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EFFECT OF POLY ETHYLENE GLYCOL (PEG) ON (FENOLES COMPOUNDS) PRODUCTION OF *OLEA EUROPAEA* L. FROM CALLUS *IN-VITRO*

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ABSTRACT

The present study in order to increase the production of some secondary metabolic compounds (Fenoles compounds) of *Olea europaea L. in vitro.* Secondary metabolites were estimated quantitatively and qualitatively using high performance liquid chromatography HPLC and compared with those in the mother plant. In order to increase the production of secondary metabolites, Poly Ethylene Glycol (PEG) used at concentrations (0, 0.5, 1.0, 1.5, 2.0) %. The results showed that the concentration 2% of PEG caused highly significant production in all of the secondary metabolites (Fenoles compounds) of callus for *Olea europaea L.*

Key Words: *Olea europaea* L., Fenoles compounds, PEG.

INTRODUCTION

The olive tree (*Olea Europea L*, Oleaceae) is one of the most important trees in Mediterranean countries. It grows through the entire Mediterranean and in most of southern European countries [1]. *Olea Europaea* L. wildly studied for its alimentary use. The fruits and oil are important components in the daily diet of a large part of the world's population, where as the leaves are important for their contents of secondary metabolites. They consist of phenolic compounds, flavonids and volatiles oils [2-3].

Plants have played a significant role in maintaining human health and improving quality of human life for thousands of years and have served human as valuable components of medicines [4]. Herbal medicine is a growing area of health care that demands attention. It is also an important branch of alternative medicine [5].

Plant tissue culture techniques inters in several applications like plant micro propagation, genetic study ,plant improvement ,production of pathogen free plant disease (bacterial and fungal), in addition the production of secondary metabolite *in vitro* is possible through plant tissue culture.

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In vitro study holds a potential for the production of high quality plant based medicines. This can be achieved through different methods including micro propagation of cell lines which are capable of producing high yield of secondary compounds. The accumulation of secondary products in plant cell culture depends on many factors including the composition of the culture medium and environmental conditions [6-7].

Plant materials and Sterilization

The explants of *O. europaea L.* from newly branches were collected from the Al- Mustansiriya University Gardens in Baghdad, Iraq on 20/03/2014. rinsed with running tap water for 1 hr., then transferred to laminar air flow-cabinet and submerged in (99)% ethanol for one minute, Washed with sterilized DDH2O, then rinsed with sodium hypochlorite at the concentrations (2.0)% for (10) min. Then washed with DDH2O three times for five minutes and planted in vials of Agriculture (universal tubes) [8].

Callus medium

Explants (leaves) of *O. europaea* L. were dissected and cultured on culture vessels containing MS medium with different concentrations of the auxin 2,4-D 0, 1, 2, 3 or 4 mg/l, Table (1), then distributed into 10

replicates which incubated at 16/8 hrs. light/dark photoperiod at the illumination intensity was 1000 lux for 16 hours a day at a temperature 25 ± 1 °C f. The percentage of callus formation was recorded after 30 days [9].

Fresh and dry weight callus measurements.

The fresh weight of callus was measured by using a sensitive balance, then the callus was dried using an electric oven at 70 °C for 24 hrs, then measured by a sensitive balance [10].

Extraction and analysis of (Fenoles compounds) from *O.europaea L.*

A quantity of 0.5 g fresh callus were crushed and extracted with petroleum ether for 4hrs in a Soxhlet apparatus. The extract 1ml was concentrated under reduced pressure. The concentrated extract was dissolved in 20 ml petroleum ether, 2 ml methanol acid and 2 ml of KOH were added. The mixture was shaken for 2 min and allowed to stand for 10 min. The upper layer was removed and washed with water. Then the mixtures were passed through 2.5um disposable filter. Then 20 µl were injected on HPLC column was analyzed by HPLC according the optimum condition [11].

High Performance Liquid Chromatography (HPLC)

Samples analyses were performed with the HPLC system equipped with two shimadzu LC-10 AT equipped with binary delivery pump model LC-10A shimadzu, the eluted peaks were monitored by UV-Vis 10 A- SPD detector shimazeu SPD-10AVP and C-R6A chromatopack data processors, the standard phenols were obtained from Sigma Chemical Co. all the solvents used in this investigation were of HPLC grade.

Column: 3 µm particle size (50 x 2.0 mm I.D) C-18 DB column.

Mobile phase: solvent were 0.01 M ammonium acetate: acetonitrile (12:88 V/V) detection UV set at 285 nm., Flow rate 1.1 ml/min., temperature 35 °C [12]. The HPLC was used to estimate the increase or the decrease of the *Olea europaea L*. secondary metabolites, then the results were compared with the intact plant. The concentration of samples was measured by comparing the area of sample with the area of the standard multiply by concentration of standard which was 25 mg/l under the same conditions by using the following formula: [13].

$$\text{Concentration of sample (µg/ml)} = \frac{\text{Area of sample}}{\text{Area of standard}} \times \text{conc. of standard} \times \text{dilution factor}$$

Experimental design and statistical analysis

A completely randomized design (CRD) was used. Least significant differences (LSD) were calculated. The difference between the test average compared according to the least significant differences (LSD) at the probability of 5% [14].

RESULTS

Effect of different concentrations of Polyethylene glycol (PEG) on callus fresh weight (mg).

The results displayed in table (2) and fig (1) show that the concentration 1.5% of PEG had the highest callus fresh weight 2650 mg while the lowest callus fresh weight 713 mg was recorded at 0.5% PEG. The all treatments had no significant differences between them., and the concentration 1.5% of PEG had the highest callus dry weight 589 mg while the lowest callus dry weight 56 mg was recorded at 0.5% PEG. And there was no significant differences between them.

Effect of different concentrations of Polyethylene glycol PEG (%) on (Fenoles compounds) production from callus.

The results in table (3) showed that different concentrations of secondary metabolites increased depending on the increasing concentrations of added Polyethylene glycol PEG compared with intact plant. The figures (2),(3),(4),(5),(6) showed the HPLC curves results by using different concentrations of PEG.

All compounds (Ferulic acid, Oleuropein, OH-tyrosol, Tyro sol, Ligesroside) gave ahighly significant values (222.13 , 111.70 , 151.65, 198.09 , 229.37) µg/ml respectively at the concentration 2% PEG while the lowest concentration recorded at mother plant (1.07,1.33,1.01,1.03,1.29) µg/ml respectively and all concentrations of PEG gave a high significant values than the mother plant.

Table 1. The medium of callus induction components

NO	Components	Concentration (mg/l.)
1	MS	Full strength
2	Sucrose	30000
3	L- Asparagine	150
4	Glycine	100
5	Benzyl adenine	0.5
6	2,4-D	0,1,2,3,4
7	Agar-Agar	8000

Table 2. Effect of different concentrations of Polyethylene glycol (PEG) on callus fresh weight (mg)

Concentrations of PEG (%)	Fresh weight(mg)	Dry Weight(mg)
Cont.	1787	159
0.5	713	56
1.0	910	73
1.5	2650	589
2.0	1621	288
LSD(0.05)	2388.7	574.1

Table 3. Effect of different concentrations of Polyethylene glycol PEG(%)on (Fenoles compounds) production from callus

Secondary Metabolites	Concentration of PEG (%)					Mother plant	L.S.D 0.05
	Cont.	0.5	1.0	1.5	2.0		
Ferulic acid	55.98	166.45	89.26	161.19	222.13	1.07	1.369
Oleuropein	29.84	76.24	58.19	80.71	111.70	1.33	1.961
OH-tyrosol	50.43	49.07	106.65	122.40	151.65	1.01	1.904
Tyro sol	47.73	60.94	114.63	123.16	198.09	1.03	1.775
Ligesroside	51.74	56.18	92.88	107.64	229.37	1.29	1.658

Fig 1. Callus induced from leaf explant cultured on MS medium supplemented with left to right (1.5,2.0, Cont, 1.0 or 0.5) % of PEG

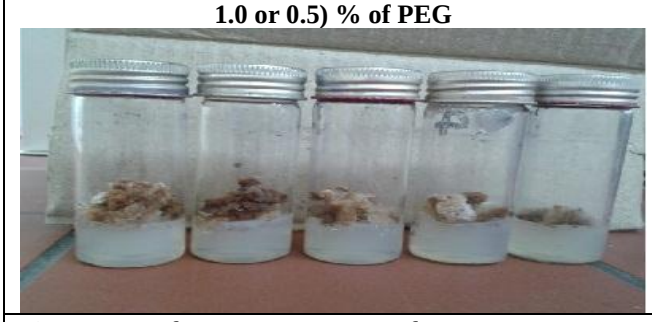


Fig 2. HPLC for control treatment of PEG

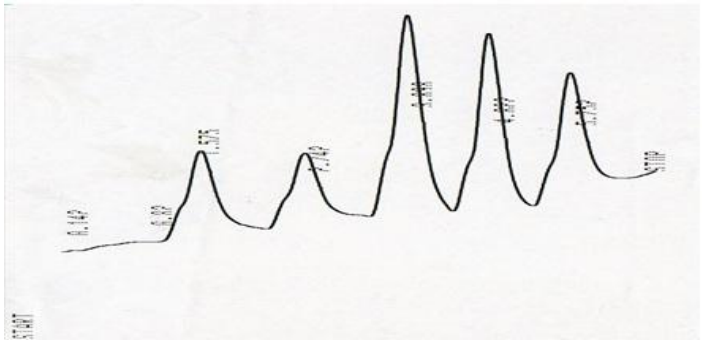


Fig 3. HPLC for 0.5% treatment of PEG

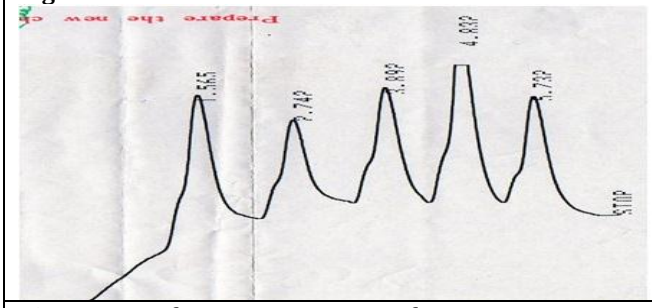


Fig 4. HPLC for 1.0% treatment of PEG

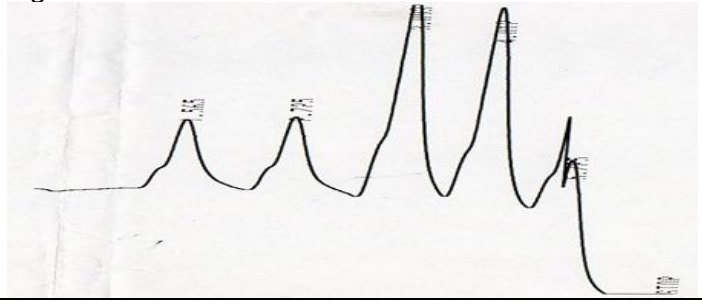


Fig 5. HPLC for 1.5% treatment of PEG

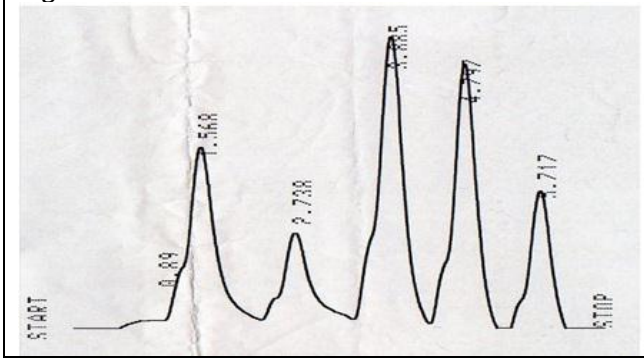
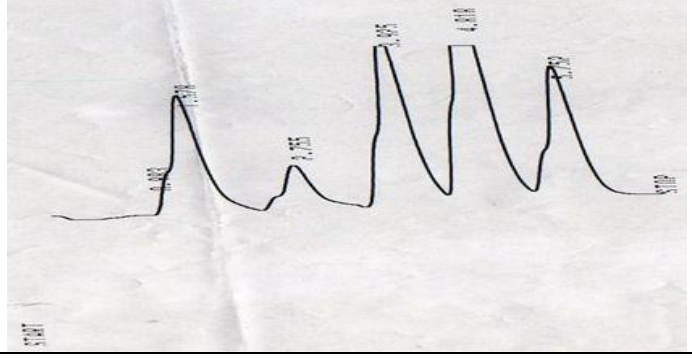


Fig 6. HPLC for 2.0% treatment of PEG



DISCUSSION

The results showed that there were no significantly differences in callus fresh and dry weight (mg) grown on maintenance medium in light. The effect of treatments (cont,0.5,1.0,1.5,2.0)% PEG on producing (Fenoles compounds) from callus by HPLC technique was very hight significant comparing with mother plant, Some studies described the effect of PEG on callus, for example callus of *Pogonatherum Paniceum* had the ability to resist the stress of PEG. The effect of PEG stress and culture conditions on the callus of *P.Paniceum* appeared mainly in two aspects, delaying regeneration time and debasing regeneration rates [15]. Many studies showed that adding PEG increased secondary metabolites in many plants, Shreedhara, (2013) said the stress is one of the factors that enhance secondary metabolites production. When use PEG with the callus culture medium resulted in an increase of Hispidulin secondary metabolite for the *Millingtonia hortensis* [16].

CONCLUSION

Adding 2% of Poly Ethylene Glycol (PEG) for callus medium caused highly significant increase in all the studied secondary metabolites (Fenoles compounds) of O.europaea L.

RECOMMENDATION

Use another stimulate to increase (Fenoles compounds) of O.europaea L.

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