Phytochemical screening on calli of *Fagonia indica* and *Fagonia bruguieri* Dc.

EMAN, A. ALAM

Botany Department, National Research Centre, Dokki, Giza, Egypt
Eman200980@hotmail.com

Abstract: Tissue culture technique was used to produce calli from the two wild economic plants (*Fagonia indica* Burm f var. *indica* and *Fagonia bruguieri* Dc.). MS medium supplemented with 5 mg/l Kinetin + 1 mg/l NAA induced calli from *F. indica* stem segments explants. In this regard, *Fagonia bruguieri* leaf explants can induce calli on MS medium supplemented with 5 mg/l Kinetin + 1 mg/l NAA also, while terminal bud explants can induce calli on this medium and MS medium supplemented with 5 mg/l Kinetin + 1 mg/l 2,4-D. While MS medium supplemented with 6 mg/l Kinetin + 2 mg/l NAA was the most suitable medium for growth of these calli of the two plants. Phytochemical screening on both calli of *F. indica* and *Fagonia bruguieri* revealed a variation in the presence/ amount of carbohydrates and / or glycosides, saponins, tannins, unsaturated sterols and/or triterpenoids, alkaloids, cardiac glycosides, cyanogenic glycosides, flavonoids, coumarins , chlorides and sulphates.

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Key words: *Fagonia*, *F. indica*, *Fagonia bruguieri*, phytochemical screening, tissue culture, calli .

1. Introduction

Genus *Fagonia* is represented in Egypt by 18 species (Tackholm, 1974), but it was represented by 15 species in Boulos, 2000. *Fagonia* species were extensively studied by many workers regarding their medicinal uses, since these plants were antitumor, antioxidant, analgesic, astringent, febrifuge and prophylactic against small-pox agents, species of *Fagonia* were also used for the treatment of cancer in the indigenous system, fever, asthma, urinary discharges, toothache, stomach troubles and kidney diseases (Ahsan *et al*., 2007 and Satpute *et al*., 2009). Species of *Fagonia* have been found to contain saponins (Abdel- Khalik *et al.,* 2001) , alkaloids (Sharawy and Alshammari, 2009), terpenoids (Perrone *et al.,* 2007), sterols (Shoeb *et al.,* 1994), flavonoids (Ibrahim *etal.,* 2008), proteins and amino acids (Sharma *et al*., 2010), coumarins (Zhang *et al.,* 2008), trace elements (Fatima *et al*., 1999). Tissue culture technique was used to cultivate different genera of Zygophyllaceae for regeneration purposes such as *Zygophyllum xanthoxylon* (Bunge) (Sun, 2008), to produce more biologically active compounds such as ascorbic acid from callus of *Fagonia cretica* (Kapoor, 2002), alkaloids, saponins, flavonoids and phenolic compounds of antibacterial activity more than the intact plant (Eman *et al*., 2010) diosgenin from callus of *Balanites aegyptiaca* (Gour and Kant, 2006) and beta -carboline and serotonin alkaloids and fatty acids from callus of *Peganum harmal*a (Piacetini *et al*., 2004).

The present study aims to produce calli from the two wild economic plants (*Fagonia indica* Burm f var. *indica* and *Fagonia bruguieri* Dc.) using tissue culture technique for the first time and to make a phytochemical screening on the chemical constituents of these calli.

2. Material and Methods

Plant materials

Samples of *F. indica* Burm f. var. *indica* (= *F. parviflora* Boiss.) and *Fagonia bruguieri* Dc. were collected from Quatamia - Suez desert road (150 Km away from Suez City). All the samples were authenticated by comparison with voucher specimens in the herbarium of Botany Department, Faculty of Science, Ain Shams University, Cairo, Egypt, where voucher specimens were deposited.

Methods

A- Tissue culture study

1- Callus induction

This experiment was carried out to study the effect of different levels of 2,4-D , NAA and Kinetin on calli induction of different explants obtaining from *F. indica* Burm f. var. *indica* (= *F. parviflora* Boiss.) and *Fagonia bruguieri* Dc. (stem, leaf and terminal bud). Explants were surface sterilized by immersion in 70 % ethanol for 30-60 seconds, then

soaked in 50% of commercial Clorox for 15-20 minutes, then washed with sterile distilled water "3 times" (Hoda, 1994). The sterilized explants were aseptically transferred to sterilized MS medium (Murashige and Skoog, 1962); supplemented with 3% sucrose, 1% agar and either 5 mg/l Kinetin + 1 mg/l 2,4-D (1) or 5 mg/l Kinetin + 1 mg/l NAA (2). Calli cultures were incubated at 25± 2°C for six weeks (Eman *et al.*, 2010).

2-Callus growth

Calli of *F. indica* (stem segments explants) and *Fagonia bruguieri* (leaf and terminal bud explants) can be maintained by subculturing on MS medium supplemented with different concentrations of auxins and cytokinins (Eman *et al.*, 2010) as follows:

Media	Hormones
a	6 mg/ l Kinetin+ 2 mg/l NAA
b	6 mg/ l Kinetin+ 2 mg/l 2,4-D
c	5 mg/ l Kinetin + 1 mg/l NAA
d	5 mg/ l Kinetin+ 1 mg/l 2,4-D
e	5 mg/l Kinetin + 2 mg/l NAA
f	5 mg/l Kinetin + 2 mg/l 2,4-D

Calli cultures were incubated at 25± 2°C for four weeks. Weight of calli was taken as an indicator of callus growth on each medium as follows:

-	no growth of calli were observed on this medium
+	≤ 1 g
++	≤ 2 g
+++	≤ 5 g

3. Results and Discusion

A- Tissue culture study

1- Calli induction

MS medium supplemented with 5 mg/l Kinetin + 1 mg/l NAA induced calli from *F. indica* stem segments explants. In this regard, *Fagonia bruguieri* leaf explants can induce calli on MS medium supplemented with 5 mg/l Kinetin + 1 mg/l NAA also, while terminal bud explants can induce calli on both this medium and MS medium supplemented with 5 mg/l Kinetin + 1 mg/l 2,4-D (Photos: 1- 4).

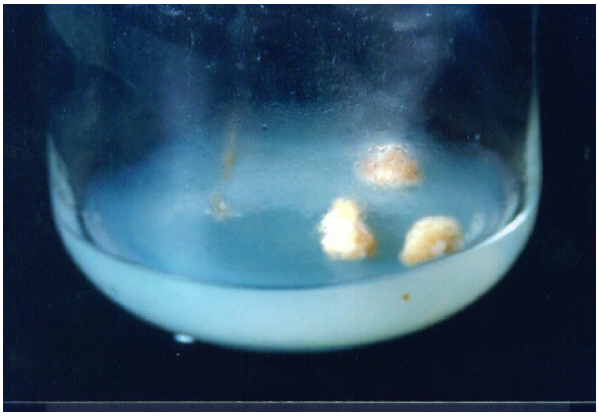

Photo (1): Callus induction of *F. indica* stem explants on MS+5mg/l Kinetin+1mg/l NAA.

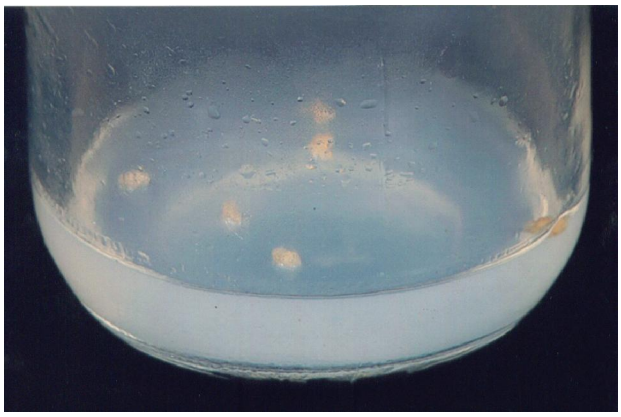

Photo (2):Callus induction of leaf of *F. bruguieri* on MS+5mg/l Kinetin+1mg/l NAA.

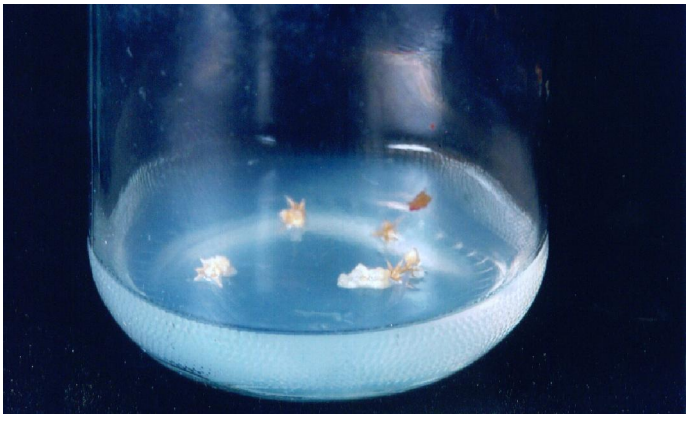

Photo (3):Callus induction of terminal bud of *F. bruguieri* on MS+5mg/l Kinetin+1mg/l 2,4-D

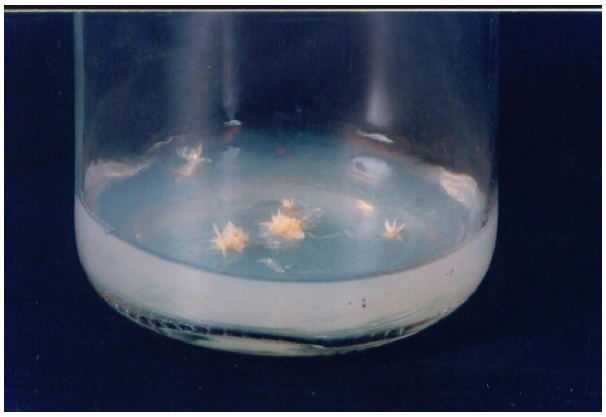

Photo (4): Callus induction of terminal bud of *F. bruguieri* on MS+5mg/l Kinetin+1mg/l NAA.

2- Callus growth of *F. indica* (stem segments explants) and *Fagonia bruguieri* (leaf and terminal bud explants)

Data in Table (1) and Photos (5-7) revealed that, callus of *F. indica* stem segments explants grew on media number a, d and e only, with special reference to that on medium number a. While *Fagonia bruguieri* leaf explants calli can grew on media number b only, terminal bud explants of *Fagonia bruguieri* can grew on media number a, c, d and f only, with special reference to that on medium number a.

Table (1): Quantitative estimation of calli of *F. indica* (stem segments explants) and *Fagonia bruguieri* (leaf and terminal bud explants) .

Media	Weight of callus of *F. indica* stem segments explants	Weight of callus of *F. bruguieri* leaf explants	Weight of callus of *F. bruguieri* terminal bud explants
a	+++	-	+++
b	-	+	-
c	-	-	++
d	++	-	++
e	+	-	-
f	-	-	+

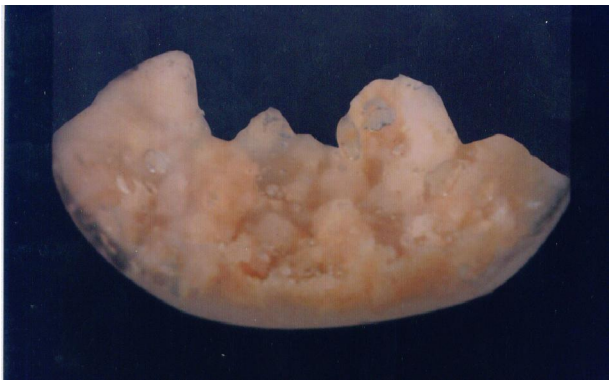

Photo (5): Callus growth of stem segments explants of *F. indica* on medium number (a).

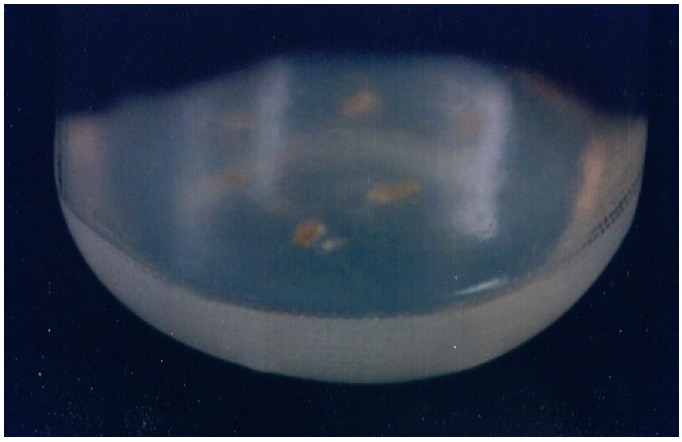

Photo (6): Callus growth of leaf explants of *F. bruguieri.* on medium number (b) .

Photo (7): Callus growth of terminal bud explants of *F.bruguieri* on medium number (a).

B-Preliminary phytochemical screening on both callus of *F. indica* hypocotyle explants and the intact hypocotyle:

Preliminary phytochemical screening (Table: 2) [on both calli of *F. indica* stem segments explants that grew on media number a, d and e (callus 1a, 1d, 1e), *Fagonia bruguieri* leaf explants calli that grew on media number b (callus 2b), terminal bud explants of *Fagonia bruguieri* that grew on media number a, c, d and f (callus 3a, 3c, 3d, 3f)] revealed that, there were variations in the presence/ amount of sapponins, flavonoids and chlorides within different calli of *F. indica* stem explants that grew on different media. Regarding *F.bruguieri* terminal bud explants there were variations also in these phytochemicals in addition to Sulphates, irodoids and cyanogenic glycosides within different calli that grew on different media, and there were also variations in these phytochemicals within calli of terminal bud and leaf explants of *Fagonia bruguieri.* So we can conclude that there were two factors controlled the *in vitro* production of phytochemicals; explants used and concentrations and types of plant growth regulators added to the medium (such as concentrations and types of auxins " NAA or 2, 4-D" in our study). So we can use these factors for directing the tissues to produce the needed phytochemicals.

Table (2): Preliminary phytochemical screening on both calli of *F. indica* (stem segments explants) and *Fagonia bruguieri* (leaf and terminal bud explants) on different media.

Experiment	Calli of *F. indica* stem explants			Calli of *Fagonia bruguieri* (leaf and terminal bud explants)				
	1a	1d	1e	2b	3a	3c	3d	3f
1- Carbohydrates and / or Glycosides	+	+	+	+	+	+	+	+
2- Saponins	-	-	+	-	+	++	-	-
3- Tannins	+	+	+	+	++	+	+	+
4- Unsaturated sterols and / or Triterpenoids	+	+	+	+	+	+	+	+
5- Alkaloids	+	+	+	+	+	+	+	+
6- Cardiac glycosides	+	+	+	+	+	+	+	+
7- Cyanogenic glycosides	+	+	+	+	-	+	+	+
8- a- Chlorides	-	-	+	-	+	-	-	-
8- b- Sulphates	+	+	+	+	-	+	+	+
9- Irodoids	-	-	-	-	-	-	+	+
10-Flavonoids	-	-	+	-	+	-	-	-
11- Coumarins	+	+	+	+	+	+	+	+
12- Anthraquinones	-	-	-	-	-	-	-	-

Corresponding author:
Eman, A. Alam
Botany Department, National Research Centre,
Dokki, El- Behoos Street, Giza, Egypt.
Eman200980@hotmail.com

Mobile: 0179469341-0187180968 (Egypt).

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