

Effect of SiO₂ NPs on increase of active compounds in leave callus of *Tagetes erecta* L. (Marigold) plant in vitro.

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Abstract

The present study was conducted in order to increase the production of some secondary metabolic compounds of Marigold plant *Tagetes erecta* L. invitro. Secondary metabolic compounds quantitative and qualitative analysis using chromatography device with high performance liquid (HPLC) and compared with the mother plant. In order to increase the production of secondary metabolites, silicon dioxide (SiO₂) nanoparticles used with concentrations (con, 50, 100,150,200 mg/l). The results showed that the most concentrations of (SiO₂) NPs led to high significant in the most secondary metabolites from leave callus of *Tagetes erecta* L.

Keywords: Increase, Compounds, Leave, Plant, Vitro

INTRODUCTION

Medicinal plants have been of great importance to the health care needs of individuals and their communities. The use of herbal preparations made from medicinal plants is widespread in developing countries. The healing powers of traditional herbal medicines have been realized since antiquities. About 65% of the world populations have access to local medicinal plant knowledge system.

Tagetes is a genus of annual or perennial, mostly herbaceous plants in the sunflower family (Asteraceae).It was described as a genus by Linnaeus in 1753.

Non-pharmacological data studies showed that *T. erecta* plant exhibited insecticidal (Sarin, 2004), larvicidal (Murchs et al., 2000), mosquitocidal (Nikkon et al., 2011) and nematocidal activity (Patrick et al., 2011). Flower extract was found to contain biologically useful lutein compounds and studied for use as nutritional supplement and as poultry food colorant (Leigh, 1999).

The petals yield a natural dye, the colorants consisting mainly carotenoid-lutein and flavonoid-patuletin, with crude extracts used for dyeing textiles. The study describes an innovative dyeing process with net enhancement of dye uptake due to metal mordanting. Results suggest a potential for industrial application (Padma et al., 2009).

Plant tissue cultures are the core of plant biology, which is important for conservation, mass propagation, genetic manipulation, bioactive compound production and plant improvement. In recent years, the applications of nanoparticles (NPS) has successfully led to the eliminations of microbial contaminants from explant and demonstrated the positive role of NPS in callus induction, organogenesis, somatic embryogenesis, somaclonal variation, genetic transformation and secondary metabolite production.

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Plant cell cultures are a preference source to whole plant for the production of high-value secondary metabolites which are usually acting lower role than primary metabolites in the plant (Karuppusamy, 2009).

Tissue culture techniques are utilized to enhance yield of secondary metabolites by trigger stress response like using elicitors, precursors and biotransformation, variation in environment conditions, change in medium constituents (Radman *et al.*, 2003).

Plant tissue cultures are exposed to stresses and stress consociation that they may not have encountered in nature in their long evolution. It is exceptional reflection on the plasticity of the plant genome that it could be converted and respond to novel in vitro stresses (American journal of plant physiology, 2011).

Nano science is one of the most superior research and development in modern science, nanotechnology is now frequently used throughout the pharmaceutical industry, medicine, and tissue engineering, the use of nanoparticle (NP) materials offers many advantages due to their unique size and physical properties (Faraji *et al.*, 2010).

AIMS OF THE PRESENT WORK

Latest study have showed interest in using chemical elicitors in plant tissue cultures techniques for increasing secondary metabolites. The aim of this study to use the nanomaterials such as (SiO₂) nanoparticle on leave explant of *Tagetes erecta* L. (Marigold) plant to increase the secondary metabolites. Results of secondary metabolites produced from callus treatment with elicitor are compared with the secondary metabolites got from the mother plant which was not subjected to elicitation.

MATERIALS AND METHODS

Plant material and sterilization

The explant of *Tagetes erecta* L. from leaves were collected from local market in Baghdad Iraq on 4/10/2021. Explants (leaves) rinsed with running tap water for 1/2 hr. Transferred to laminar air flow cabinet and submerged in (99%) ethanol for one minute, washed with sterilized DDH₂O for 5 minutes then rinsed with Sodium hypochlorite at the concentration (1%) 10 minutes, then washed with sterilized DDH₂O 3 times for (5) minutes and planted in universal tubes. (Pierik, 1987)

Callus induction

The sterilized explants (leaves) were dissected and cultured on culture Vessels containing Murashige and skoog, (1962) (MS) medium with different concentrations of the auxin 2,4-dichloro fenoxiacetic acid (2,4-D) (0,1, 2, 3,4) mg/l, table(1) then distributed into ten replicates for each concentration which incubated at dark period at a temperature 25 +1 Co. The percentage of callus formation

was recorded after (30) days (Ramawat, 2008).

Fresh and dry weight of callus measurement

The fresh weight of callus induced from leave explants 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 sensitive balance (Hopkins and Huner, 2004)

Extraction and analysis of secondary metabolites from leaves and callus of *Tagetes erecta* L.

The marigold leaves residues (20g) were first defatted by Soxhlet method with n-hexane at 80°C for 2h. The defatted marigold leaves residue from plant and 1gm callus were extracted with ethanol aqueous solution (70%, v/v) ethanol for 1.5 h. At the end of extraction, the crude extract was filtered through SPE Solid phase extraction, and the liquid was concentrated with stream of liquid nitrogen (bubbling). The concentrated extract (7g) was recovered and dispersed in distilled water (extract/ water ratio 1:10) and stirred for 30 min.

The suspension was then centrifuged (3000xg, 20 min ambient temperature) separated and rewashed distilled water. The triple washed extract was freeze dried to yield approximately 0.89 yellow powder and stored at 4°C. The purified marigold residues extract (PMRE) was dissolved with 70% ethanol aqueous solution before used. The PMRE (0.8 mg/l in 70% ethanol aqueous solution) filtered by Millipore filter (0.45µm) was injected into HPLC Shimadzu.

HPLC analysis

High – performance liquid chromatography (HPLC) Shimadzu-10. The main compound was separated on a FLC (first Liquid Chromatography) Column under the optimum condition. Column; phenomen C 18. 3µm particle size (50 X 4.6 mm I.D) column. Mobile phase; linear gradient of methanol (solvent A) and methanol/water/ formic acid (20:80: 0.25, V/V/V; Solvent B) from 0% B for 15 minutes as mobile phase flow rate 1.2ml/min detection: UV 280 nm. The separation occurred on liquid chromatography Shimadzu 10AV-LC equipped with binary delivery pump model LC-10 A shimadzu, the eluted peaks were monitored by UV-Vis 10 ASPD Spectrophotometer.

Estimate the increase or decrease in the secondary metabolites compounds by device (HPLC)

High-performance liquid chromatography (HPLC) was used, the samples were performed with the HPLC system equipped with two shimadzu reciprocating pumps a variable UV-VIS detector shimadzu data processors, to estimate the increase or the decrease in the secondary metabolites compounds of *Tagetes erecta* L. and compare these increases or decreases with the mother plant (Okoh, *et al.*, 2007) The readings were measured at the wavelengths and by the time of the retention of the Rt solutions to the standard samples under study. The

concentrations of active substances was quantified by comparing the area of package material standard package with an area of the model under the same conditions by using the following law:

Concentration Area of sample

Concentration of sample (µg/ml) = -----
-----X conc. of standard X dilution factor

Area of standard

Experimental design and statistical analysis

A completely randomized design (CRD) was used. Least significant differences (LSD) were calculated. The difference between the test averages compared according to least significant differences (LSD) at the probability of 5% (Salkind and Ramsey, 2007).

RESULTS

The effect of different concentrations of (2, 4-D) on fresh and dry weight of leave callus on MS medium after four weeks of culture.

The results of fresh weight of callus in (Table2) showed that the concentration (1 mg/l) of (2,4-D) had high significant differences than other treatments which reached to (365.00 mg) and there is no significant differences with treatments (3,2mg/l) of (2,4-D) which reached to (172.00,131.00 mg) respectively. Also there is no significant differences between treatments (4 mg/l) and cont. which reached to (77.00, 33.00 mg) respectively. In the same table the results of dry weight of callus showed that the concentration of (1mg/l) of (2, 4-D) gave high significant differences than other treatments which reached to (25.00 mg).

Effect of different concentrations of SiO₂ nanoparticles on fresh and dry weight of leave callus on MS medium after four weeks of culture.

The results in (Table3) showed that adding of SiO₂ NPS. gave high significant differences of fresh weight of callus on treatments (50,cont, 100 mg/l) which gave (630.00, 525.00,517.00 mg) respectively.

The lowest fresh weight of callus found in treatment (200mg/l) of SiO₂ NPS which reached to (378.00mg).

In the same table the effect of adding of SiO₂NPS on dry weight of callus showed high significant differences in treatments (50, cont., 100 mg/l) of SiO₂ NPS which reached (50.70, 46.70, 38.70 mg) and there is no significant differences in these treatments, the lowest dry weight of callus in treatment (150 mg/l) of SiO₂NPS which reached to (27.30 mg).

The effect of different concentrations of SiO₂ nanoparticles (mg/l) on secondary metabolites of leave callus of *Tagetes erecta* .

The results in (Table 4) showed adding of SiO₂ NPS effect on secondary metabolites of leave callus, the treatment of (200 mg/l) of SiO₂ NPS gave high significant differences of gallic acid than other treatments which reached to (107.30 µ g/ml) but the lowest concentration of gallic acid found in the treatment of mother plant which reached to (33.40 µ g/ml).

In the same table the treatments(200, 150 mg/l) of SiO₂ NPS gave high significant differences than the other treatments in concentration of syringic acid which reached to (169.40, 154.20 µ g/ml) respectively and the lowest concentration of syringic acid found in treatment of cont. which reached to (58.60 µ g/ml).The treatment of (200,150 mg/l) of SiO₂ NPS gave high significant differences than other treatments which reached to (95.2, 86.80 µg/ml) of Ellagic acid.The lowest concentration of Ellagic acid found in cont. treatment which reached to (36.80 µ g/ml).

The same table showed the high concentration of Quercetin in (150,200 mg/l) treatments of SiO₂ NPS which recorded (62.00, 54.90 µ g/ml) respectively with high significant differences than other treatments.

The concentration of Quercetagenin reached to (232.80, 201.60 µ g/ml) in treatments (200, 150 mg/l) of SiO₂ NPS with high significant differences than other treatments.

The adding of SiO₂ NPS caused increasing of lutein concentration in treatments (200,150 mg/l) of SiO₂ NPS which reached to (151.00, 137.80 µ g/ml) respectively which showed high significant differences than other treatment except the treatments of (cont., 100 mg/l) which reached to (112.00, 111.60 µ g/ml) respectively.

The adding of SiO₂ NPS caused high significant differences in treatments of (200, 150 mg/l) of SiO₂ NPS which reached to (126.90, 123.50 µ g/ml) of kaempferol respectively than other treatments except the treatment of (100 mg/l) SiO₂ NPS which reached to (95.40 µ g/ml).

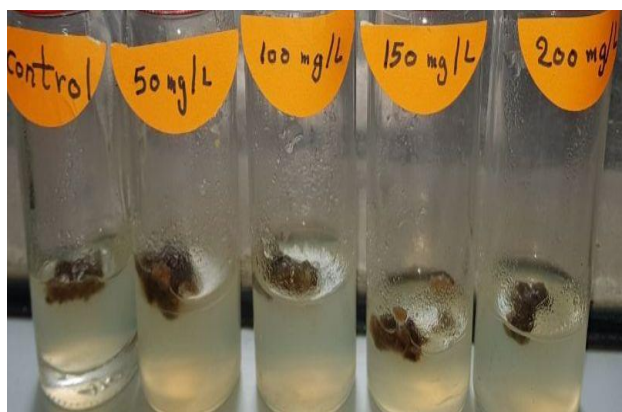


Fig 1. The effect of different concentrations of SiO₂NPS on callus fresh weight (mg/l) from left (cont. 50, 100, 150, 200).

Figures below showed the effect of different concentrations of (SiO₂) NPS (mg/l) on producing secondary metabolites from callus by HPLC technique.

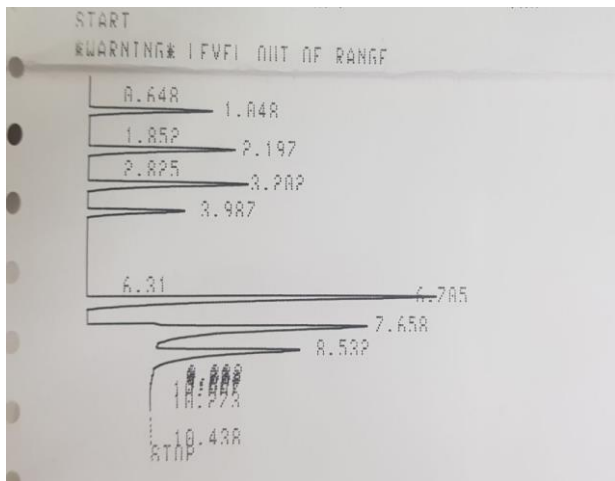


Fig.2. HPLC for control treatment of (SiO₂) NPS

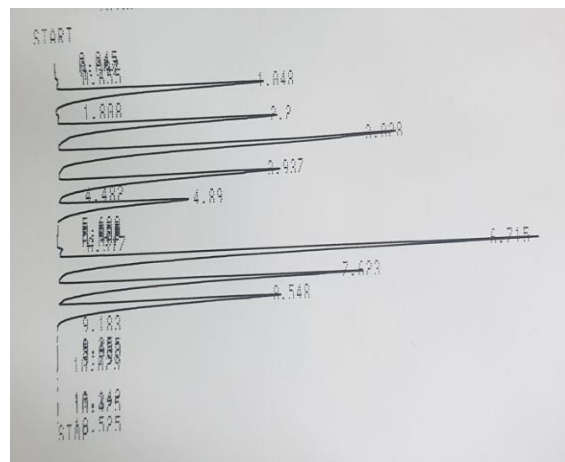


Fig.5.HPLCfor 150 mg/l of treatment of (SiO₂) NPS

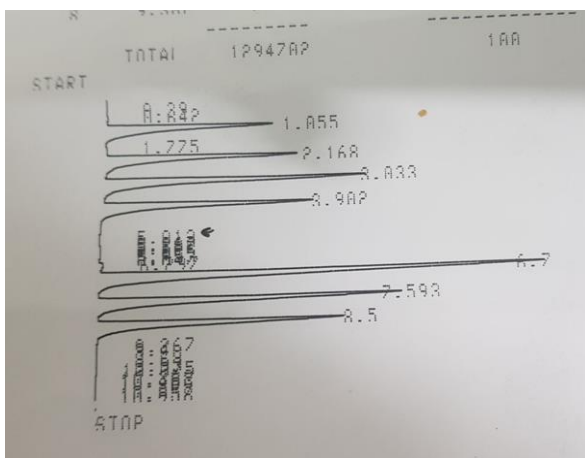


Fig.3 HPLC for 50 mg/l treatment of (SiO₂) NPS

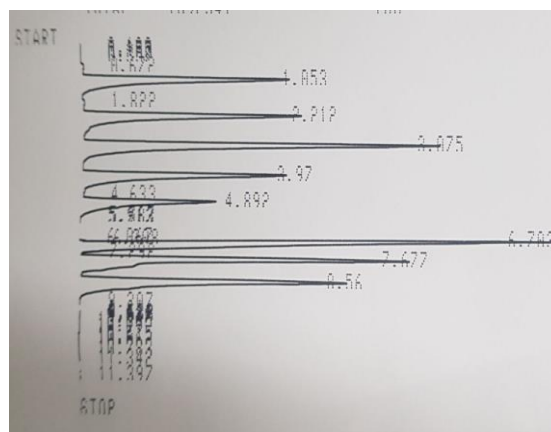


Fig.6. HPLC for 200mg/l treatment of (SiO₂) NPS

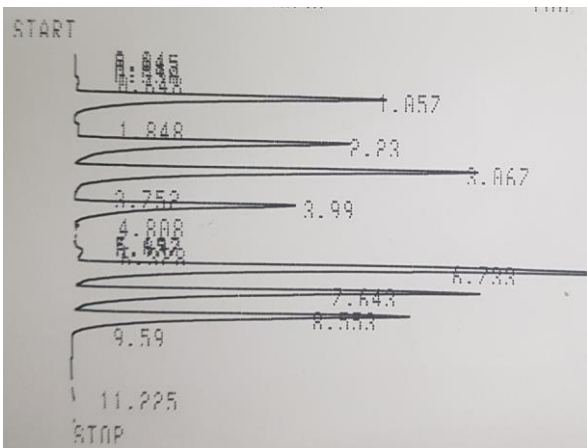


Fig.4. HPLC for 100 mg/l treatment of (SiO₂) NPS

DISCUSSIONS

2, 4-Dichloro phenoxy acetic acid (2, 4-D) is a synthetic auxin, which is a class of plant growth regulators. Mechanism of action of (2, 4-D) mimics the action of auxin. It is commonly used in plant tissue culture as a plant growth regulator, Auxins induce cell division, cell elongation and formation of callus in culture .It is one of the most commonly added auxin in plant cell cultures.The optimum concentration of (2,4-D) were to be effective when they were supplemented at low levels. High concentration of (2, 4-D) levels may cause inhibition in cell division within the explant. (Radhakrishnan et al., 2001) said that cells used up (2, 4-D) amounts as required and any excess began to actively exhibit the herbicidal effects therefore decelerating down the callus induction process. (Nguyen et al., 1997) reported that cultivar differences played an important role in determining the amount of callus that formed. The development of callus from younger leaves explants is directly linked with (2, 4-D) which is a suitable growth hormone important for callus induction in most plant species in plant tissue culture work. This is similar to the findings of (Mamun et al., 2004 and Baskaran et al., 2006).

Nanomaterial enhancement of secondary metabolites plant are rich source of various bioactive secondary metabolites, which play significant role in the survival of plants in their respective environment.

In vitro plant cell and organ culture has been proven to be advantageous for the production secondary metabolites .The content of secondary compounds in cell and organ cultures was significantly enhanced by optimizing the composition of the culture medium, incorporation of precursors and elicitors and providing appropriate culture conditions.NPS added to the plants in vitro culture medium may act as nutrient source and elicitor.

The results indicated that the addition of SiO₂ NPs could enhance the proportion of live cells by weakening oxidative stress. Treatment with SiO₂ NPs could maintain the integrity of the cell, increase the thickness of the cell wall and the pectin. In addition, the pectin content, cation exchange capacity (CEC) and pectin methylesterase (PME) activity were also increased in the SiO₂ NPs-pretreated cells, leading to a decreased degree of pectin methylesterification and an improved mechanical force of the cell walls.(Doo *etal*.2017).

This results agree with (Al-Oubaidi and Al-Khafagi , 2018) when they used NPs to increasing tanins and phenols of *Punica granatum* L. in callus.

CONCLUSION

Adding SiO₂ nanoparticle for callus medium caused highly significant increase in all

the studies secondary metabolites (Gallic acid, Syringic acid, Ellagic acid, Quercetin , Quercetagetin, Luteinand Kaempferol) of *Tagetes erecta* L. in all the concentrations of the previous NPS materials

Recommendation

- 1- Use another nanoparticles or elicitors on the same plant to examine its effect to increasing medicinal compounds.
- 2- Used the same nanoparticles on another medicinal plant.
- 3- Further studies on detection of mutations (somaclonal variation) by using DNA markers

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Table 1: The medium of callus induction components

<i>NO.</i>	<i>components</i>	<i>Concentration (mg/l)</i>
1	MS	4400
2	Sucrose	30000
3	L_Aspargine	150
4	Glycine	100
5	2,4_D	0,1,2,3,4
6	Agar_Agar	8000
7	BA	0.5

Table 2: Effect of different concentrations of (2, 4-D) on fresh and dry weight of leave callus on MS medium after four weeks of culture .

<i>Characters</i>	<i>concentrations of 2,4-D (mg/l)</i>					<i>L.S.D</i>
	<i>Cont.</i>	<i>1</i>	<i>2</i>	<i>3</i>	<i>4</i>	
Fresh Weight of callus	33.00b	365.00a	131.00ab	172.00ab	77.00b	252.7
Dry Weight of callus	5.30b	25,00a	13.70b	13.30 b	8.00b	11.09

Table 3: Effect of different concentrations of SiO₂ nanoparticles on fresh and dry weight of leave callus on MS medium after four weeks of culture.

characters	concentrations of SiO ₂ (mg/l)					L. S. D
	Cont.	50	100	150	200	
Fresh Weight Of callus	525.00a	630.00a	517.00a	387.00b	378.00b	171.50
Dry Weight Of callus	46.70a	50.70a	38.70ab	27.30b	30.00b	13.38

Table 4: Effect of different concentrations of SiO₂nanoparticles (mg/l) on secondary metabolites of leave callus of *Tagetes erecta*.

Secondary Metabolites	concentrations of SiO ₂ (mg/l)					Mother Plant	L.S.D
	Cont.	50	100	150	200		
Gallic acid	48.80d	72.40c	79.30c	93.00b	107.30a	33.40e	12.77
Syringic acid	58.60d	96.30c	123.40b	154.20a	169.40a	82.70c	18.83
Ellagic acid	36.80c	56.40b	57.60b	86.80a	95.2a	43.20c	11.57
Quercetin	6.10c	7.80c	7.80c	62.00a	54.90a	28.20b	12.11
Quercetagetin	125.90c	146.20bc	175.80b	201.60a	232.80a	113.50c	36.52
Iutein	112.00ab	88.60b	111.60ab	137.80a	151.00a	74.30b	46.93
Kaempferol	75.20bc	74.80bc	95.40ab	123.50a	126.90a	42.00c	38.02

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