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INCREASING (GLYCOSIDES COMPOUNDS) OF *OLEA EUROPAEA* L. FROM SHOOT TIPS USING $AgNO_3$ NANO PARTICLE *IN VITRO*

Wasan Mohammed Mousa Al-Sowaidi and Hashim K. Mohammed Al-oubaidi*

*Biology Department-Collage of Science, University of Al- Mustansiriyah, Baghdad, Iraq.

ABSTRACT

The present study in order to increase the production of some secondary metabolic compounds(Glycosides compounds) in shoot tips of *Olea europaea L. in vitro.* (Glycosides compounds) 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**,** Silver nitrite nanoparticles $AgNO_3$NPs used at concentrations (0, 0.5, 1.0, 1.5, 2.0) mg/l. The results showed that the concentration 2mg/l of AgNO3 NPs caused highly significant production in most of the secondary metabolites (Glycosides compounds) of shoot tips for *Olea europaea L*. while the concentration 1.0 mg/l of $AgNO_3$ NPs caused highly significant value in OH- Tyrosol.

Key words: *Olea europaea* L., Glycosides compounds, $AgNO_3$ NP.

INTRODUCTION

The olive *(Olea Europaea L.*) meaning "olive from Europe is a species of small tree in the family Oleaceae, found in much of Africa, the Mediterranean Basin from Portugal to the Levant" **(**Fabrizia, 2012).

Olive tree parts, and olive oil, have a number of common compounds that might possess positive health effect to the human body, as well as a good source of Vitamin E. The processing of olives (fruit) especially affects their phenolic content, but other parts of the plant also contain phenolics, such as leaves and bark.(Gooch**,** 2005).

Olive fruit contain several types of polyphenols, mainly tyrosols, phenolic acids, flavonols and flavones, and for black olives, anthocyanins. During the crushing, kneading and extraction of olive fruit to obtain olive oil, the glycosidic oleuropein, demethyloleuropein and ligstroside **(**Omar**,** 2010).

The production of the secondary metabolites *in vitro* is possible through plant tissue culture (Barnum, 2003). The production of medicinal plants by tissue culture provides industrial source of different necessary metabolic compounds , including alkaloid ,phenol , terpenoid ,vitamins and other of compounds which are necessary in medical uses (Shengwei and Jingsam,2000). The production of medicinal and pharmaceutical compounds using tissue culture is affected by several factors, such as the explant that is used in the culture and its source, the amount of explant, the component of nutrient media used in the culture and the condition of storage like light, temperature and aeration (Mantell and Smith, 1984).

Corresponding Author

Hashim K. Mohammed Al-oubaidi
Email: hashimkadhum@yahoo.com

MATERIALS AND METHODS

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. Then rinsed with running tap water for 1 hr., 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) (Pierik, 1987).

Growth medium

Explants (Shoot tips)of *O. europaea L*. were

dissected and cultured on culture vessels containing MS medium with concentrations of the cytokinen BA 2 mg/l, Table (1), then distributed into 10 replicates which incubated at 16/8 hrs. light photoperiod at the illumination intensity was 1000 lux for 16 hours a day at a temperature 25 ± 1 ℃ f. The percentage of shoot tips growth was recorded after 30 days (Mohan and Hely, 2007).

Measuring fresh and dry weight of shoot tips

The fresh weight of small plants was measured by using a sensitive balance, then the small plants was dried using an electric oven at 70 °C for 24 hrs, then measured by a sensitive balance (Hopkins and Huner, 2004).

Extraction and analysis of Glycosides compounds from *O.europaea L.*

A quantity of 0.5g fresh shoot tips 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 (Lawrence *et al*., 1986).

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 shimadzu 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 ^{o}C. (Perineau, and Goepfert, 1990). 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: (Budhiraja, 2004).

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

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% (Salkind and Ramsey, 2007).

RESULTS

Effect of different concentrations of silver nano particles (AgNo3) on shoot tips fresh and dry weight (mg).

The results displayed in table (2) and fig (1) show that the control had the highest shoots fresh weight 137 mg which is no significantly differences between all treatment (cont,0.5,1.0,1.5,2.0)mg\l AgNo3 NPs. The results also showed that the highest shoots dry weight was in control 29.0 mg and all treatments of AgNo3 NPs no significant differences between them.

Effect of different concentrations of silver nano particles AgNo3 (mg/l)on Glycosides compounds production from shoot tips

The results in table (3) showed that different concentrations of increased depending Glycosides compounds on the increasing concentrations of added AgNo3 NPs compared with intact plant.

The Oleuropein gave a highly significant values 156.99 µg/ml at the concentration 2mg/l of AgNo3 NPs while the lowest significance at mother plant which gave 0.50 µg/ml. The OH-Tyrosol gave high significant values 152.23 µg/ml at the concentration 1.0 mg\l of AgNo3 NPs while the lowest significance at mother plant which gave 0.71 µg/ml. The Ligustroside was high concentration at treatments 1.5, 2.0 mg\l AgNo3 NPs(4.59,4,59) µg/ml which they no significant different with treatment of (cont, 0.5, 1.0) while the lowest significance at mother plant which gave 0.12 µg/ml.High concentration of Oleacein in treatments 1.5, 2.0mg\l AgNo3 NPs which gave 6.32 µg/ml while the lowest concentration was at mother plant which gave 0.27µg/ml.

DISCUSSION

The results showed that there were no significantly differences in shoot tips fresh and dry weight (mg) grown on a maintenance medium in light. The effect of treatments(cont,0.5,1.0,1.5,2.0)mg\l AgNo3 NPs on producing secondary metabolites from shoot tips by HPLC technique was very high significant comparing with mother plant. The effect of AgNo3 Nps studied by many researchers, Ozudogru *et al* (2005) said AgNo3 NPs promote shoot tips growth but used the low concentration AgNo3 NPs cause delayed resulting in growth of the proliferated shoot tips Vasudevan *et al*., (2004) said treatment of the plant tissues with AgNO3 NPs can be suggested as good strategies for increasing of secondary components and improve of medicinal properties.

Table 1. Components of media for growth shoot tips

No	Components	Concentration (mg/l.)
1	MS	Full strength
2	Sucrose	30000
3	Glycine	100
4	NAA	0.2
5	Thiamin	0.5
6	BA	2
7	Agar-Agar	8000

Table 2. Effect of different concentrations of silver nano particles AgNo3 on shoot tips fresh and dry weight (mg) grown on a maintenance medium in light after 30 days

Fresh weight(mg)	Concentration of AgNo3(mg\l)	Dry weight(mg)
137	**Cont.**	**29.0**
80	**0.5**	**18.3**
97	**1.0**	**24.0**
61	**1.5**	**15.3**
66	**2.0**	**16.3**
89.6	**LSD(0.05)**	**18.79**

Table 3. Effect of different concentrations of silver nano particles AgNo3 (mg/l) on Glycosides compounds production from shoot tips

Secondary Metabolites	Concentration of AgNO3 (mg/l) NPs					mother plant	L.S.D 0.05
	Cont.	0.5	1.0	1.5	2.0		
Oleuropein	39.28	83.47	91.03	88.56	156.99	0.50	1.55
OH-Tyrosol	54.11	81.48	152.23	101.77	118.62	0.71	1.59
Ligustroside	4.09	3.55	3.07	4.59	4.59	0.12	1.83
Oleacein	5.16	3.22	4.22	6.32	6.32	0.27	0.97

Figure 1. Shoot tips growth on MS medium supplemented with left to right (cont., 1.0, 0.5, 2.0or 1.5) mg/l of AgNo3 NPs.

Figure 2. HPLC for control treatment of AgNo3 NPs

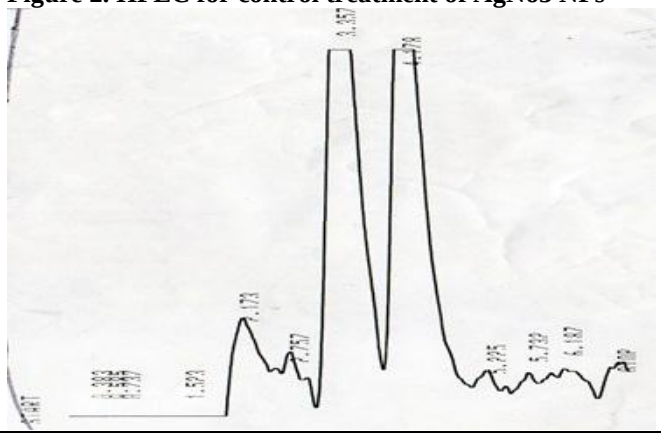


Figure 3. HPLC for 0.5mg/l treatment of AgNo3 NPs

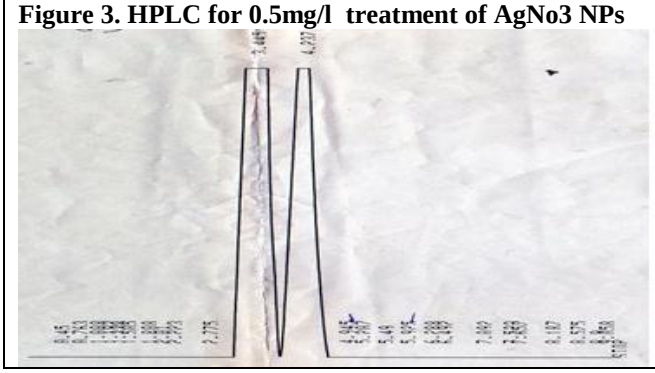


Figure 4. HPLC for 1.0mg/l treatment of AgNo3 NPs

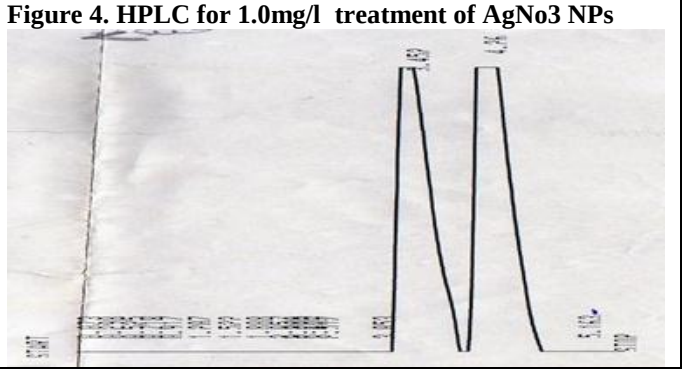


Figure 5. HPLC for 1.5mg/l treatment of AgNo3 NP

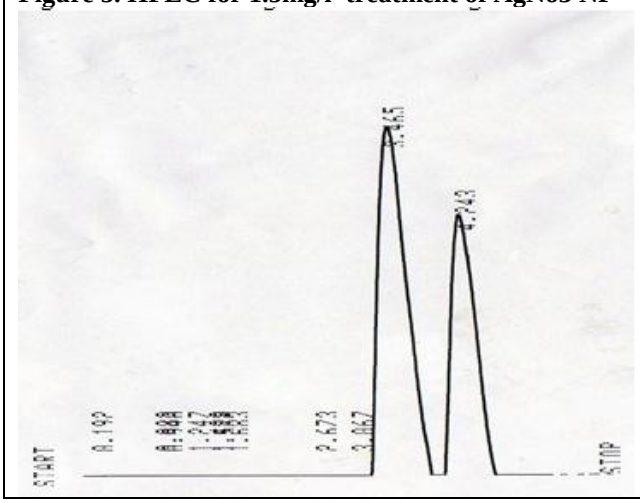
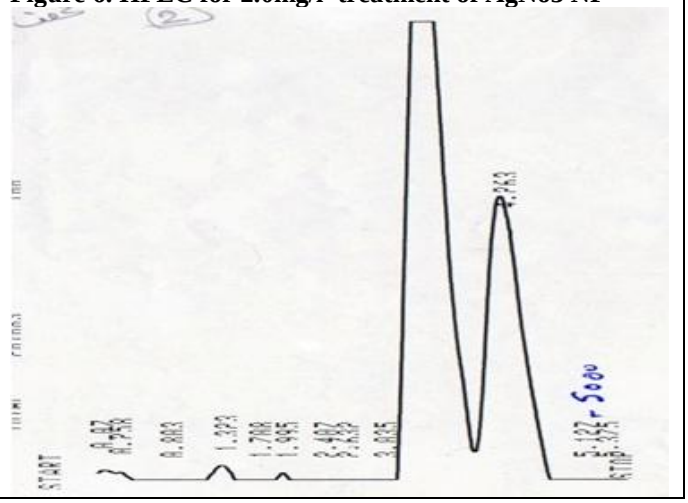


Figure 6. HPLC for 2.0mg/l treatment of AgNo3 NP



CONCLUSION

Adding 2.0 mg/l of silver nanoparticles (AgNO3) for Shoot tips medium caused highly significant increase in most the studied secondary metabolites (Glycosides compounds) of *O.europaea* L.

RECOMMENDATION

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

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