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The effect of TiO_2 and SiO_2 nanoparticles and salinity stress on expression of genes involved in parthenolide biosynthesis in Feverfew (*Tanacetum parthenium* L.)

Mahshid Khajavi[1], Mahdi Rahaie[2,*], Asa Ebrahimi[1]

[1] *Department of Biotechnology and Plant Breeding, Faculty of Agriculture, Science and Research Branch of Islamic Azad university, Tehran, Iran*
[2] *Department of Life Science Engineering, Faculty of New Sciences and Technologies, University of Tehran, Tehran, Iran*
* Corresponding author: Tel: +98-21-86093408; Fax: +98-21-88497324; Email: mrahaie@ut.ac.ir

Abstract. Medicinal plants can produce various chemical compounds as secondary metabolites that have benefit to human. Feverfew (*Tanacetum parthenium* L.) is a medicinal plant belongs to the Asteraceae family. This plant due to have parthenolide compounds has attracted much attention for medicinal value and pharmacological activity. Due to the economic importance of the plant metabolite in cancer and migraine treatment, application of approaches for increasing the metabolite was the objective of this study. For this purpose, after cultivation in greenhouse, plants were treated with TiO_2 and SiO_2 nanoparticles and salinity stress at different times and concentrations. Real Time PCR used to evaluate the expression of *TpGAS,* COST and *TpCarS* genes which involved in secondary metabolites biosynthesis pathway (parthenolide and β-caryophyllen). It was found, SiO_2 NPs can increase the expression of *TpCarS*, COST and *TpGAS* in the concentration of 25mM with increasing time from 6 to 24h. In this concentration (25mM), TiO_2 treatment, up-regulated the *COST* and *TpGAS* in contrast, down-regulated the *TpCarS* with increasing time from 6 to 24h. Salinity treatment affected the expression of all three genes, so that with increasing time, the expression of all three genes was elevated. In conclusion, according to above and HPLC results, it was shown the nanoparticles and salinity treatments can increase parthenolide synthesis in whole plant of Feverfew and then they can be used as elicitor for more production of the metabolite.

Keywords. *Tanacetum parthenium* L., Nanoparticle, SiO_2, TiO_2, Salinity stress, Gene expression analysis.

Abbreviations: *GAS*, Germacrene A Synthase; *COST*, Costunolide Synthase; *CarS*, Cariophyllene Synthase; PTL, Parthenolide; NP, Nanoparticle.

INTRODUCTION

Medicinal plants have a specific role in treatment and prevention of many human diseases. These plants are attracting more attention for produc-

ing safer medicine because it is believed that they rarely have side effects compared to chemical drugs. Feverfew (*Tanacetum parthenium* L.) (Asteraceae), is a diploid (2n=2x=18) and perennial medicinal plant. This plant has medical applications on a wide range of disease such as migraine headaches, stomach aches, toothaches, insect bites, rheumatoid arthritis and infertility (Pareek et al. 2011). These medicinal properties are due to the existence of chemical compounds which called secondary metabolites. Terpenoids are the largest class of plant secondary metabolites (Croteau et al. 2000). Sesquiterpene lactones are the main group of terpenoids and frequently are derived from Mevalonic acid (MVA) pathway (Van Klink et al. 2003). *T. parthenium* (L.), contains many sesquiterpene lactones and parthenolide has the most concentration (comprises up to 85%) among the total sesquiterpenes (Pareek et al. 2011).

Parthenolide is used for the treatment of migraine and shows specifically anticancer and anti-inflammatory activity, as well (Tassorelli et al. 2005; Walsh et al. 2011; Mathema et al. 2012; Al-Fatlawi et al. 2015; Wang and Li, 2015). β-caryophyllene is another sesquiterpene lactone distributed in the essential oil of various plants. This compound has represented several biological activities, such as anti-inflammatory, antibiotic, antioxidant (Legault and Pichette, 2007), anticancer (Tundis et al. 2009) and antiproliferative activity (Amiel et al. 2012).

Sesquiterpenes, like parthenolide and β-caryophyllene are synthesized via farnesyl diphosphate (FPP) in Mevalonate pathway. TpGAS and COST are two genes involved in parthenolide production. At the first step, *TpGAS* converts farnesyl diphosphate to germacrene A, COST converts germacrene A acid to costunolide, and parthenolide is one of the deratives of costunolide. It has been revealed that costunolide synthase is a cytochrom P450 enzyme. *TpCars* is another sesquiterpene synthase in Feverfew which is responsible for the production of β-caryophyllene. This enzyme converts farnesyl diphosphate to β-caryophyllene directly. (Majdi et al. 2011; Liu et al. 2011; Menin et al. 2012; Basha et al. 2016).

Several factors affect the production of secondary metabolites, and elicitors are one of the most efficient ones (Zhao et al. 2005). Plants and plant cells (*in vitro* culture) show the morphological, biochemical and physiological reactions to biological, chemical or physical factors, which is considered as "elicitors." In fact, Elicitation is an induced or enhanced synthesis process of secondary metabolites by the plants and it is a way to ensure their survival, persistence, and competitiveness (Karuppusamy, 2009; Kiong et al. 2005). Elicitors are biological or nonbiological agents that cause biosynthesis and accumulation of secondary metabolites in plants through induction of defense responses (Ramirez-Estrada et al. 2016). The components of microbial cells and poly and oligosaccharides, chemicals such pesticides, heavy metals, and the signaling compounds in plant defense responses (growth factors, e.g. jasmonate) and physical factors such as hyperosmotic stress, UV, cold shock, ultrasound, and pulsed electric field can act as stimulators for hyperproduction of secondary metabolites (Zhao et al. 2010; Gueven and Knorr, 2011; Lin and Wu, 2002).

Nanoparticles (NPs) are new materials which show unique properties related to their physical size. The nanoparticles used in present work are SiO_2 and TiO_2 which are among the most used nanomaterials (Servin et al. 2012; Siddiqui and Al-Whaibi 2014). Titania (TiO_2) has a wide range of applications such as cosmetics (Anselmann, 2001), cancer treatment (Kalbacova et al. 2008), sunscreens or food (Lan et al. 2013). Silica (SiO_2) is another popular metal oxide NPs used in multiple varieties of applications such as disease labeling, drug delivery, photodynamic therapy (Ohulchanskyy et al. 2007), cancer therapy (Cheng et al. 2010; Rosenholm et al. 2010), fertilizer and pest control (Sakr, 2017; Tripathi et al. 2014; Laing et al. 2006).

The effects of TiO_2 NPs on different biological characters of the plant have been done in several studies. Among them, it could be pointed out to the effect of TiO_2 on the growth and microRNA expression profile of tobacco (Frazier et al. 2014), effects of nano-TiO_2 on seed germination (Castiglione et al. 2011), development and mitosis of root tip cells of *Vicia narbonensis* L. and *Zea mays* L. (Castiglione et al. 2011), The effect of SiO_2 and TiO_2 nanoparticles on the expression of GPPS gene (involved in thymoquinone biosynthetic pathway) in *Nigella sativa* L. (Kahila et al. 2017) and finally, Mandeh et al. (2012) which studied *in vitro* influences of TiO_2 nanoparticles on barley tissue culture and quantitative and qualitative characteristics of calli were analyzed after each subculture. In the field of agriculture, SiO_2 has an inhibition effect on the pest (HongShu et al. 2009), Carriers in drug delivery and absorption and transport of nutrients in plants (Liu et al. 2006). The role of nano-SiO_2 in the characteristics of seed germination of tomato has also investigated (Siddiqui and Al-Whaibi, 2014).

Salinity stress is a high concentration of soluble salts in soil and water. Most common soil salinity is caused by high sodium (Na^+) and chloride (Cl^-) (Tavakkoli et al. 2010). Salt stress as an environmental factor is another factor that influences on gene expression (Siddiqui and Al-Whaibi 2014).

The effect of salinity on secondary metabolites in plants is various. There are different examples in this case. For example, during NaCl stress in *Swertia chirata,* significant increases ($p \leq 0.05$) was occurred in secondary metabolites at 50mM and initial increase in 100mM NaCl, which falls back to normal levels at the seventh day (Abrol et al. 2012).

In the present work, to evaluate the elicitation role of nanoparticles and abiotic stress on hyperproduction of parthenolide, it was investigated the effect of two NPs (TiO_2 and SiO_2) and salinity stress on the expression of three related genes, *TpGAS*, *COST* and *TpCarS* that are involved in the biosynthetic pathway of PTL and β-caryophyllene in Feverfew.

MATERIALS AND METHODS

Plant material and growth conditions

The seeds of Feverfew were provided by the medicinal plant institute of Shahid Beheshti University, Tehran, Iran. Germinated seeds in pots were grown under controlled circumstances with a 16:8h photoperiod light/dark, 25/18 °C for day/night and supplied with a photosynthetic photon flux density of 3000 lux (Fig. 1).

Fig. 1. The grown plants in pots in green house under controlled condition.

Nanoparticles characterization

The SiO_2 nanoparticles were purchased from TECNAN Inc. (Tecnología Navarra de Nanoproductos S.L., Spain). A size of 10–15 nm for NPs was estimated (Fig. 2). The XRD measurement clearly showed that the SiO_2 NPs were amorphous. The elemental analysis of the nano-powder by ICP-MS technique (Thermo Elemental VG PQ-ExCell) showed a purity of 99.999%.

The TiO_2 nanoparticles were provided from Degussa Inc., Germany. The analyzed data from XRD showed ~25nm diameter and specific area equal to 55 $m^2g^{-1}$for the nanoparticles (Ave. of 24.5 nm in diameter, a mixture of anatase and rutile with more proportion of anatase (89.2 %),).

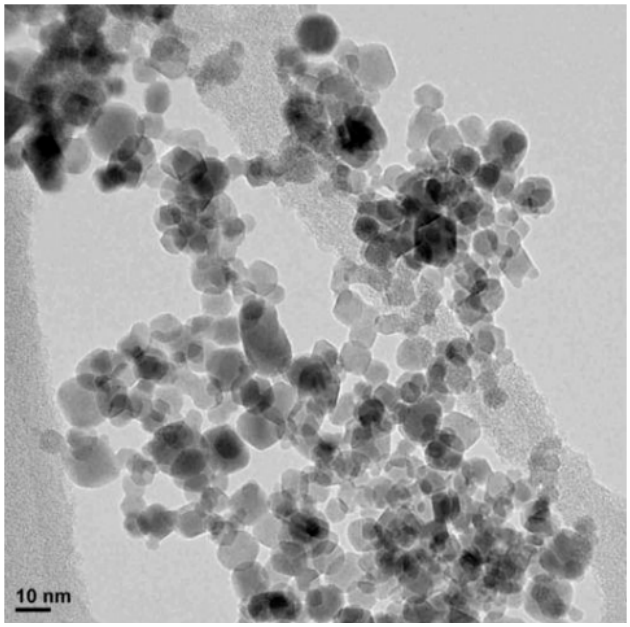

Fig. 2. The TEM micrograph of SiO_2 Nanoparticles.

Plant treatment with NPs and Salt

The SiO_2 and TiO_2 NPs, were separately prepared in two concentrations (25 and 50 mg/l) and the solution was used for watering of each pot. For each pot, 80 ml of 0.3M NaCl solution was applied for doing salinity stress treatment. All tissues were collected at the certain times (6, 24 and 48h after irrigation) for gene expression analysis and phytochemical studies and were instantly flash frozen in liquid nitrogen then stored at -80°C until RNA extraction.

HPLC analysis

The chromatography assay was performed on a 25 cm×4.6 mm with pre-column, Eurospher 100-5 C18 ana-

lytical column provided by KNAUER (Berline, Germany) reversed phase matrix (5µm) (Waters) and elution was carried out in a gradient system with acetonitrile as the organic phase (solvent A) and distilled water (solvent B) with the flow-rate of 1 mL min-1. The Peaks were monitored at 220 nm wavelength. Injection volume was 20 µL and the temperature was maintained at 25°C. All injections were repeated three times (n=3). Calibration graphs were plotted subsequently for linear regression analysis of the peak area with concentration 1, 10, 25, 50, 80, 120, 150 and 200 mg L^{-1}.

RNA extraction

Total RNA was isolated from the leaf tissue by using the Isolation total RNA Kit (Denazist Asia Inc., Mashhad, Iran), according to the manufacturer's instructions. The quantity and quality of extracted RNA were determined respectively by using a spectrophotometer instrument (NanoDrop2000c, Thermo scientific, USA) and Agarose gel electrophoresis. RNA samples were stored at −80 °C until further analysis.

Gene selection and primer designing

In the present work, *TpGAS* and Cost genes that involved in biosynthesis of PTL and *TpCarS* as mediator in biosynthesis of β-caryophyllen at Mevalonate pathway in Feverfew (Majdi et al. 2011), plus β-Actin as a house keeping gene were selected and their sequences retrieved from the gene bank, NCBI (http://www.ncbi.nih.gov). Four pairs of primers including β-Actin (-5'- AGCATGGTATTGTGAGCAACT-3', R-5'- TGGGTCATCTTCTCTCTGTTAGC-3'), *TpGAS* (F-5'-TACCAGTTTGAGCGTGAAAGA- 3', R- 5'-CAATCATGATCTTGAGCTCGT- 3'),*TpCarS* (F-5'-GAGCATGTCCACAAAGTATTTCAC-3', R-5'- GCATCGGAATATCTTTACACACAG-3') and Cost (F- 5'- GAGACACAAGAAGAAGTGAGATCAG-3', R- 5'- AAAGGTGTAGGAGCATGTAACCTC-3') were designed using primer 3 free software (http://biotools.umassmed.edu/bioapps/primer3_www.cgi).

Primers confirmation was performed by three criteria, including BLAST search in Gene Bank, single peak in qPCR and single band on gel electrophoresis.

cDNA synthesis

cDNA synthesis was performed by Revert Aid First strand cDNA synthesis Kit (Thermo Scientific, USA) with 200 U of M-MLV RT enzyme, oligo-dT and random Hexamer primers according to manufacturer's instruction.

Quantitative PCR

Quantification of *COST*, *TpGAS* and *TpCarS* genes expression levels in the samples were measured by qPCR using Hot Firepol EvaGreen qPCR master mix (solis BioDyne, Estonia). qPCR was performed according to manufacturer's instruction in Qiagen Real-time PCR System (Rotor-Gene Q, Germany) using above primers. It should be mentioned that before qPCR, the specificity of all primers and optimization of PCR reactions was done with conventional PCR. The relative expression levels were calculated according to Pfaffl method (Pfaffl 2001).

Data Statistics

Expression levels were calculated from the Ct values obtained from triplicate biological samples. Statistical significance analysis of relative gene expression level compared with the reference gene (β-*Actin*) was performed with completely randomized design (CRD). Mean values of relative expression levels were compared with LSD test (P=0.05) using SPSS ver. 21.0 software.

RESULTS

In this study, the effects of TiO2 and SiO2 nanoparticles and salinity stress on expression of *TpGAS*, and *COST* genes that are involved in the biosynthetic pathway of PTL in Feverfew (Fig. 3) were investigated. Also change of *TpCarS* gene expression which produces β-caryophyllen was analyzed. The present study focuses on elicitation effects of nanoparticles and salinity on parthenolide and β-caryophyllen productions in Feverfew.

Gene Expression Analysis

COST Gene

Two concentrations (25 and 50mM) of SiO_2 NP, in two periods of time (6 and 24h) were used. The results showed that, in both 25 and 50mM concentrations, the expression of *COST* gene was enhanced with increasing of time. While in both times, the gene expression in 25mM, was more than 50mM treatment; furthermore, the

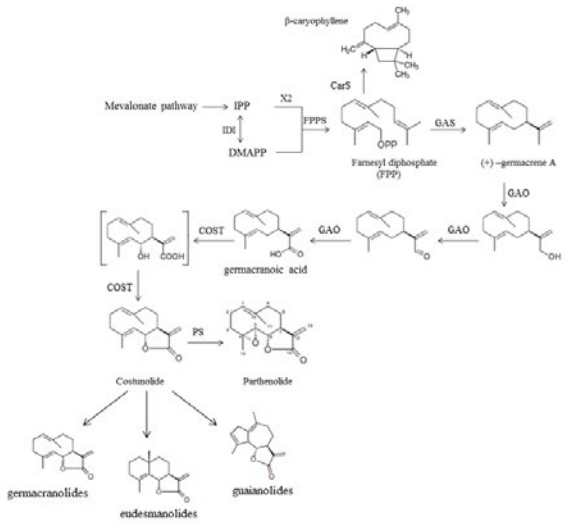

Fig. 3. The suggested biosynthetic pathway for parthenolide and β-caryophyllen synthesis in feverfew (Majdi et al., 2011). CarS: caryophyllen sybthase; GAS: germacrene A synthase; GAO: germacrene A oxidase; COST: costunolide synthase; PS: Parthenolide synthase.

expression of the gene in 25mM and after 24h was very high and more than 7-folds in comparison to 6h (Fig. 4a).

The result of TiO_2 NP was similar to SiO_2 treatment at 25mM concentration and increased the expression of COST gene. In 25mM Concentration of TiO_2 NP with increasing time from 6h to 24h, elevation of the gene expression by more than 6-fold (Fig.4b) was observed. In opposite, the gene expression in 50mM of TiO_2 NP was decreased by rising time until close to zero.

As Fig. 4 (a and b) shows, Although the ratio of expression level for SiO2 and TiO_2 nanoparticles in 6h/24h is nearly the same, but the absolute expression of the *COST* gene in TiO_2 treated samples (133.8 fold) is significantly more than SiO_2 treated plants (20.2 fold).

The effect of salinity treatment on expression of *COST* gene were also measured; after 6h and 48h at

0.3 M concentration A gradual increasing trend (2-folds) in gene expression was observed (Fig. 4c).

TpGAS Gene

TpGAS is a gene involved in the parthenolide biosynthesis pathway. The expression analysis of the gene transcript pointed that, it is affected by SiO_2 and TiO_2 NPs and salt stress treatment. The results represented that SiO_2 NPs in 25mM concentration at 24h, significantly increased the expression of GAS compared to 6h treatment (24.5 fold). In opposite, the TiO_2 NPs treatment didn't have a significant effect on the expression of

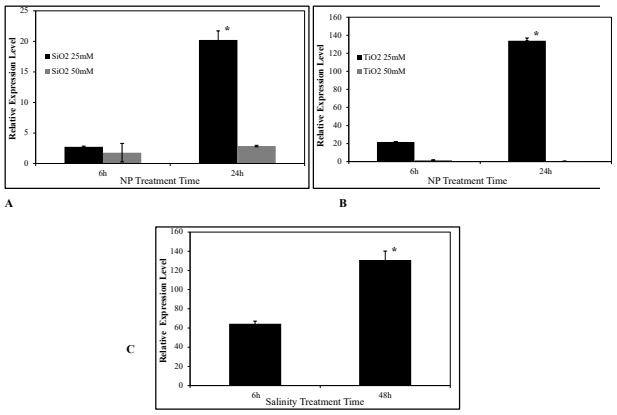

Fig. 4. The effect of elicitors on expression of *COST* gene in feverfew: A) SiO_2 NP treatment; B) TiO_2 NP treatment; C) salinity stress.

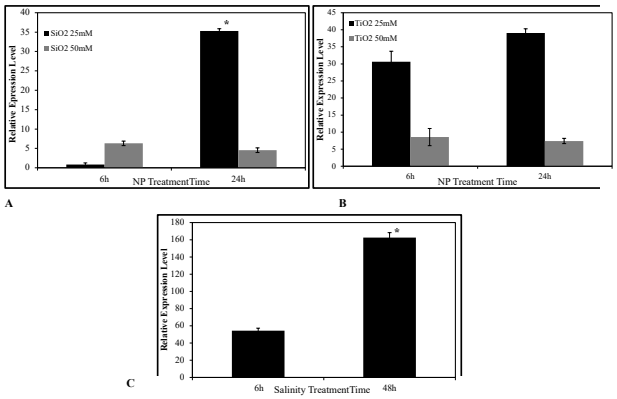

Fig. 5. The effect of elicitors on expression of *TpGAS* gene in feverfew. A: SiO_2 NP treatment; B: TiO_2 NP treatment; C: Salinity stress.

the gene in both time and concentrations (Fig. 5a and b).

The effect of salinity stress was investigated with the same concentration used above. As shown in the Fig.5c, the Expression of *TpGAS*, was statistically enhanced in 48h in comparison to 6h treatment, (~3 fold).

TpCarS Gene

TpCarS is another gene which was analyzed in our experiment. The results of expression analysis showed that the gene was affected by SiO_2 and TiO_2 NPs and salinity treatment, as well. As it can be seen in the Fig. 6a and b, an increase (1.4 fold) and decrease (6 fold) in expression of *TpCarS* in 25mM of SiO_2 and TiO_2 NPs treatment were observed respectively, from 6h to 24h. In contrast, in the 50mM concentration of NPs, no significant changes were detected during the time period.

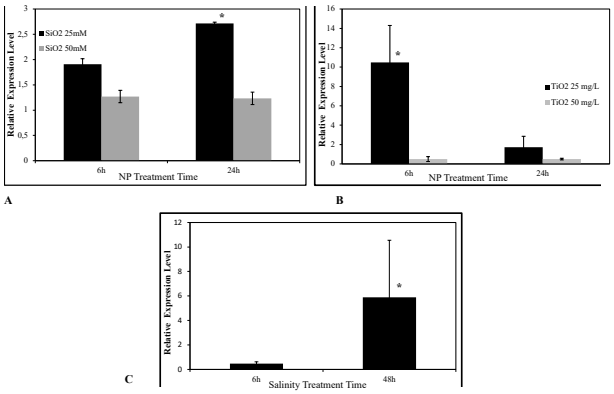

Fig. 6. The effect of elicitors on expression of *TpCarS* gene in feverfew. A: SiO_2 NP treatment; B: TiO_2 NP treatment; C: Salinity stress.

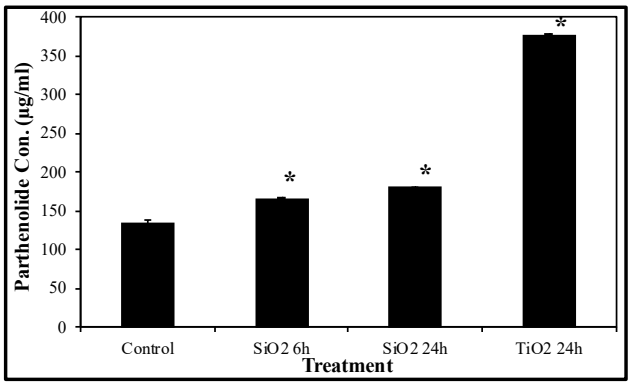

Fig. 8. Parthenolide concentration in *Tanacetum parthenium* leaves at different treatments.

The effect of salinity stress as an environmental elicitor with 300mM of NaCl in two times, including 6h and 48h was investigated. As shown in Fig. 6c, the 48h salt stress elevated the expression of *TpCarS* gene significantly (~12 fold) compared to 6h and 24h treatments.

Parthenolide concentrations in feverfew under different treatments

To assay the parthenolide concentration in different treatments, leaves tissue were analyzed by HPLC (Fig. 7). There were significant differences between parthenolide concentrations ($P< 0.05$) in different treatments (Fig. 8).

The highest and least amount of parthenolide was observed in 25mM of TiO_2 after 24h and control plants with 378.61µg/mg and 136.02µg/mg, respectively. All treatments increased the concentration of parthenolide in Feverfew leaves compared to control plants ($P<0.05$). The SiO_2 (25mM) after 6 and 24h treatments increased the leaves parthenolide content by 1.23 and 1.37 fold compared to control. The TiO_2 (25mM, 24h) raised the parthenolide amount in leaves far more than SiO_2 NPs with 2.78 fold.

DISCUSSION

Nanoparticles are a new class of men made materials which are emerging in these years. In hence, a new trend in biological sciences is toward investigation of interaction of the synthetic agents to organisms including plants. There are many reports which study the effect of them (TiO_2 and SiO_2) on plants in different kinds of morphological and molecular levels (Siddiqui and Al-Whaibi 2014; Castiglione et al. 2011; Frazier et al. 2014; Kahila et al. 2017), as well *in vivo* culture (Mandeh et al. 2012). A number of studies also prove the key role of different elicitors, including chemical compounds (Van Fürden et al. 2005; Esmaeilzadeh Bahabadi et al. 2011; Esmaeilzadeh Bahabadi et al. 2014;

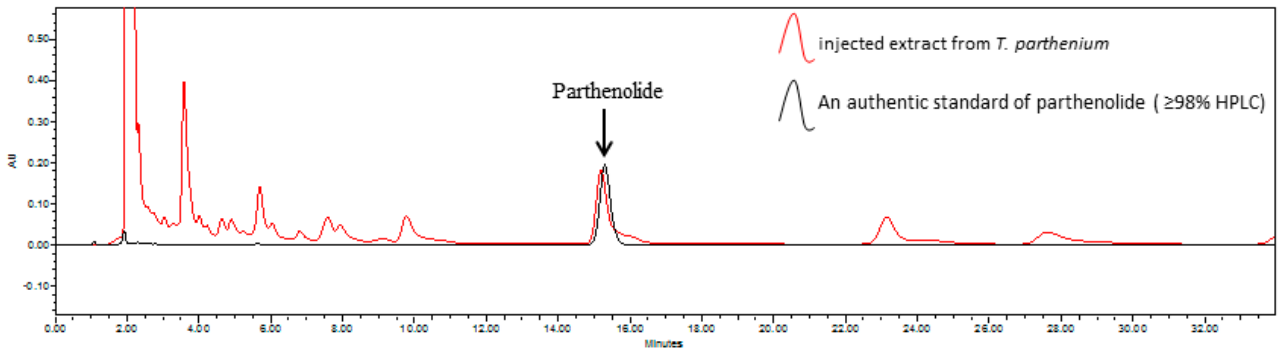

Fig. 7. The HPLC analysis of extract from *T. parthenium* and 110 ppm of standard partenolide from Sigma-Aldrich company (USA). Minutes: Run time, AU: Absobtion intensity.

Wang et al. 2007; Marsh et al. 2014), biotic (Kim et al. 2001; Jeong et al. 2005; Savitha et al. 2006; Wu et al. 2007; Kang et al. 2009; Gao et al. 2011; Swaroopa et al. 2013; Ahmed and Baig. 2014) and abiotic (Akula and Ravishankar 2011; Chan et al. 2010; Szakiel et al. 2011) on metabolite production in different plants. However, nearly most of studies have been conducted on cell or hairy root culture and investigation on *in vivo* whole plant elicitation is limit. The aim of the present work was to determine the elicitation role of nanoparticles and salt stress on metabolite hyperproduction through gene expression in whole plant.

NPs and salinity treatments affect the parthenolide biosynthesis genes

The effect of different kind of elicitors was investigated to find a clear view about the impact of elicitors on the expression of genes involved in the secondary metabolites biosynthesis pathway in *T. parthenium*. It seems that SiO_2 NPs in low concentration can have a positive effect on parthenolide production in Feverfew. In opposite to SiO_2, TiO_2 NPs treatment created a different expression pattern of the *TpGAS*, *COST* and *TpCarS* genes.

Dimkpa et al. (2012) demonstrated the beneficial effects of CuO and ZnO NPs on IAA production *in vitro* in the soil isolate *P. chlororaphis* O6. An increased IAA production with exposure to sublethal levels CuO NPs was observed in their study. They explained an ion release and nonspecific mechanism for increased level of IAA due to CuO and ZnO NPs treatment, respectively.

The salt stress up-regulated all the mentioned genes in our study. It seems, this is an evolutionary mechanism in medicinal plant to tolerance abiotic stress with producing of metabolites including PTL compound in Feverfew.

A comparison between the salts stress and the nanoparticles effect on the genes and hyperproduction of parthenolide, mentioned that, the NPs are more effective than salt stress, because they affected two key genes, including *TpGAS* and *COST* which catalyzes two steps of parthenolide biosynthesis pathway compared to salt stress which affect only on *TpCarS* gene.

The drought and salt stresses cause common reactions in plants. Cellular dehydration then osmotic stress and transfer of water from the cytoplasm to vacuoles are a result of both stresses (Akula and Ravishankar, 2011).

Salinity stress often causes both ionic and osmotic stress in plants, which increases or decreases specific secondary metabolites in plants (Mahajan and Tuteja, 2005). For example, Parida and Das (2005) found that anthocyanins are increased in response to salt stress. Oppositely, Daneshmand et al. (2010) demonstrated that salinity stress decreases the anthocyanin level in the salt-sensitive species. As the Plant polyamines are involved in plant response to salinity, changes of free and bound polyamines levels has been reported in sunflower (*Helianthus annuus* L.) roots under salinity stress (Mutlu and Bozcuk, 2007). The endogenous JA (Jasmonic acid) under salt stress in tomato cultivars has been found (Pedranzani et al. 2003). Polyphenols are another group of metabolites which their synthesis and accumulation is usually is stimulated as a reaction to biotic or abiotic stresses (Dixon and Paiva, 1995; Muthukumarasamy et al. 2000). In some of plants such red peppers (Navarro et al. 2006); a raise in polyphenol content with increasing level of salt in different tissues has also been reported (Parida and Das, 2005).

Positive correlation between TpGAS and COST expression and parthenolide concentration under NPs Treatments

A positive correlation between *TpGAS* and *COST* expression and parthenolide concentration was observed in SiO2 and TiO_2 treatments (25mM and 24h) compared to control plants, in contrast to *TpCarS* of which its expression had a negative correlation with increasing treatment time. Although few studies have revealed the genotoxic potential of TiO_2 nanoparticles in the plant systems (*A. cepa* and *N. tabacum*) with DNA damaging effect (Ghosh et al. 2010), in this work, TiO_2 nanoparticles had a positive effect on parthenolide production. *TpGAS* encodes the enzyme that highly likely catalyzes the first step in parthenolide biosynthesis, germacrene A synthase (Fig.3) (Majdi et al. 2011). Then, it seems that the Feverfew plant responds to the NPs by up-regulation of the responsive genes and then metabolite production.

TpCarS (β-Caryophyllene synthase) encodes β -Caryophyllene which is an anti-inflammatory (Martin et al. 1993; Tambe et al. 1996) and anti-carcinogenic (Kubo et al. 1996; Zheng et al. 1992) compound and a common and quite widely distributed sesquiterpene in plants (Knudsen et al. 1993; Kubo et al. 1996).

Similar to *Artemisia annua* plant (Chen et al. 2011), in *Tanacetum parthenium* (Bouwmeester et al. 2002) farnesyl diphosphate (FPP) (Fig.3) is an initial point and serves as a basic precursor to synthesize the various classes of sesquiterpenes with divergent structures and functions by different synthases such *TpCarS* through competitive pathways. Therefore, depending on their competition with the available FPP pool, the critical step catalyzed by different sesquiterpene synthases shows a metabolic regulating to direct the cellular carbon flux

towards parthenolide or other sesquiterpenes. Therefore, it seems, the nanoparticles directly and exclusively induce the genes toward more production of parthenolide compared to β-Caryophyllene.

Different studies have proved hyperproduction of secondary metabolite related to use of elicitors, including Jasmonate (Walker et al. 2002; Zhao et al. 2010; Tocci et al. 2012; Tocci et al. 2011; Gadzovska et al. 2013; Cui et al. 2014). It has been found that Jasmonic acid (JA) and its methyl esters, methyl jasmonate (MeJA), are important signaling compounds in the process of elicitation leading to the hyper production of various secondary metabolites (Walker et al. 2002). This compound plays a key role in signal transduction processes involved in defense responses in plant and has shown that is effective to induce the production of secondary metabolites in cell cultures (Walker et al. 2002; Zhao et al. 2010; Tocci et al. 2012; Tocci et al. 2011; Gadzovska et al. 2013; Cui et al. 2014).

The effect of the elicitors on flavonoid production was reported by Wang et al. (2015). They showed that the Flavonoid content is promoted by MeJA and SA induction, which were 2.1 and 1.5 times higher in comparison to control cultures, respectively.

A list of reports on different plant species have demonstrated a positive and strong correlation between terpene content and the level of the related mRNA transcripts, which proves the terpenoid biosynthesis is majorly regulated at the transcript level (Nagegowda, 2010).

CONCLUSION

The results of our work, showed a significant effect of nanoparticles in low concentration and salinity stress on the expression of two genes involved in partheonlide biosynthesis pathway, also *TpCarS* as a caryophyllen synthase in Feverfew. It seems due to the positive effect of SiO_2 NP compared to TiO_2, on the expression of all key genes for parthenolide and β-caryophyllen productions in Feverfew, these nanoparticles can be used as efficient elicitors and useful additives to soil for increasing of secondary metabolite production in the whole plant, practically. As it was mentioned above, the metabolites are used as medicinal compounds for patients with migraine disease and different type of cancers; in hence, our results can suggest a simple and cost effective technique for mass production of them. However, due to a dominant view among researchers about the destructive role of nanoparticles in the environment, it is necessary for more research for investigation about side effect of the nanoparticles on plant growth and development in future. In conclusion, in this work, it was tried to explain the role of nanoparticle by this point of view which the nanomaterial, can also be valuable for more providing of human medicinal necessities from herbs and then, it opened a new window toward nanoparticle application for medicinal plants studies. it should be mentioned that, in this project the short-term effects of elicitors on secondary metabolite production were investigated and further studies are needed to determine the long-term impact of these elicitors on plants gene expression.

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AUTHOR CONTRIBUTION STATEMENT

MR conceived and designed research. MK conducted experiments. MR contributed new reagents or analytical tools. MR analyzed data. MK, MR and AE wrote the manuscript. All authors read and approved the manuscript.

AVAILABILITY OF DATA AND MATERIALS

The datasets during and/or analyzed during the current study available from the corresponding author on reasonable request.

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