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Harmine in *Peganum harmala* L. *in Vitro* Cultures

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ABSTRACT

Explant type and choice of plant growth regulators significantly affected callus growth, shoot regeneration and harmine production in *Peganum harmala* callus cultures produced from excised hypocotyl, leaf and root explants of seedlings germinated *in vitro*. From a range of auxins and cytokinins tested, Murashige and Skoog (MS) medium supplemented with 5 mg/l Kinetin and 1 mg/l 1-naphthaleneacetic acid (NAA) resulted in the greatest callus mass and best growth parameters from all three explant types while MS medium supplemented with 5 mg/l N_6-benzylaminopurine (BAp) and 0.1 mg/l of zeatin and NAA, resulted in greatest shoot production. MS medium supplemented with 3 mg/l BAp and 1 mg/l 2,4-D, however, resulted in higher harmine accumulation (0.962 mg/g dry weight) from root-derived calli than from callus derived from leaves or hypocotyls. Maximum levels of harmine (1.45 mg/g dry weight) were produced from shoots regenerated from hypocotyl-derived calli.

Keywords: calli cultures, shoot regeneration, harmine content, HPLC
Abbreviations: 2,4-D, 2,4-dichlorophenoxyacetic acid **BAp**, N_6 benzylaminopurine; **Kin**, kinetin, 6-furfurylaminopurine; **MS**, Murashige and Skoog medium; **NAA**, 1-naphthaleneacetic acid

INTRODUCTION

Plant cell culture technology has many advantages over traditional field cultivation and chemical synthesis, particularly for many natural compounds that are either derived from slow-growing plants or difficult to be synthesized with chemical methods. It is like an industrialized biological factory for production of high quality natural products under strictly controlled conditions (Zhao and Verpoorte 2007).

Peganum harmala L. (Syrian rue) is a perennial herb (about 50-100 cm in height) that belongs to the family Zygophyllaceae (Kotb 1983). This plant has many medicinal uses in folk medicine all over the world. Its mature seeds are used as an antiheamoroidal, helmicide, and in Turkish folk medicine it is used as a stimulating agent for the central nervous system (Kartal *et al*. 2003). It is used in India for the treatment of chronic malaria (Kotb 1983). However, it is used in Morocco to treat hypertension and mellitus (Tahraoui *et al*. 2007). In Jordan, it is used systemically internally for its antispasmodic, diuretic, sedative and analgesic effects and externally for its antirheumatic effects (El-Dwari and Banihani 2007). In Egypt, this plant is used to treat asthma, rheumatic pains and some skin diseases (Boulos 1983).

P. harmala has gained great attention in recent few years as it is a source of β-carboline alkaloids. Its seeds contain 2.5-4% β-carboline alkaloids, mainly harmaline, harmine and harmalol (Kotb 1983). Harmine has recently drawn attention due to its antitumor activity. Many recent studies have shown that harmine has antitumor (Johanian *et al*. 2005; Zakar *et al*. 2007) antimicrobial (Shahverdi *et al*. 2005) and antileishmanial (Lala *et al*. 2004) activity. Moreover, harmine has antioxidant activity and inhibits the human low-density lipoprotein oxidation (Berrougui *et al*. 2006a) and also has vasorelaxant effects on the rat aorta (Berrougui *et al*. 2006b).

This study aimed to establish, *in vitro*, a protocol for callus production and shoot regeneration from different explants of *P. harmala*. Furthermore, we aimed to enhance the biosynthesis and accumulation of β-carboline alkaloid (harmine) as a pharmaceutical compound in their cultures.

MATERIALS AND METHODS

Plant materials

Seeds of *Peganum harmala* were kindly obtained from the Institute of Horticulture Research, Agricultural Research Centre, Giza, Egypt. Seeds were surface sterilized under aseptic conditions of laminar flow hood, using 70% EtOH for 30 sec, then transferred to a solution of 50% Clorox (containing 5.25% NaOCL) for 15 min. Then these seeds were aseptically germinated onto basal Murashige and Skoog (MS) medium with 3% sucrose and 0.7 % agar, pH 5.8. Cultures were incubated in a control growth room at 26°C and 2000 Lux (27.027 μmole/m^2/s) with a 16 h photoperiod. Seeds germinated within 10 days. One month old germinated seedlings were used as mother plants to excise explants (hypocotyl, leaf and root tissues) under sterile conditions to serve as a source for callus production.

Callus production

MS medium supplemented with of 1,3,5 mg/l of N_6 benzylaminopurine (BAP) or 6-furfuryl amino purine (Kin) (as cytokinins) and in combination with 1 mg/l either 1-naphthalene acetic acid (NAA) or 2,4-dichlorophenoxy acetic acid (2,4-D) (as auxins) were used for callus production as follows:

MS basal medium (free of plant growth regulators (PGRs)) (MS_0)
MS + 1 mg/l NAA + 1 mg/l BAp (MS_1)
MS + 1 mg/l NAA + 3 mg/l BAp (MS_2)
MS + 1 mg/l NAA + 5 mg/l BAp (MS_3)
MS + 1 mg/l 2,4-D + 1 mg/l BAp (MS_4)
MS + 1 mg/l 2,4-D + 3 mg/l BAp(MS_5)
MS + 1 mg/l 2,4-D + 5 mg/l BAp (MS_6)
MS + 1 mg/l NAA + 1 mg/l Kin (MS_7)
MS + 1 mg/l NAA + 3 mg/l Kin (MS_8)
MS + 1 mg/l NAA + 5 mg/l Kin (MS_9)
MS + 1 mg/l 2,4-D + 1 mg/l Kin (MS_{10})

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MS + 1 mg/l 2,4-D + 3 mg/l Kin (MS_{11})
MS + 1 mg/l 2,4-D + 5 mg/l Kin (MS_{12}).

Three segments of hypocotyl, leaf and root were cultured in glass jars (175 ml) containing 25 ml of MS-solid medium. Five replicates were used for each type of medium. All cultures were incubated in a growth room under the same conditions used for seed germination. When calli had completely formed from any medium (after 4 weeks), they were subcultured onto the same, fresh medium. Subcultures were repeated every 4 weeks.

Determination of callus growth parameters

The percentage of callus formation, fresh, dry weights and dry matter content (%) were determined for each treatment after 4 weeks of cultivation.

Shoot regeneration

Three pieces about 250 mg/ for each piece of hypocotyl, leaf and root derived calli from MS-medium supplemented with 1 mg/l NAA +5 mg/l Kin were cultured onto the following regeneration media:
MS basal medium (free of PGRs) (MS0)
MS + 0.1 mg/l NAA + 0.1 mg/l zeatin + 1 mg/l BAp (MS1)
MS + 0.1 mg/l NAA + 0.1 mg/l zeatin + 3 mg/l BAp (MS2)
MS + 0.1 mg/l NAA + 0.1 mg/l zeatin + 5 mg/l BAp (MS3)
MS + 0.1 mg/l NAA + 0.1 mg/l zeatin + 7 mg/l BAp (MS4).

All cultures were incubated at 26°C and 3000 Lux (40.54 µmole/m^2/s) under cool white inflorescent lamps (Philips, Germany) in a controlled growth room. After one month of culture, percentage and number of formed shoots were recorded.

Total alkaloids: extraction and determination

Total alkaloids: extraction

Total alkaloids were extracted according to the method described by Kartal *et al.* (2003).

Harmine determination

Two grams of dried *P. harmala* samples (intact plants, derived calli or regenerated shoots) were used for extraction. The extracted alkaloid solution was passed through a 0.45 µm filter pore (Whatman, USA) and 20 µl was used for HPLC analysis using a Beckman HPLC apparatus. For harmine standardization, 10 µl of a 500 µg/ml harmine standard solution was calibrated and evaluated. According to the method described by Kartal *et al.* (2003) samples and harmine standard solutions were detected at 240 nm. Separation of total alkaloids compounds were carried out using an ODS column (5 µm × 250 × 4.5 mm) at a flow rate of 1 ml/min. The mobile phase was constructed as follows: isopropyl alcohol: acetonitrile: water: formic acid (100:100:300:0.3) (v/v/v/v) and the pH was adjusted to 8.6 with triethylamine. Twenty µl of each sample were injected into the column. The run procedure was carried out for 10 min at 35°C.

Statistical analysis

All experiments were statistically analyzed using the F-test and statistical analysis was conducted according to Steel and Torrie (1960). ANOVA was determined and the LSD was calculated at P=0.05. The data presented are the means of five replicates ± standard error (SE).

RESULTS AND DISCUSSION

Callus production and growth parameters

Percentage of callus formation

The highest percentage of callus formation was 97, 92 and 85% were recorded for hypocotyl, leaf and root explants, respectively (**Table 1**). The optimum auxin: cytokinin combination for callus formation was 1 mg/l NAA + 5 mg/l Kin.

Table 1 Effect of supplemented MS-medium with different combinations and concentrations of auxins and cytokinins on percentage of callus formation from hypocotyl, leaf and root explants of *Peganum harmala* L. cultured under light condition at 26 ± 1°C.

Types of MS media	*Peganum harmala* L. explants		
	Hypocotyl	Leaf	Root
MS_0	---	---	---
MS_1	75 ± 6.9 NS	68 ± 6.5 NS	65 ± 6.1 NS
MS_2	83 ± 8.5 c	71 ± 7.8 c	69 ± 5.9 d
MS_3	87 ± 8.9 c	75 ± 7.3 NS	72 ± 6.5 d
MS_4	69 ± 7.6 d	61 ± 5.8 NS	54 ± 4.9 NS
MS_5	73 ± 5.8 b	64 ± 6.1 c	57 ± 5.6 c
MS_6	75 ± 6.2 d	69 ± 6.4 c	66 ± 6.4 b
MS_7	84 ± 9.4 b	75 ± 7.3 c	63 ± 6.6 b
MS_8	93 ± 8.2 b	83 ± 7.9 c	74 ± 7.8 a
MS_9	97 ± 8.5 a	92 ± 8.9 b	85 ± 7.8 c
MS_{10}	62 ± 6.6 NS	58 ± 5.4 NS	53 ± 4.7 NS
MS_{11}	67 ± 5.7 c	56 ± 4.8 NS	54 ± 4.9 d
MS_{12}	70 ± 6.5 b	54 ± 4.5 NS	50 ± 4.5 NS

Data were expressed as means of five replicates ± SE (standard error). Means with the same letter in each column or line are significantly different at P = 0.05 according to the *f*-test NS, not significant.

Only MS_9 medium was significantly different to other media. Hypocotyl explants showed a more significant reaction compared with leaf and root explants.

Table 2 Effect of supplemented MS-medium with different combinations and concentrations of auxins and cytokinins on calli fresh weight (g/jar) derived from hypocotyl, leaf and root explants of *Peganum harmala* L. cultured under light conditions at 26 ± 1°C.

Types of MS-media	*Peganum harmala* L explants		
	Hypocotyl	Leaf	Root
MS_0	---	---	---
MS_1	1.74 ± 0.12 NS	1.54 ± 0.09 NS	1.25 ± 0.15 d
MS_2	1.85 ± 0.15 d	1.63 ± 0.22 NS	1.34 ± 0.16 c
MS_3	2.09 ± 0.21 d	1.85 ± 0.17 d	1.45 ± 0.15 c
MS_4	1.39 ± 0.15 NS	1.25 ± 0.25 c	1.05 ± 0.07 d
MS_5	1.54 ± 0.19 c	1.39 ± 0.16 d	1.12 ± 0.13 d
MS_6	1.63 ± 0.12 a	1.50 ± 0.08 a	1.23 ± 0.15 b
MS_7	2.15 ± 0.16 d	1.94 ± 0.11 c	1.75 ± 0.10 b
MS_8	2.24 ± 0.22 b	2.05 ± 0.15 d	1.86 ± 0.13 c
MS_9	2.35 ± 0.18 a	2.17 ± 0.14 b	1.93 ± 0.08 a
MS_{10}	0.95 ± 0.05 NS	0.85 ± 0.12 NS	0.66 ± 0.07 NS
MS_{11}	1.08 ± 0.06 b	0.94 ± 0.02 a	0.75 ± 0.05 NS
MS_{12}	1.15 ± 0.11 NS	1.09 ± 0.09 NS	0.84 ± 0.09 c

Data were expressed as means of five replicates ± SE (standard error). Means with the same letter in each column or line are significantly different at P = 0.05 according to the *f*-test. NS, not significant.

Table 3 Effect of supplemented MS-medium with different combinations and concentrations of auxins and cytokinins on calli dry weight (g/jar) derived from hypocotyl, leaf and root explants of *Peganum harmala* L. cultured under light condition at 26 ± 1°C.

Types of MS-media	*Peganum harmala* L explants		
	Hypocotyl	Leaf	Root
MS_0	---	---	---
MS_1	0.134 ± 0.05 NS	0.118 ± 0.03 NS	0.098 ± 0.05 NS
MS_2	0.145 ± 0.01 NS	0.123 ± 0.04 NS	0.105 ± 0.06 NS
MS_3	0.195 ± 0.02 c	0.137 ± 0.06 b	0.118 ± 0.03 a
MS_4	0.113 ± 0.02 d	0.102 ± 0.03 NS	0.091 ± 0.02 b
MS_5	0.125 ± 0.03 d	0.109 ± 0.05 d	0.094 ± 0.01 c
MS_6	0.127 ± 0.06 b	0.114 ± 0.06 b	0.098 ± 0.05 c
MS_7	0.198 ± 0.01 c	0.175 ± 0.09 a	0.135 ± 0.03 a
MS_8	0.205 ± 0.04 c	0.188 ± 0.05 b	0.146 ± 0.05 d
MS_9	0.225 ± 0.07 a	0.200 ± 0.08 b	0.175 ± 0.09 b
MS_{10}	0.087 ± 0.05 NS	0.075 ± 0.01 NS	0.046 ± 0.03 NS
MS_{11}	0.095 ± 0.01 NS	0.083 ± 0.02 NS	0.063 ± 0. 05 NS
MS_{12}	0.103 ± 0.05 c	0.094 ± 0.01 d	0.077 ± 0.02 b

Data were expressed as means of five replicates ± SE (standard error). Means with the same letter in each column or line are significantly different at P = 0.05 according to the *f*-test. NS, not significant.

Table 4 Effect of supplemented MS-medium with different combinations and concentrations of auxins and cytokinins on dry matter contents (%) derived from hypocotyl, leaf and root explants of *Peganum harmala* L. cultured under light condition at 26 ± 1°C .

Types of MS-media	*Peganum harmala* L explants		
	Hypocotyl	Leaf	Root
MS_0	---	---	---
MS_1	7.70 ± 0.65 NS	7.66 ± 0.79 NS	7.84 ± 0.66 NS
MS_2	7.84 ± 0.77 NS	7.55 ± 0.65 NS	7.83 ± 0.56 NS
MS_3	9.33 ± 0.86 c	7.41 ± 0.73 b	8.14 ± 0.73 c
MS_4	8.13 ± 0.79 b	8.16 ± 0.77 d	8.67 ± 0.84 d
MS_5	8.12 ± 0.83 d	7.84 ± 0.85 c	8.39 ± 0.76 d
MS_6	7.79 ± 0.77 d	7.60 ± 0.66 b	7.97 ± 0.69 b
MS_7	9.21 ± 0.82 b	9.02 ± 0.74 c	7.71 ± 0.75 b
MS_8	9.15 ± 0.88 a	9.17 ± 0.88 c	7.85 ± 0.83 b
MS_9	9.59 ± 0.93 a	9.22 ± 0.91 a	9.07 ± 0.85 a
MS_{10}	9.16 ± 0.81 NS	8.82 ± 0.86 b	6.97 ± 0.54 c
MS_{11}	8.80 ± 0.77 b	8.83 ± 0.92 NS	8.4 ± 0.79 NS
MS_{12}	8.96 ± 0.82 b	8.63 ± 0.77 b	9.17 ± 0.95 NS

Data were expressed as means of five replicates ± SE (standard error). Means with the same letter in each column or line are significantly different at P = 0.05 according to the *f*-test test. NS, not significant.

Callus growth parameters

The addition of 1 mg/l NAA and 5 mg/l Kin to MS medium gave the highest values of calli fresh and dry weights (mg/ jar) (**Tables 2, 3**). The highest percentage of dry matter content (**Table 4**) was observed for hypocotyl (**Fig. 1**) explants. The resulting calli from this treatment were induced to form shoots. Furthermore, highly significant fresh, dry weights and dry matter content were recorded on MS_9 compared with other treatments. Hypocotyl explants were more significant in fresh, dry weights and dry matter content compared with leaf and root explants, respectively. Callus production and growth varied depending on the explant type and PGR choice.

Shoot regeneration

Hypocotyl-derived calli resulted in the highest percentage of shoot formation (95%; **Table 5**) and the greatest number of shoot (48 shoots per calli clump; **Fig. 2**). MS3 was the best medium for shoot regeneration from different types of calli cultures.

The synergistic effect of BAp and auxin on enhancement of shoot regeneration has been demonstrated in *Santolina canescens* (Casado *et al*. 2002), *Bupleurum fruticosum* (Fraternale *et al.* 2002), *Acacia nilotica* (Sane *et al*. 2001) and *A. albida* (Gassama *et al*. 1986).

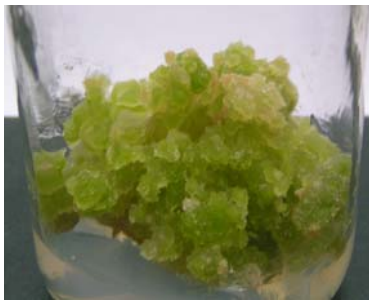

Fig. 1 Calli production from hypocotyl explants of *P. harmala* after 4 weeks of culture on MS medium supplemented with 5 mg/l Kin and 1 mg/l NAA in the light.

Fig. 2 *In* vitro shoot regeneration from hypocotyl explants of *P. harmala* after 4 weeks on MS medium containing 5 mg/l BAP + 0.1 mg/l of both Zeatin and NAA.

Table 5 Effect of MS medium supplemented with 0.1 (mg/l) each of NAA and zeatin in combinations with 1,3,5 and 7 (mg/l) BAp on shoot regenerated parameters derived from hypocotyl, leaf and root calli cultures of *Peganum harmala* L. cultured under light condition at 26 ± 1°C.

Type of MS media	*Peganum harmala* L. calli cultures					
	Hypocotyl		Leaf		Root	
	Shoots (%)	№ of shoots	Shoots (%)	№ of shoots	Shoots (%)	№ of shoots
MS(0)	17	15 ± 1.5 NS	12	9 ± 1.1 NS	9	5 ± 0.8 NS
MS(1)	76	39 ± 3.8 NS	67	28 ± 2.5 NS	50	17 ± 1.2 NS
MS(2)	87	45 ± 4.1 b	81	35 ± 3.2 b	58	20 ± 2.6 NS
MS(3)	95	48 ± 4.5 a	86	37 ± 2.9 c	64	25 ± 2.5 a
MS(4)	82	42 ± 3.8 NS	75	33 ± 3.1 c	53	19 ± 1.7 NS

Data were expressed as means of five replicates ± SE (standard error). Means with the same letter in each column or line are significantly different at P = 0.05 according to the *f*-test. NS, not significant.

Table 6 Effect of MS-medium supplemented with different growth regulators on harmine alkaloid contents (mg/g dry weight) of hypocotyl, leaf and root calli cultures after one subculture and their relative percentage to harmine content in *in vivo* mature seeds* of *P. harmala* L.

Types of MS media	*Peganum harmala* calli cultures					
	Hypocotyl		Leaf		Root	
	Callus	Relative (%)	Callus	Relative (%)	Callus	Relative (%)
MS_0	0.00	0.00	0.00	0.00	0.00	0.00
MS_1	0.423 ± 0.04 NS	8.90	0.496 ± 0.035 NS	10.42	0.538 ± 0.049a	11.30
MS_2	0.493 ± 0.042 NS	10.36	0.525 ± 0.041 NS	11.03	0.587 ± 0.053c	12.33
MS_3	0.532 ± 0.039c	11.18	0.567 ± 0.052b	11.91	0.635 ± 0.058 NS	13.34
MS_4	0.619 ± 0.054b	13.00	0.763 ± 0.069c	16.03	0.865 ± 0.077d	18.17
MS_5	0.653 ± 0.055a	13.72	0.876 ± 0.076a	18.40	0.962 ± 0.093**c**	20.21
MS_6	0.625 ± 0.058b	13.13	0.851 ± 0.079d	17.88	0.907 ± 0.085**c**	19.05
MS_7	0.387 ± 0.029 NS	8.13	0.425 ± 0.039 NS	8.93	0.485 ± 0.038 NS	10.19
MS_8	0.476 ± 0.035 NS	10.00	0.485 ± 0.033 NS	10.19	0.583 ± 0.048**c**	12.25
MS_9	0.458 ± 0.042 NS	9.69	0.463 ± 0.038 NS	9.73	0.537 ± 0.051 NS	11.28
MS_{10}	0.512 ± 0.049 NS	10.76	0.535 ± 0.051a	11.24	0.653 ± 0.058**b**	13.73
MS_{11}	0.583 ± 0.052 c	12.25	0.594 ± 0.062d	12.48	0.732 ± 0.069**b**	15.38
MS_{12}	0.537 ± 0.048c	11.28	0.545 ± 0.053d	11.45	0.697 ± 0.065 NS	14.64

* Harmine content = 4.76 mg/g dry weight of *in vivo P. harmala* mature seeds.

Data were expressed as means of five replicates ± SE (standard error). Means with the same letter in each column or line are significantly different at P = 0.05 according to the *f*-test. NS, not significant.

Table 7 Effect of MS-medium supplemented with different growth regulators on harmine alkaloid contents (mg/g dry weight) of hypocotyl, leaf and root regenerated shoots after one subculture, and their relative percentage to harmine content in *in vivo* intact seeds* of *P. harmala* L.

Types of MS media	Regenerated shoots from *Peganum harmala* L explants					
	Hypocotyl		Leaf		Root	
	Shoots	Relative %	Shoots	Relative %	Shoots	Relative %
MS(0)	1.25 ± 0.12 NS	26.3	1.05 ± 0.09 NS	22.1	0.97 ± 0.08 NS	20.4
MS(1)	1.32 ± 0.15 b	27.7	1.15 ± 0.11 NS	24.2	1.09 ± 0.12 NS	22.9
MS(2)	1.38 ± 0.13 c	29.0	1.25 ± 0.15d	26.3	1.19 ± 0.15b	25.0
MS(3)	1.45 ± 0.14 a	30.5	1.37 ± 0.14d	28.8	1.35 ± 0.12a	28.4
MS (4)	1.40 ± 0.17 a	29.4	1.28 ± 0.13b	26.9	1.22 ± 0.09c	25.6

* Harmine content = 4.76 mg/g dry weight of *in vivo P. harmala* mature seeds.

Data were expressed as means of five replicates ± SE (standard error). Means with the same letter in each column or line are significantly different at P = 0.05 according to the *f*-test. NS, not significant.

Determination of harmine alkaloid

Total alkaloids were extracted from hypocotyls, leaf, and root-derived calli, from regenerated shoos as well as from *in vivo* mature seeds.

Harmine content was generally affected by both the explant type and choice of PGRs (**Table 6**). Harmine accumulation more on MS media supplemented with 2,4-D + BAp, 2,4-D + Kin, NAA + BAp or NAA + Kin than on PGR-free MS medium. MS_{11} resulted in highest harmine content while hypocotyls-derived calli resulted in the highest harmine production and maximum relative harmine content (**Table 6**).

Highest harmine content (µg/g dry weight) as well as highest relative percentage of harmine content was recorded for shoots regenerated from hypocotyl-derived calli and from *in vivo* intact seeds (**Table 7**).

Explant type and PGR choice thus affected calli growth, shoot regeneration and also played a role in harmine accumulation. Reduced levels of 2,4-D led to an increase in digitoxin accumulation in cell cultures of *Digitalis purpurea* (Hagimori *et al*. 1982). Low levels of both 2,4-D and Kin also favored diosgenin production of cultures of *Costus speciosus* (Jain *et al*. 1984) and anthraquinone production in *C. ledgeriana* (Harkes *et al*. 1985).

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REFERENCES

Berrougui H, Isabelle M, Cloutier M, Hmamouchi M, Khalil A (2006a) Protective effects of *Peganum harmala* L. extract, harmine, and harmaline against human low-density lipoprotein oxidation. *Journal of Pharmacognosy and Pharmacology* **58**, 967-974

Berrougui H, Martin-Cordero C, Khalil A, Hmamouchi M, Ettaib A, Marheuenda E, Herrera MD (2006b) Vasorelaxant effects of harmine, and harmaline extracted from *Peganum harmala* L. seeds in isolated rat aorta. *Pharmacology Research* **54**, 150-157

Boulos L (1983) Zygophyllaceae family. In: Boulos L (Ed) *Medicinal Plants of North Africa*, References Publication Inc., Michigan, USA, p 195

Casado JP, Navarro MC, Utrilla MP, Martinez AL, Jiménez JK (2002) Micropropagation of *Santolina canescens* Lagasca and *in vitro* volatiles production by shoot explants. *Plant Cell, Tissue and Organ Culture* **69**, 147-153

El-Dwari QA, Banihani SM (2007) Histo-functional effects of *Peganum harmala* on male rats spermatogenesis and fertility. *Neurology and Endocrinology Letters* **28**, 305-310

Fraternale D, Giamperi L, Ricci D, Rocchi M (2002) Micropragation of *Bupleurum fruticosum*: the effect of triacontanol. *Plant Cell, Tissue and Organ Culture* **69**, 135-140

Gassama YK, Duhoux EP (1986) Micropropagation d'*Acacia albida* Del. (Légumineuses) adulte. Bulletin de l'IFAN. Cheik Anta Diop:T. 46, Ser A. N° 34, pp 315-320

Hagimori M, Matsumoto T, Obi Y (1982) Studies on the production of *Digitalis cardenolides* by plant tissue culture. II. Effect of light and plant growth substances on digitoxin formation by undifferentiated cells and shoot-forming cultures of *Digitalis purpurea* L. grown in liquid media. *Plant Physiology* **69**, 653-656

Harkes PA, Krijbolder L, Libbenga KR, Wijnsma R, Verpoorte R, Nsengiyaremge T, Baecheim-Svendsen A (1985) Influence of various media constituents on growth of *Cinchona ledgeriana* tissue cultures and production of alkaloids and anthraquinones therin. *Plant Cell, Tissue and Organ Culture* **4**, 199-214

Jain M, Rathore AK, Khanna P (1984) Effect of tricontanol on the micropropagation of *Costus speciosus*. *Agricultural and Biological Chemistry* **48**, 1197-1201

Johanian F, Ebrahimi SA, Rahbar-Roshandel N, Mahmoudian M (2005) Xanthomicrol is the main cytotoxic component of *Dracocephalum kotschyii* and a potential anti-cancer agent. *Phytochemistry* **66**, 1581-1592

Kartal M, Altun ML, Kurucu S (2003) HPLC method for the analysis of harmol, harmalol, harmine, and harmaline in the seeds of *Peganum harmala* L. *Journal of Pharmaceutical and Biomedical Analysis* **31**, 263-269

Kotb FT (1983) Therapeutic medical. In: Fabre J (Ed) *Medicinal Plants in Libya*, Arab Encyclopedia House, Beirut Press, Lebanon, pp 322-386

Lala S, Pramanick S, Mukhapadhyay S, Bandyopadhyay S, Basu MK (2004) Harmine evaluation of its antileishmanial properties in various vesicular delivery systems. *Journal of Drug Target Applied* **12**, 165-175

Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue culture. *Physiologia Plantarum* **15**, 473-497

Sane R, Jaiwal PK (2001) *In vitro* multiplication of *Peganum harmala* an important medicinal plant. *Indian Journal for Experimental Biology* **38**, 499-503

Shahverdi AR, Monsef-Esfahani HR, Nickavar B, Bitarafan L, Khodaee S, Khoshakhlagh N (2005) Antimicrobial activity and main chemical composition of two smoke condensates from *Peganum harmala* seeds. *Zeitschrift für Naturforschung* **60**, 707-710

Steel RD, Torrie JH (1960) *Principles and Procedures of Statistics*, McGraw Hill, New York, 134 pp

Tahraoui A, El-Hilaly J, Israili ZH, Lyoussi B (2007) Ethnopharmacological survey of plants used in the traditional treatment of hypertension and diabetes in South-Eastern Morocco (*Errachidia province*). *Journal of Ethnopharmacolgy* **110**, 105-117

Zakar F, Oody A, Arjmand A (2007) A study on the antitumoral and differentiation effects of *Peganum harmala* derivatives in combination with ATRA on leukamic cells. *Archive for Pharmaceutical Research* **30**, 844-849

Zhao J, Verpoorte R (2007) Manipulating indole alkaloid production by *Catharanthus roseus* cell cultures in bioreactors: from biochemical processing to metabolic engineering. *Phytochemistry Reviews* **6**, 435-457