

## Comparison of Different Genotypes of Fennel (*Foeniculum vulgare* Mill.) in Terms of Chemical Compounds Extracted from Seeds and in the Callus Induced from Tissue Culture

<sup>1</sup>Mahran Mokhtar El Nagar and <sup>2</sup>Enas Mahmoud Mekawi

<sup>1</sup>Horticulture Department, Faculty of Agriculture, Benha University, Egypt.

<sup>2</sup>Agricultural Biochemistry Department, Faculty of Agriculture, Benha University, Egypt.

### ABSTRACTS

A laboratory study was conducted to evaluate the essential oil composition, total phenolic compound, total flavonoid compound and the antioxidant activity in seed and callus induced from tissue culture of two fennel genotypes. The influence of genotype, explants type and growth regulators concentrations on callus induction was investigated.

Results strongly showed that there were significant differences between two fennel genotypes. Generally the highest values of all parameters were obtained from Balady genotype. Callus was induced from two explants type on MS medium containing different combinations of 2,4-D and Kinetin.

The essential oil characterization by GC revealed that seed oil had the highest amount of trans-Anethol of two genotypes. On the other hand, callus oil from Balady genotype had the highest amount of  $\alpha$ -Pinene while Romanesco genotype had the highest amount of p-Cymene. Balady genotype had the highest amount of total phenolic and flavonoid content in seed as well as callus induced from tissue culture compared with Romanesco genotype. The antioxidant activities of both genotypes were evaluated and Balady genotype showed the highest DPPH\_ scavenging activity expressed as IC<sub>50</sub> compared with Romanesco genotype.

**Key words:** *Foeniculum vulgare* Mill. tissue culture, callus induction, oil, antioxidant activity.

### Introduction

Recently, an increasing interest in the cultivation and the production of untraditional vegetable crops has been noticed in Egypt in order to cover the increasing demand of the local consumption as well as export purposes. Among them sweet fennel (*Foeniculum vulgare* Mill.) is one of the most promising crop belong to family *Apiaceae* (Umbelliferae) in Egypt (Salama *et al.*, 2014). It is considered as the most important economic medicinal and aromatic plant grown within the Mediterranean region (Kirici *et al.*, 2010; Khodadadi *et al.*, 2013). The cultivated area in Egypt is about 2000–3000 fed and its export value amounts to 10 million US \$ according to the Ministry of Agriculture Statistics. Due to medicinal and aromatic compound's content of fennel, it is widely used in culinary preparation as a flavoring agent, food and beverages and perfumery industries, scenting soaps and cosmetics industries as well as in confectionery (Cao *et al.*, 1998; Telci *et al.*, 2009; Rather *et al.*, 2012). Due to unique and preferred flavor and aroma, the swollen bases of fennel are freshly consumed in salad or cooked as kitchen vegetable (Atta-Aly, 2001). Many researches have been carried out on fennel oils composition from various origins (Damjanovic *et al.*, 2005; Singh *et al.*, 2006; Napoli *et al.*, 2010). It has been found that the principal constituents are trans-anethol and fenchone (Akgül and Bayrak, 1988; Zoubiri *et al.*, 2014).

*In vitro* culture of cells, tissues or organs provides the accessibility to important secondary metabolites. Production of these metabolites by plant cell and tissue culture has many advantages such as standardization and improvement of products quality (Anzidei *et al.*, 1996; Zobayed *et al.*, 2004). In addition, Plant cell culture is considered as an effective system for the study of the biological importance of bioactive metabolites *in vitro* (Yanpaisan *et al.*, 1999; Afify *et al.*, 2011). Despite the cost needed to improve biotechnology techniques for producing aromatic compounds, there are several factors that support the idea. Nature-oriented consumers are concerned about the possible side effects of artificial food additives. Thus, natural products are increasingly preferred. In addition to that aromatic compounds of plant tissue culture systems, microbial fermentation or biological transformations should be more natural than their synthetic types (Lawless, 1992). Consumers are also concerned about pesticide and herbicides residues which are commonly found in agricultural food. Consumer acceptance is not the only crucial factor in pushing the industries to find biotechnology ways for as providing raw materials due to erratic weather conditions, seasonal changes, natural disasters or political instability in plant growth areas are also involved (Hunault, 1981 and 1984; Hunault *et al.*, 1989; Hunault and Maatar, 1995).

The importance of phenolic and flavonoid compounds as antioxidant in the maintenance of health and protection against coronary heart disease and cancer is also of raising interest among scientists, food

**Corresponding Author:** Mahran Mokhtar El Nagar, Horticulture Department, Faculty of Agriculture, Benha University, Egypt.  
E-mail: mahran.ashry@fagr.bu.edu.eg

manufactures and consumers (Löliker, 1991; Carbonneau *et al.*, 1998; Serafini *et al.*, 1998; Goupy *et al.*, 1999). This trend leads the future toward functional food with specific health effects (Carbanaro *et al.*, 2002; Faudale *et al.*, 2008; Gross *et al.*, 2009; Ghasemzadeh *et al.*, 2012).

The purpose of present study was to evaluate the essential oil composition, total phenolics, total flavonoids and the antioxidant activity in seed and callus induced from tissue culture of two fennel genotypes.

## Material and methods

### Plant material:

Two genotypes of fennel (*Foeniculum vulgare* Mill.) the local genotype Balady and Italian genotype Romanesco were obtained from the Preservation Germplasm Laboratory of the Department of Horticulture, Faculty of Agriculture, Benha University.

### Establishment of aseptic plants:

For establishing aseptic cultures of fennel growing *in vitro*, dry mature seeds were surface sterilized. Sodium hypochlorite a common disinfectant surface sterilize plant tissues was used. Seeds of the fennel genotypes were immersed in a 2.5% sodium hypochlorite for 15 min which is present in commercial bleach solutions (Clorix). Then they were rinsed 3 times with sterile distilled water for 5 min each. During immersion and rinsing the solution was stirred on a shaker at 200 rpm under the laminar air flow hood. The sterilized seeds were placed into sterile tissue culture jars containing a half concentrated basal MS medium (Murashige and Skoog, 1962) supplemented with B5 vitamins (Gamborg *et al.*, 1968), 3.0% sucrose and solidified with 0.7% Oxoid-Agar. The medium was adjusted to pH 5.8 before autoclaving at 121°C and 1.2 kg/cm<sup>2</sup> to 1.3 kg/cm<sup>2</sup> pressure for 20 min. All cultures were incubated at 25°C ±1°C under florescent light (2000 LUX) and a 16 h photoperiod.

### Induction of callus:

Explants of stem and petiole pieces (1cm) of two fennel genotypes were taken from aseptic plants 15 days old after *in vitro* germination of the seed and cultured horizontally on the MS medium. Explants induction media for all experiments contained 7 g/l agar and 30 g/l sucrose. Explants were sub-cultured every 4 weeks. The MS media were adjusted to 5.8 pH prior autoclaving at 121°C for 20 minutes. Cultures were incubated on MS medium containing different plant growth regulators as the following: 0.5, 1, or 2 mg/l 2, 4- D as auxin alone or in combination with 0.5, 1 or 2 mg/l kinetin. Callus cultures were maintained on the same medium and sub cultured every 4 weeks on a fresh medium to get the required amount of callus.

## Chemical investigation:

### Preparation of powder extract:

Seeds and callus induced from tissue culture of two fennel genotypes under study were extracted by liquid nitrogen. Ten grams of frozen tissues were ground in mortar with pestle and extracted 100 ml of 80% methanol by maceration (48 h).

### Oil extraction:

The essential oil of callus tissues and fennel seed was extracted by hydro-distillation according to Guenther (1960). The GC analysis of the oil was carried out using Gas chromatograph, (Hewlett packard GC. Model 5890) equipped with a flame ionization detector (FID). A fused silica capillary (HP-5), (30 m length x 0.53 mm internal diameter (i.d.) x 0.88 um film thickness), was used for the separation in the GC.

The following are the operating conditions of GC instrument: Injector and detector temperature were 250 and 270 °C, initial oven temperature, 50 °C for 2 min., raised at 6 °C per min, and then hold at 150 °C for 5 min, then raised at 5 °C per min. then hold at 190 °C the carrier gas was nitrogen at 4 ml per min. and hydrogen and air were used for the combustion at 30 and 300 ml per min., respectively.

The identification of the different constituents was achieved by comparing their retention times with those of the authentic samples.

### Determination of total phenolics:

The total phenols of powdered fennel seeds and callus were spectrophotometrically determined by Folin Ciocalteu reagent assay according to Singleton and Rossi (1965), using gallic acid as a standard compound for the preparation of calibration curve (20–120 mg/l). Total phenolics content of samples was measured at 670 nm and expressed as mg gallic acid equivalents (GAE)/g dry weight. All samples were analyzed in triplicates.

### Determination of total flavonoid content:

Total flavonoids content of fennel extracts was spectrophotometrically determined by the aluminum chloride method using quercetin as a standard (Zhishen *et al.*, 1999). After incubation at room temperature

samples were measured at 512 nm and expressed as mg quercetin equivalents (QE)/g fresh weight. Samples were analyzed in triplicates.

#### *Antioxidant activity (DPPH assay):*

The method described by Blois (2002) was used to assess the (1,1-diphenyl-2-picrylhydrazyl) DPPH<sub>•</sub> radical scavenging activity of sweet fennel methanolic extract. 1 ml of 0.1 mM DPPH<sub>•</sub> was added to 3 ml of fennel methanolic extracts. The mixture was shaken vigorously and allowed to stand at room temperature for 30 min. The butylated 4-hydroxytoluene (BHT) was used as a positive control; and negative control contained the entire reaction reagent except the extracts. Then the absorbance was measured at 517 nm against blank (methanol pure). Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. The capacity to scavenge the DPPH<sub>•</sub> radical was calculated using the following equation:  
 DPPH<sub>•</sub> scavenging effect (Inhibition %) =  $[(Ac - AS)/Ac] \times 100$   
 where Ac was the absorbance of the control reaction and As the absorbance in the presence of the plant methanolic extract. IC<sub>50</sub>: its concentrations for 50% inhibition.

#### *Experimental design and Statistical analysis:*

Experiments were arranged in a completely randomized block design with 3 replications. Data were estimated as the mean and its standard error of the different traits. The calculations were done using Microsoft Excel 2010 program.

## **Results**

### *Callus formation:*

It is advantageous to clonally multiply tissue by initiation of callus growth from different explants type. Experiments were performed to monitor the rate of callus tissues formation which are a good source for secondary plant production. Dry mature seeds were surface sterilized with sodium hypochlorite for establishing aseptic cultures of fennel growing *in vitro* (Figure 1). Explants from stem and petiole pieces of aseptic fennel plants were used to obtain a high amount of callus (Figure 1). Different growth regulators in various concentrations were tested for their ability to induce callus. The growth/size of callus tissues that had developed from different explants was recorded six weeks after tissue culture initiation.

The results presented in table 1, revealed that callus formed from stem and petiole pieces of each of the two fennel genotypes. Higher number of callus was obtained with the two genotypes when the primary culture was conducted on MS medium containing 2,4-D as a plant growth regulators. In stem explants derived from Balady and Romanesco genotypes, different 2,4-D concentrations were tested alone or in combination with kinetin. Results in table 1 indicated that 2 mg/l 2,4-D with 0.5 mg/l kinetin was the best concentration giving the biggest size of callus as well as the highest percentage of callus formation (100%). In regard to stem explants derived from Balady genotype, the biggest size of callus was 10.4 mm and 8.8 mm of stem explants derived from Romanesco genotype. Reducing 2,4-D concentration in the presence or in the absence of kinetin resulted in decreasing of callus induction as well as callus size of both genotypes.



**Fig. 1:** Establishing aseptic cultures of fennel Balady genotype and formation of calli derived from different explants types.

*Callus was observed two month after initiation of the cultures. a: sterilized dry mature fennel seed, b: aseptic fennel plant after 15 days of seed germination, c: stem segments, d: petiole peaces, e: callus formation on MS medium containing 2 mg/l 2,4-D and 0.5 mg/l kinetin.*

Hundred percent of callus formation was also obtained when petiole explants cultured on media contained 0.5, 1.0 or 2 mg/l of 2,4-D alone or in combination with 0.5 mg/l kinetin for Balady and Romanesco genotypes. The biggest size of callus derived from petiole explants was 6.1 mm and 7.6 mm of Balady and Romanesco genotypes respectively. Reducing 2,4-D concentration in the presence or in the absence of kinetin

resulted in decreasing of callus induction as well as callus size of both genotypes (Table 1). Generally, growth of the callus of stem pieces was more vigorous than that formed from explants of petiole pieces. Additionally, Formation of callus was observed on stem explants derived from genotype Balady after 4 weeks, and for genotype Romanesco after 5 weeks of tissue culture initiation.

**Table 1.** Effect of plant growth regulators on callus induction in different types of explants of Balady and Romanesco fennel genotypes.

Growth regulator (mg/l)		Balady				Romanesco			
2,4-D	Kinetin	Stem		Petiole		Stem		Petiole	
		Callus size (mm)	Callus formation %						
0.5	0	6.0 ± 0.9	98	4.5 ± 0.5	94	5.8 ± 1.1	98	4.3 ± 0.6	93
1	0	7.0 ± 0.9	100	4.9 ± 0.1	100	5.3 ± 0.2	100	5.0 ± 0.9	98
2	0	8.6 ± 0.5	100	5.9 ± 0.1	100	8.0 ± 1.0	100	6.0 ± 0.9	100
0.5	0.5	3.7 ± 0.3	100	3.9 ± 0.2	97	3.9 ± 1.0	96	3.8 ± 0.3	97
1	0.5	4.9 ± 0.1	100	4.0 ± 0.0	99	3.8 ± 0.1	99	2.7 ± 0.4	98
2	0.5	10.4 ± 0.5	100	6.1 ± 1.1	100	8.8 ± 1.0	100	7.6 ± 0.5	100
0.5	1	3.8 ± 0.2	95	2.6 ± 0.7	90	3.1 ± 0.4	95	2.8 ± 0.2	91
1	1	4.7 ± 0.2	99	3.7 ± 0.3	98	3.8 ± 0.2	98	3.7 ± 0.2	97
2	1	5.8 ± 1.1	100	3.9 ± 0.2	99	4.0 ± 0.3	99	3.9 ± 0.1	99

All values of callus size are the mean ± standard deviation in three replicates.

### Chemical analysis:

#### Chemical compositions of the fennel oils from callus and seed:

Data presented in table 2 indicate that the oil content and composition of the two different fennel genotypes were different. In addition, the qualitative and quantitative oil composition derived from callus tissues and seed were varied. Seed oil of Balady genotype had the highest amount of trans-Anethol (69.9%), Fenchone (12.2%), Limonene (5.5%) and the lowest amount (0.1 %) of Sabinene, 1,8-Cineol, 3-Carene and Fenchyl acetate. Seed oil of Romanesco genotype had the highest amount of trans-Anethol (66.0%), Fenchone (11.7%), Estragol (5.7%), Limonene (5.3%) and the lowest amount (0.2 %) of Sabinene, 3-Carene, Fenchyl acetate and (0.8%) 1,8-Cineol (Table 2). Oil from callus induced of Balady genotype had the highest amount of  $\alpha$ -Pinene (29%), p-Cymene (21.7%),  $\beta$ -Pinene (20%) and the lowest amount of Estragol (0.2%). Concerning the oil composition derived from callus of Romanesco genotype, the p-Cymene had the highest amount (22.2) followed by  $\alpha$ -Pinene and Fenchone (14.3%) and the lowest amount was 0.3% of 1,8-Cineol and  $\beta$ -Myrcene (Table 2). Moreover, Cis-anethol was not observed in the callus oil of the two fennel genotypes.

**Table 2:** Percentage composition of the oil from seed and callus of two fennel genotypes.

Compound	Balady		Romanesco	
	Seed	Callus	Seed	Callus
$\alpha$ -Pinene	2.2	29.0	2.0	14.3
Camphene	0.9	3.8	0.7	4.1
Sabinene	0.1	0.5	0.2	2.8
$\beta$ -Pinene	1.2	20.0	0.9	13.5
$\beta$ -Myrcene	1.3	0.5	1.7	0.3
p-Cymene	1.5	21.7	2.5	22.2
Limonene	5.5	3.1	5.3	4.9
1,8-Cineol	0.1	0.2	0.8	0.3
3-Carene	0.1	1.3	0.2	3.2
Fenchone	12.2	7.1	11.7	14.3
Camphor	0.2	5.5	0.4	12.7
Estragol	3.7	0.2	5.9	0.5
Fenchyl acetate	0.1	0.3	0.2	0.6
Cis-anethol	0.4	0	0.3	0
Trans-Anethol	69.6	6.0	66.0	5.5

All values are in percentage of three replications.

#### Total phenolics and flavonoids content:

Results in table 3 showed that total phenolic content was varied according to the genotype. The total phenolic content of Balady genotype was 5.9 and 3.6 mg/g DW in seed and callus tissues, respectively. However, in Romanesco genotype total phenolic content was 5.1 and 3.4 mg/g DW in seed and callus tissues, respectively.

Regarding the total flavonoid content of two fennel genotypes, it was obvious that seed of Balady genotype had the highest amount of total flavonoid content 3.6 mg/g DW comparing with 3.0 mg/g DW of Romanesco genotype. Moreover, the total flavonoid content in callus tissues was 2.6 and 2.3 mg/g DW of Balady and Romanesco genotype, respectively (Table 3).

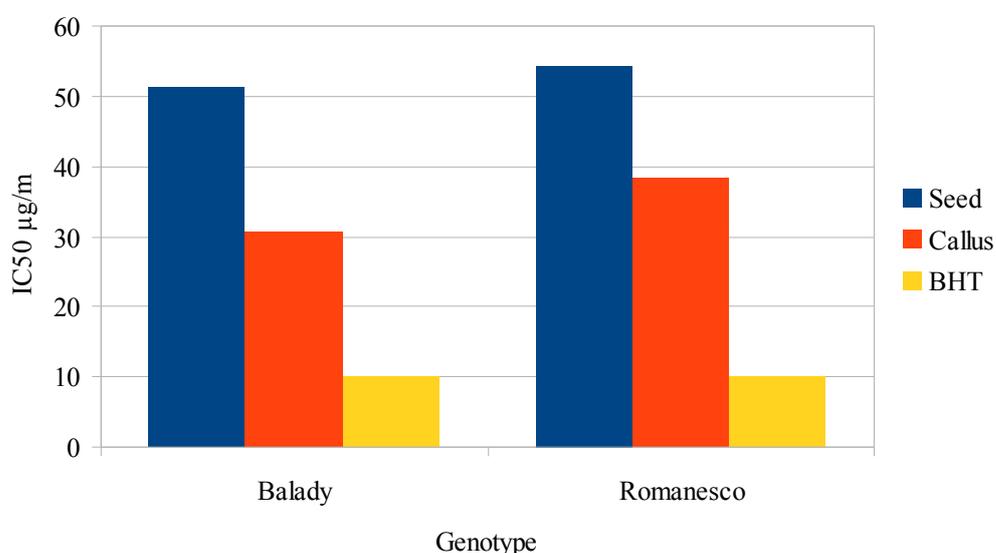
**Antioxidant activity:**

The antioxidant activity using DPPH of seed and callus tissues of Balady and Romanesco genotypes has been determined. The main values of radical DPPH scavenging activity expressed as IC<sub>50</sub> against DPPH radical of two fennel genotypes under study compared to BHT as a synthetic antioxidant agent are illustrated in figure 2. Romanesco genotype demonstrated the highest IC<sub>50</sub> (54.3 and 38.3 µg/ml of seed and callus tissues, respectively). In addition, the values of IC<sub>50</sub> for methanolic extracts of Balady genotype was 51.2 and 30.6 µg/ml of seed and callus tissues, respectively) while, BHT exhibited the highest scavenging activity (10.07 µg/ml).

**Table 3:** Determination of total phenolics and flavonoids content in seed and callus of two fennel genotypes.

Genotype	Total phenolic content (mg/g DW)		Total flavonoid content (mg/g DW)	
	Seed	Callus	Seed	Callus
Balady	5.9 ± 0.13	3.6 ± 0.15	3.6 ± 0.23	2.6 ± 0.20
Romanesco	5.1 ± 0.12	3.4 ± 0.13	3.0 ± 0.21	2.3 ± 0.15

All values are the means ± standard deviation of three replicates.



**Fig. 2:** IC<sub>50</sub> of DPPH free radical of 80% methanolic extract from seed and callus of two fennel genotypes.

**Discussion:**

The most important finding of this study is the development of a high amount of callus, and its utilization in production of secondary plant compounds of *Foeniculum vulgare* Mill. Hore, 1979 reported that the poor seed-set of various aromatic umbelliferae is the result of bad pollen quality. He also stated that fennel may present some self-incompatibility. Tissue culture and plant cells methods have been done to produce plants of better compatibility and efficient performance based on human needs (Afify *et al.*, 2011).

**Callus Induction:**

In *Foeniculum vulgare* Mill., tissue cultures has been attained using hypocotyl explants (Anzidei *et al.*, 1996; Khodadadi *et al.*, 2013) or stem and petiole segments (Hunault, 1981), and the present study showed that using explants derived from, stem and petiole segments are a viable alternative for callus induction in *Foeniculum vulgare* Mill. Our results confirm that fennel has a high a capacity to produce callus as already shown by several authors (Paupardin *et al.*, 1980; Hunault, 1981 and 1984; Anzidei *et al.*, 1996; Khodadadi *et al.*, 2013). Callus induction and morphogenic response of different genotype tested were controlled by interaction between genotype, explants type and growth regulators.

**Growth regulators:**

Callus formation are normally begun in culture medium containing high auxin levels (mainly 2,4-D). In *Foeniculum vulgare* Mill., the most commonly used auxin is 2,4-D, alone or in combination with kinetin or NAA with kinetin or with BAP (Paupardin *et al.*, 1980; Hunault, 1981 and 1984; Hunault and Maatar, 1995;

Anzidei *et al.*, 1996; Afify *et al.*, 2011; Khodadadi *et al.*, 2013). The presented results showed that the highest number of callus obtained from various explant types and different genotypes were induced on MS medium supplemented with 2 mg/l 2,4-D with 0.5 mg/l kinetin or 2,4-D alone. These results indicated that stress induction through high auxin concentration, especially 2,4-D, is required for induction of callus in fennel. In general, results in agreement with (Hunault 1984; Anzidei *et al.*, 1996; Afify *et al.*, 2011; Khodadadi *et al.*, 2013).

#### Genotypic differences:

Effects of different types and concentrations of growth regulators and genotypes on the callus induction of *Foeniculum vulgare* Mill. are shown in tables 1, 2, 3 and figure 2. The main effect of genotypes showed that Balady genotype yielded callus and different chemical compositions higher than Romanesco genotype. Similar results were reported by Bennici *et al.*, 2004 and Khodadadi *et al.*, 2013 who reported that type and concentrations of growth regulators with genotypes influenced the frequency of callus formation. Hunault *et al.* 1989 and Hunault and Du Manoir 1992 found differences in morphological characters, essential oil composition and fruit production in somatic embryo-derived clones, when compared to the original clone. Miura *et al.* 1987 have shown that in *F. vulgare* the coefficient of variation between plants obtained via embryogenesis was lower than plants obtained from seeds. On the other hand, Bennici *et al.*, 2004 indicated that the tissue culture in their Francia Pernod fennel does not induce somaclonal variation on the regenerated plants and that the pattern of regeneration (organogenic or embryogenic) and growth regulators do not influence the genetic stability and uniformity.

#### Essential oil:

According to the results in table 2, the chemical composition of the seed oil was different from that determined in callus oil. Seed oil had the highest amount of trans-Anethol, Fenchone, Limonene and the lowest amount of Sabinene, 1,8-Cineol, 3-Carene and Fenchyl acetate. On another hand, callus oil of Balady genotype had the highest amount of  $\alpha$ -Pinene, p-Cymene,  $\beta$ -Pinene and the lowest amount of Estragol. Concerning the oil composition derived from callus of Romanesco genotype, the p-Cymene had the highest amount followed by  $\alpha$ -Pinene, Fenchone and the lowest amount was 1,8-Cineol and  $\beta$ -Myrcene. Moreover, Cis-anethol was not observed in the callus oil of the two fennel genotypes. Production of anethole has not been observed in most studies that have been conducted on Fennel tissue culture (Kirici *et al.*, 2010; Khodadadi *et al.*, 2013). But Afifi *et al.* (2011) observed Trans-anethole in the callus tissue culture. Furthermore, in a study by Kirici *et al.* (2010), the results of the GC/MS in regenerated plants showed that monoterpene materials including Pinene and Limonene and had the highest percentage. The essential oil composition of *F. vulgare* exhibits considerable chemodiversity depending upon the method of extraction and geographical origin. The accumulation of these volatile compounds inside the plant is variable, appearing practically in any of its parts viz. roots, stem, shoots, flowers and fruits (Diaaz-Maroto *et al.*, 2006; Gross *et al.*, 2009). Rather *et al.*, 2012 reported that the essential oil content and composition varies during the different maturation stages of *F. vulgare*. The essential oil content was reported to decline with fruit maturity. The content of trans-anethole, the main component, varied between 81.63% and 87.85% (Telci *et al.*, 2009). However, present results confirm that fennel has a high capacity to produce oil from seed and callus as already shown by several authors (Akgül and Bayrak, 1988; Damjanovic *et al.*, 2005; Fang *et al.*, 2006; Singh *et al.*, 2006; Telci *et al.*, 2009; Napoli *et al.*, 2010; Atta-Aly, 2011; Khodadadi *et al.*, 2013; Zoubiri *et al.*, 2014).

#### Phenolic and flavonoids compounds:

The other classes of phytochemicals present in *F. vulgare* are phenolics and flavonoids compounds. Results in table 3 showed that Balady genotype had the highest amount of total phenolic and flavonoids content in seed as well as callus induced from tissue culture compared with Romanesco genotype. These results are in agreement with those obtained by Liu *et al.*, 2008; Yoo *et al.* 2008; and Salama *et al.*, 2014) who found that the total phenolic content of fennel differed according to genotype and plant tissues.

#### Antioxidant activity:

The antioxidant activity of wild, edible and medicinal fennels from different Mediterranean countries has been determined. Wild fennel has been found to exhibit a radical scavenging activity higher than that of both medicinal and edible fennels (Faudale *et al.*, 2008). DPPH<sub>•</sub> has been widely used for the determination of antioxidant activity of different plants, vegetables, and fruit extracts (Goupy *et al.*, 1999; Yu, 1994; Oktay *et al.*, 2003; Blois, 2002; Salama *et al.*, 2014). The main values of radical DPPH<sub>•</sub> scavenging activity expressed as IC<sub>50</sub> against DPPH<sub>•</sub> radical of two fennel genotypes Balady and Romanesco compared to BHT as a synthetic antioxidant agent are illustrated in Figure 2. IC<sub>50</sub>: Its concentration for 50% inhibition. Balady genotype yielded the highest IC<sub>50</sub> in comparison with Romanesco genotype. In addition, BHT exhibited the highest scavenging activity (10.07  $\mu$ g/ml). The methanolic extracts of both fennel genotypes were able to scavenge DPPH<sub>•</sub> radical.

These results revealed that Balady genotype exhibited the highest activity when compared with, Romanesco genotype. Mata et al. (2007) evaluated the antioxidant activity of ethanol and water extracts of fennel plant. The ethanolic extract showed the highest radical scavenging activity value ( $IC_{50} = 12 \text{ lg/ml}$ ) while, butylated hydroxytoluene (BHT) which was used as standard gave  $IC_{50} = 15.7 \text{ } \mu\text{g/ml}$  and water extract gave  $IC_{50} = 48.0 \text{ } \mu\text{g/ml}$ . The results illustrated in Figure 2 revealed that total phenolic and flavonoid compounds strongly contributed to the antioxidant scavenging activity of fennel methanolic extracts. These findings further supported the positive relationship between total phenolic, total flavonoid and antioxidant activity of different plant species. Ghasemzadeh et al. (2012) reported the strong positive relationship between total phenol and antioxidant activity that appears to be the trend in many plant species. Serafini et al. (1998); Carbonneau et al. (1998) and Carbanaro et al. (2002) also suggested that flavonoids and phenolics have a preventive role in the development of cancer and heart disease. Consumption of controlled diets high in the fruits and vegetables increased significantly the antioxidant capacity of plasma (Cao *et al.*, 1998). Moreover, epidemiological studies showed that significant negative correlation was found between the intake of fruits and vegetables and heart disease mortality (Knekt *et al.*, 1996). Carbanaro *et al.*, 2002 showed that the presence of phenolics in food is particularly important for their oxidative stability and antimicrobial protection. From clinical point of view, there is numerous evidence of sweet fennel to alleviate diseases.

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