24	1	2	2	1.7	4	4.7	1.9	3.7	15	20	8.4	14.3	32	24	40	32
√%	47	17	12	25.2													35	27	40	34.1
CCC. 25.0mg/l%	72	24	20	38.7	2	2	1	1.5	4	13	6.6	7.9	12	22	11	15.1	52	56	54	54
√%	59	30	28	39													47	50	49	48.5
CCC. 2.5 mg/l %	76	28	32	45.3	2	2	1	1.6	4	10	5.7	6.7	12	20	15	15.7	36	48	60	48
√%	68	33	35	45.2													38	45	52	44.9
Mean period (P.) %	72	29	22		2	2	1		3	6.7	5.7		9.9	15	10		37	37	38	
√%	62	31	23														36	34	34	
L.S.D. 5 % T	11				0.3				1.9				2.9				9.2			
P	7.2				N.S.				1.2				1.9				N.S.			
T x P	19				0.6				3.3				4.9				15.9			

AC.(activated charcoal),M.(mannitol),CCC.(cycocel)

1.2. Chemical constituent of Populus alba conserved explants:

Data in Table (2) indicated that, the pigments of chlorophyll (–a and –b) and carotenoids generally decreased significantly when the conservation period was increased. Activated charcoal, mannitol at 9.1 g/l and cycocel 2.5 mg/l gave the highest level of chlorophyll (–a and –b) and carotenoids recorded (122.4, 59.6 and 134.8), (117.9, 58.3 and 128.6) and (133.7, 56.3 and 109.3 mg/100 g f.w.) respectively as the means of conservation periods. Also, the content of total soluble sugars showed the same trend recorded the highest values (102.3 and 103.1 mg/100 gm f.w.) for each (AC at 3g/l) and (cycocel at 2.5 mg/l). However, the lowest value of chlorophyll and carotenoids was recorded when the explants treated with mannitol at (45.5 g/l) or cold treatments at 4ºC after 4 months of storage.

It is quite clear from the data in Table (3) that highest indoles and phenols contents were declined significantly when the storage periods was increased. Also, supplementation the conservation media with (AC), mannitol at 9.1 g/l and cycocel at 2.5 mg/l raised the content of indoles and phenols in the conserved explants. On the other hand, generally low temperature and mannitol at 45.5 g/l gave the lowest value of indoles and phenols content in explant compared with other conservation treatments.

In conclusion, low temperature induces low growth by lowering the overall cell metabolic activity which remains more or less uniform through the culture period, similar effect was found by, El-Sayed (2000) on *Ceratonia siliqua* and El-Saka (1996) on *Naecissus tazzetta.*

On the contrary, may be activated charcoal adsorbs harmful gases and substances, hence, reducing its concentration and hence the harmful effect in the ambient atmosphere of the culture.

1.3. Micropropagation characters of explants conserved:

The Data in Table (4) indicated that after 4 months of storage the conserved explants recorded the highest means of survival percentage, shootlet number and leaves number per new explant recorded(90 %, 2 shootlets and 7.1 leaves/shootlet) respectively. Also, the significant effect treatments which, increased the characters of survival percentage, shootlet length, leaves number per explant on rooting percentage were both treatments of cycocel at (25 or 2.5 mg/l) and (AC) at 3 g/l which recorded the highest level of growth characters compared to

other conservation treatment which gave, (97.3, 94.7 and 94.0 %) for shootlet survival, (1.9, 2.0 and 2.2) shootlet length cm, (8.2, 8.5 and 10.3) leaves number per explant and (36.0, 29.3 and 34.0 %) of rooting percentage respectively.

In addition, the lowest value of shoot length, leaves number and rooting percentage obtained on the explants resulted by using either low temperature or high level of mannitol.

Table 2: Effect of some conservation treatments on some chemical constituents (mg/100gfw)of *Poulus alba* explants *in vitro.*

Period months (P.) Treatments (T.)	Chlorophyll -a				Chlorophyll –b				Carotenoids				Total sugars			
	4	8	12	Mean (T.)	4	8	12	Mean (T.)	4	8	12	Mean (T.)	4	8	12	Mean (T.)
Control	173	80.3	9.1	87.3	79.7	26	9.1	38.1	184	81.1	24	96	23	135	14	57.1
Cold 4ºC	89.1	17.9	22	42.9	34.6	4.1	15	17.8	82.6	14.8	26	40.97	36	220	16	90.5
AC. 3 g/l	198	159	10	122.4	117	53	8.5	59.6	216	148	41	134.8	24	266	16	102.3
M . 45.5 g/l	32.4	0	0	10.8	19.7	0	0	6.57	30.7	0	0	10.23	39	0	0	12.83
M . 9.1 g/l	287	62.1	4.7	117.9	162	8.7	3.8	58.3	305	72.7	7.9	128.6	43	211	9.4	87.8
CCC. 25.0mg/l	102	71.1	1.6	58.3	114	20	3.1	45.8	160	91.1	11	87.5	28	177	7.9	70.9
CCC. 2.5 mg/l	178	218	5.8	133.7	103	61	4.5	56.3	150	165	13	109.3	26	276	7.1	103.1
Mean period (P.)	155	101	8.8		90	29	7.2		161	95.5	20		31	214	12	
L.S.D. 5 % T	44				18.9				42.7				19.8			
P	28.8				12.3				27.9				13			
T x P	76.1				32.6				73.9				34.3			

AC.(activated charcoal),M.(mannitol),CCC.(cycocel)

Table 3: Effect of some conservation treatments on some chemical constituents (mg/100gfw)of *Poulus alba* explants *in vitro.*

Period months (P.) Treatments (T.)	Total Indoles				Total Phenols			
	4	8	12	Mean (T.)	4	8	12	Mean (T.)
Control	241	92.7	114	149.2	231	131	118	160
Cold 4ºC	128	59.4	48	78.5	107	45.1	93.8	81.8
AC. 3 g/l	225	285	180	230.4	207	231	160	200
M . 45.5 g/l	71.8	0	0	23.93	158	0	0	52.6
M . 9.1 g/l	306	245	79.2	210	230	185	118	177.3
CCC. 25.0mg/l	238	315	20.3	191	259	195	63	172.3
CCC. 2.5 mg/l	243	346	30	206.5	254	243	55.2	184
Mean period (P.)	208	224	78.7		207	172	101	
L.S.D. 5 % T	44				52.3			
P	28.8				34.4			
T x P	76.1				90.5			

AC.(activated charcoal),M.(mannitol),CCC.(cycocel)

2.1. Shooting behavior of Melaleuca ercifolia conserved explant:

Survival percentage:

As shown in Table (5) the data revealed that *M. ercifolia* explants remained survive on MS medium at almost conservation treatments for a year, but the survival percentage declined sharply after 8 months , i.e. it was (80 %) after 4 months and reached to (37.1 %) after 12 months. Conserved explant on control MS medium or under low temperature at 4ºC or provided the medium with (AC) at 3 g/l recorded the highest significant percentage (69.3, 78.7 and 73.3%) respectively regardless the period of conservation, while using mannitol at (45.5 g/l) gave the lowest percent (13.3%). The interaction between the conservation treatments and period gave the highest survival rate(96.0%) after 4 months when the explants was storage on MS provide with activated charcoal at (3 g/l) or cycocel (2.5 mg/l) for each one.

On the other hand, supplementation the medium with mannitol at (45.5 g/l) failed to the explant survival more than 4 months; the survival percent record (0.0 %) after 8 and 12 months.

In this concern, the positive effect of storage at low temperature were shown also by Anabela and Maria (1999) on *Quercus suber,* El-Sayed (2005) on *Sequuoia sempervieres* and Ivaylo *et al.* (2009) on white poplar, moreover, the negative effects of mannitol was shown by Sawsan and Gabr (2010) on *Deutzia scabra* Thunb.

Table 4: Effect of some conservation treatments on *Poulus alba* regeneration growth characters**.**

Period months (P) Treatments (T.)	Survival %				Shootlet No.				Shootlet length (cm)				Leaves No.				Rooting %			
	4	8	12	Mean (T.)	4	8	12	Mean (T.)	4	8	12	Mean (T.)	4	8	12	Mean (T.)	4	8	12	Mean (T.)
Control %	100	92	68	86.7	1	1	1	1.1	1	1.9	1.3	1.5	7	9.8	6.3	7.7	10	26	12	16
√%	91	80	58	76.3													15	31	17	21
Cold 4ºC %	100	36	20	52	2	2	1	1.8	1	0.7	0.5	0.6	6.1	5.7	4.6	5.5	4	0	0	1.3
√%	91	38	22	50.1													6.3	1	1	2.8
AC. 3 g/l %	100	82	100	94	1	1	2	1.3	1	2.7	1.6	1.9	6.8	7.6	10	8.2	46	48	14	36
√%	91	69	91	83.6													43	45	20	36.2
M . 45.5 g/l %	28	0	0	9.3	2	0	0	0.7	1	0	0	0.2	5.1	0	0	1.7	0	0	0	0
√%	30	1	1	10.6													1	1	1	1
M. 9.1 g/l %	100	54	54	69.3	2	1	1	1.5	1	0.8	0.7	0.9	7.6	4.7	4.7	5.7	2	6	18	8.7
√%	91	48	47,8	62.4													4.7	10	23	12.7
CCC.25.0mg/l%	100	84	100	94.7	2	1	1	1.3	1	2.9	1.8	2	7.9	8.5	9.1	8.5	14	56	18	29.3
√%	91	71	91	84.2													20	50	23	31
CCC.2.5 mg/l %	100	96	97	97.3	1	1	2	1.3	1	2.4	3.3	2.2	8.9	9.4	13	10.3	8	58	36	34
√%	91	84	84	86.1													14	51	37	33.9
Mean period (P.) %	90	63	83		2	1	1		1	1.6	1.3		7.1	6.5	6.8		12	28	14	
√%	82	56	56														15	27	18	
L.S.D. 5 % T	7.6				0.3				0.5				1.4				8.3			
P	5				1				0.4				0.9				5.4			
T x P	13.2				0.5				0.9				2.5				14.3			

AC.(activated charcoal),M.(mannitol),CCC.(cycocel)

Shoot number per explant:

Data in Table (5) illustrated that the number of shootlet was significantly decreased by the storage period increased, the number declined from (2 to 1) shootlet per explant after 4 to 12 months of conservation. The largest number of shootlet (1.9) recorded on conserved media provided with mannitol at (9g/l) and cycocel either two concentrations (25 and 2.5g/l). The significant highest value (3) shootlet per explant was scored after 4 months, when the storage medium was provided with cycocel at(2.5 mg/l), also supplemented the media with mannitol at(9.1 g/l) and cycocel at (25 mg/l) gave increasing in the number of shootlet recorded (2)shootlet compared to other all storage treatments. However, using low temperature at 4ºC declined the shootlet number per explant for each three periods of storage.Similar results were obtained by Kishi and Takagi (1997) on orchids when the explants were preserved under low temperature at 4ºC.

Shootlet length (cm):

It can be noticed from the data in Table (5) that shootlet length of conserved explant increased significantly from (1.9 to 3.8 cm) when the storage period was increased from 4 to 12 months respectively. The longest shootlet (4.7 cm) was obtained with control treatment. Also, the interaction between the conservation periods and treatments gave the same trend; after 12 months conserved explant on control MS medium gave the longest shootlet recorded(7.3 cm). However, using low temperature at 4ºC caused significantly decreased in the shootlet length to (0.4 cm) after same period.

Number of leaves / shootlet.:

According to the data presented in Table (5) the number of leaves/shootlet was correlated positively with the conservation period; i.e. with the periods of storage increased the number of leaves increased significantly

recorded (19, 25 and 30 leaves/shootlet) after 4,8 and 12 months respectively. All the conservation treatment increased the leaves number significantly compared with control treatment except either using low temperature or high concentration of mannitol (45.5 g/l) recorded significantly decline in the leaves number to (3.11 and 2.7). In the same trend the interaction between conservation treatments and periods gave the same result.

Rooting percentage:

From the data in Table (5) it can be illustrated that increasing storage periods had no significant effect on rooting percentage while, the conservation treatments gave significantly effect. In generally, control treatment, (AC) at 3g/l and cycocel for each high and low concentration (25 and 2.5 mg/l) recorded the highest rooting ability during the three storage especially at the last period. However, rooting capacity of conserved explant was absent at low temperature treatments at all periods of storage, also addition of mannitol at 45.5 g/l to conservation media recorded significantly declined on the rooting capacity at all conservation periods compared with other conservation treatments. In this regard, El-Shafey et al. (1997) on Acacia albida and Jouira et al. (1997) on *Ulmus pumila X U. japonica* found that addition of (AC) to the culture media promoted the root induction of explant in vitro.

Table 5:. Effect of some conservation treatments on the survival percentage and some growth characters of *Melaleuca ercifolia* explants *in vitro*.

Period months (P) Treatments (T.)	Survival %				Shoot let No.				Shoot let length (cm)				Leaves No.				Rooting %			
	4	8	12	Mean (T.)	4	8	12	Mean (T.)	4	8	12	Mean (T.)	4	8	12	Mean (T.)	4	8	12	Mean (T.)
Control %	92	84	32	69.3	2	2	2	1.6	2.4	4.5	7.3	4.7	21	28	36	28.4	96	100	96	97.3
√%	83	76	40	66.1													86	91	86	87.5
Cold 4ºC %	68	92	76	78.7	1	1	1	1.3	0.5	3.8	0.4	0.4	3.6	2.7	2.9	3.11	0	0	0	0
√%	57	61	48	55.4													1	1	1	1
AC 3 g/l %	96	64	60	73.3	2	1	2	1.5	3	4.7	5.4	4.3	25	37	39	33.8	92	92	96	93.3
√%	86	57	55	66													80	80	86	85.2
M . 45.5 g/l %	40	0	0	13.3	2	0	0	0.5	0.5	0	0	0.2	8	0	0	2.7	44	28	12	28
√%	34	1	1	12													43	30	14	28.8
M . 9.1 g/l %	80	32	24	45.3	2	2	2	1.9	2.1	4.8	4.7	3.8	23	36	44	34.4	76	88	92	85.3
√%	70	32	27	43.3													68	78	80	75.3
CCC.25.0mg/l%	88	68	40	65.3	2	1	2	1.9	2.5	5.2	4.1	3.9	27	38	43	35.9	80	88	96	88
√%	78	57	40	58.3													67	75	86	75.9
CCC.2.5 mg/l %	96	68	28	64	3	2	2	1.9	2.3	4	4.5	3.6	24	34	41	33.2	92	88	96	92
√%	86	57	37	57.4													80	75	86	80.4
Mean period (P.) %	80	58	37		2	1	1		1.9	3.9	3.8		19	25	30		69	69	70	
√%	71	49	34														61	61	63	
L.S.D. 5 % T	12.6				0.3				0.9				2				11.1			
P	8.3				0.2				0.6				3.8				N.S.			
T x P	21.8				0.5				1.7				10.3				19.3			

AC.(activated charcoal),M.(mannitol),CCC.(cycocel)

2.2. Chemical constituents of Melaleuca ercifolia conserved explant:

It can be noticed from Table (6) that there was significant reduction in the pigments and total carotenoids compounds of the shootlet resulting with prolonging of increasing storage periods, i.e. minimal amount of these pigments recorded at the last storage period. However, mannitol at (9.1 g/l) or control treatments caused significantly increased in either chlorophyll-a and –b especially at the first period of conservation, while the lowest value of the chlorophyll- a and –b resulted from the explant treated with cycocel at 2.5 mg/l. For carotenoids compound of shootlet, the control treatment and mannitol at (9.1g/l) gave the highest level of carotenoids in conserved shootlet compared to other treatments of conservation.

Concerning the total sugar amount in conserved explant, storage the shootlet under low temperature achieved high level of total sugar indigenous explant compared with other all treatment and the significant amount was recorded after the second period of conservation.

Table 6: Effect of some conservation treatments on some chemical constituents(mg/100gfw)of *Melaleuca ercifolia* explants *in vitro*.

Period months (P.) Treatments (T.)	Chlorophyll -a				Chlorophyll –b				Carotenoids				Total sugars			
	4	8	12	Mean (T.)	4	8	12	Mean (T.)	4	8	12	Mean (T.)	4	8	12	Mean (T.)
Control	196	73.9	33	101.2	97	36	27	53.6	308	180	83	190.3	37	133	15	61.8
Cold 4ºC	140	135	48	107.7	76	83	19	59.1	142	90	51	94.2	69	237	17	107.9
AC. 3 g/l	87.3	49.5	43	59.9	51	41	44	45.3	279	164	72	171.5	24	132	7.6	54.6
M . 45.5 g/l	67.7	0	0	22.57	46	0	0	15.17	81.3	0	0	27.1	81	0	0	27
M . 9.1 g/l	202	112	85	133	100	35	46	59.9	306	122	62	163.2	44	126	9.6	59.7
CCC. 25.0mg/l	74.2	79.2	44	65.7	47	39	34	39.8	191	161	51	134.2	23	129	8.5	53.3
CCC. 2.5 mg/l	76.5	81.9	15	58.4	48	40	6.7	31.6	162	120	26	103	22	125	8.7	51.8
Mean period (P.)	121	88.5	45		66	46	29		210	140	57		43	147	11	
L.S.D. 5 % T	24.9				22.2				30.4				8			
P	16.3				14.5				19.9				5.3			
T x P	43.2				38.5				52.7				13.9			

AC.(activated charcoal),M.(mannitol),CCC.(cycocel)

Data in Table (7) indicated that there was no significant effect in the total indoles in shootlet storage affected by increasing storage periods, while the conservation treatments showed significant different in the amount of indoles in shootlet. Addition of mannitol at (45.5 g/l) raised the level of indoles and phenoles significantly especial after the first period of conservation.

In the same trend, using low temperature raised the level of indoles in the shootlet significantly compared to other treatments. However, the level of indoles was declined by using the control , (AC)and cycocel at(2.5 mg/l) treatments.

The same trend in the effect of low temperature was found by El-Sayed Hwida (2000) on *Ceratonia siliqua*. Also, on *Sequoia sempriverns* the positive effect of mannitol addition was recorded by El-Sayed (2005) and on *Azadirachta indica* by Youssef and Helmy (1998).

Concerning total phenols compounds in conserved explant the data in Table (7) revealed that, increasing the period of conservation caused significantly decreased in the endogenous phenols in explants.In general, the high amount of phenols was recorded after 4 months of storage, but the amount varied from treatment to anther; the control treatment raised the level of phenol significantly after 12 months of storage compared to other treatments, while the lowest level of phenols results from shootlets treated with cycocel at (2.5 mg/l) for 12 months.

Table 7: Effect of some conservation treatments on some chemical constituents(mg/100gfw)of *Melaleuca ercifolia* explants *in vitro*.

Period months (P.) Treatments (T.)	Total Indoles				Total Phenols			
	4	8	12	Mean (T.)	4	8	12	Mean (T.)
Control	67.4	452	105	208.1	301	159	307	255.6
Cold 4ºC	308	216	406	309.8	193	193	194	193.3
AC. 3 g/l	146	388	194	242.7	217	149	164	176.2
M . 45.5 g/l	476	0	0	158.6	866	0	0	288.5
M . 9.1 g/l	644	124	257	341.7	408	180	189	259
CCC. 25.0mg/l	206	291	247	248.2	259	195	175	165.8
CCC. 2.5 mg/l	117	263	84.9	155.1	204	142	59.3	134.8
Mean period (P.)	280	289	216		338	161	181	
L.S.D. 5 % T	145.7				33.9			
P	95.4				22.2			
T x P	252.3				58.7			

AC.(activated charcoal),M.(mannitol),CCC.(cycocel)

2.3. Micropropagation characters of explant conserved:

Data presented in Table (8) showed that, as the periods of conservation increased the survival percentage was significantly decreased; the survival rate were (87, 70 and 60 %) after 4, 8 and 12 months of storage respectively. Control, (AC) and cycocel at (25 mg/l) treatments resulted in the highest survival percentage (92.7, 92.7 and 91.3 %) respectively. However, mannitol at high concentration due to significant decrease in the survival percent, especially after the two last period of storage, which caused a harmful effect on survival of shootlets and died when transferring to the recovery media. Concerning, shootlet number per explant, the data in Table (8) stated that, cold treatment at 4ºC gave significant increased in the number of shootlet compared to other treatments. As the same result in survival percentage, addition of mannitol at (45.5 g/l) to conserve media recorded the lowest number of shootlet after 4 months of storage. However, low temperature and cycocel at (25 mg/l) treatments raised the number of shootlet especially after 12 months storage.

It is quite evident from data in Table (8) that, there is a significant influence of each of conservation treatments and periods on the micropropagation shootlet length and leaves number per shootlet were recorded after the second period of conservation with a significant influence of each of the two characters. Generally, the same trend was found in the two characters and the effect of treatments; ***i.e.*** each of control, (AC), mannitol at (9.1 g/l) and cycocel at(25 mg/l) resulted the highest shootlet length and number of leaves compared with other treatments especially after 8 months of storage. However, low temperature, mannitol at (45.5 g/l) and cycocel at(2.5 mg/l) treatments recorded significant decreased in these characters at each of storage periods.

Concerning, rooting percentage, also the second period of conservation gave significant increased in the rooting capacity of shootlet, while the third periods recorded significant declined in the rooting percentage. While, addition of mannitol at (9.1 g/l) in the media of conservation caused raising in the ability of rooting percentage especially at first period of conservation compared to other treatment. However, increasing the concentration of mannitol to (45.5 g/l) due to the shootlet failed to rooting after the three period of storage.

Finally, the present work confirmed that we can conserve *Populus alba* and *Melaleuca ercifolia* germplasm under low temperature at 4 **ºC** for 12 months with maintain the viability of explants high compared to the other conservation treatments under investigation , while to achieve the highest micropropagation ability of conserved explants addition of (AC) at the conserve media gave the aim.

Table 8: Effect of some conservation treatments on *Melaleuca ercifolia regeneration* growth characters.

Period months (P) Treatments (T.)	Survival %				Shootlet No.				Shootlet length (cm)				Leaves No.				Rooting %			
				Mean				Mean				Mean				Mean				Mean
	4	8	12	(T.)	4	8	12	(T.)	4	8	12	(T.)	4	8	12	(T.)	4	8	12	(T.)
Control %	100	98	80	92.7	1	2	3	1.8	0.8	1.9	0.9	1.2	7.8	17	7	10.7	36	80	16	44
√%	91	87	68	81.9													37	65	22	41.3
Cold 4ºC %	100	54	52	68.7	2	2	4	2.5	0.7	1.3	0.6	0.9	7.3	10	6.5	8.1	30	46	4	26.7
√%	91	48	44	61.1													34	43	4.7	27.1
AC. 3 g/l %	100	98	80	92.7	2	2	2	1.8	0.8	1.7	1.1	1.2	8.3	14	13	11.5	50	82	24	52
√%	91	87	68	81.9													46	69	25	47.2
M . 45.5 g/l %	16	0	0	5.3	1	0	0	0.4	0.7	0	0	0.2	10	0	0	3.5	0	0	0	0
√%	20	1	1	7.2													1	1	1	1
M . 9.1 g/l %	100	72	86	86	2	2	2	1.6	1	0.8	1.4	1.1	9	10	13	10.7	88	40	68	65.3
√%	91	62	77	76.6													75	40	57	57.2
CCC.25.0mg/l%	96	98	80	91.3	2	2	2	1.8	1.1	1.1	0.9	1	9.9	11	11	10.4	68	76	4	49.3
√%	84	87	64	78.5													58	62	8.4	42.8
CCC.2.5 mg/l %	96	72	44	70.7	2	2	2	1.6	0.7	0.9	0.8	0.8	7.2	11	9.4	9.1	24	48	16	29.3
√%	84	60	40	61.9													29	45	17	30.3
Mean period (P.) %	87	70	60		2	1	2		0.8	1.1	0.8		8.6	10	8.4		42	53	19	
√%	79	62	52														40	46	20	
L.S.D. 5 % T	10.8				0.4				0.2				1.4				8.3			
P	7.1				0.2				0.1				0.9				5.4			
T x P	18.7				0.6				0.3				2.4				14.3			

AC.(activated charcoal),M.(mannitol),CCC.(cycocel)

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