International Journal of Horticultural Science and Technology
Journal homepage: https://ijhst.ut.ac.ir

Impact of Foliar Application of Fullerene Nanoparticles on the Development, Productivity, and Synthesis of Secondary Metabolites in Feverfew (*Tanacetum parthenium* L.) during Two Distinct Harvest Phases in a Pot Experiment

Seyede Zahra Ahmadi*

Department of Horticulture Sciences, Faculty of Agriculture, Lorestan University, Khorram abad, Iran

ARTICLE INFO

Article history:

Received: 28 February 2025,
Received in revised form: 31 May 2025,
Accepted: 2 June 2025,

Article type:

Research paper

Keywords:

Abiotic elicitors,
Essential oil,
Feverfew,
Nanotechnology

*Corresponding author's email: Ahmadi.z@fa.lu.ac.ir

ABSTRACT

Biostimulants are compounds that enhance plant productivity by activating metabolic processes, resulting in increased growth and synthesis of secondary metabolites through the stimulation of plant defense mechanisms. This study aimed to evaluate the effects of carbon nanoparticles (fullerene) on the growth and yield of feverfew (*Tanacetum parthenium* L.) during two harvest periods in 2021. The research was conducted using a factorial arrangement within a completely randomized design with three replications in an open-field environment at the Faculty of Agriculture, Lorestan University. Results showed that treatment with fullerene nanoparticles significantly increased the fresh and dry weights of aerial parts, the number of flowers, essential oil concentration, and parthenolide content in the plants. Additionally, the harvest stage had a significant impact on morphological traits, with the first harvest stage producing the best growth characteristics. These findings could help in developing effective strategies for enhancing secondary metabolite production in feverfew.

Introduction

Feverfew is a medicinal plant from the Asteraceae family. It is a short, bushy, aromatic perennial that contains bioactive compounds such as sesquiterpene lactones, particularly parthenolide. This compound is found in glandular leaf trichomes (0.2 to 0.5%) and constitutes approximately 85% of the total terpene content (Pareek et al., 2011; Izadi et al., 2013). Feverfew has been traditionally used for treating fever, migraines, rheumatoid arthritis, stomach pain, toothaches, insect bites, infertility, menstrual issues, and labor pain (Pareek et al., 2011).

Biostimulants are substances that enhance plant efficiency by stimulating metabolism and metabolic processes (Starck, 2005). The binding of elicitors to plasma membrane receptors initiates a hypersensitive response in the plant or cell. This process involves chloride and potassium ion efflux, rapid protein phosphorylation changes, and the activation of protein kinases and G proteins. Subsequent stages include cytoplasmic pH reduction and the activation of NADPH oxidase, responsible for the production of ROS. The generation of second messengers (e.g. jasmonates and salicylic acid) and the activation of enzymes such as chalcone synthase and phenylalanine ammonia-lyase ultimately result in the production of secondary metabolites like phytoalexins (Ghorbanpour and Hadian, 2015).

Certain nanoparticles (NPs) with unique physicochemical properties act as biostimulants, enhancing plant growth. The effects of nanoparticles depend on their physicochemical characteristics, application method (foliar, hydroponic, soil-based),

and concentration (Zhao et al., 2020). The history of nanocarbons dates back to 1985 (Pyrzynska, 2011), and carbon nanoparticles have been reported to improve photosynthetic activity, plant development, and water absorption (Mukherjee et al., 2016).

Soil application of fullerene (500 to 5000 mg kg^{-1}) has been reported to reduce soybean biomass by 40%, maize biomass by 44%, and tomato biomass by 10%, while also inhibiting pumpkin root growth (Kelsey and White, 2013; De La Torre-Roche et al., 2013). Fullerene C60 blocks magnesium channels, leading to reduced magnesium ion uptake, chlorophyll content, and photosynthetic products in green algae (Tao et al., 2014). Furthermore, Lin et al. (2009) reported that rice seedlings grown in hydroponic culture absorbed fullerene C70, as evidenced by its accumulation in vascular and intercellular spaces. The application of carbon nanoparticles in bell pepper plants increased total phenol, antioxidant activity and total flavonoid content (Ahmadi et al., 2024).

To date, no study has investigated the effect of fullerene nanoparticles on the growth and performance of feverfew across two harvest stages. This research is the first to examine feverfew's growth and performance under fullerene nanoparticle treatment in two harvest stages in a pot experiment. The findings may contribute to developing strategies for enhancing secondary metabolite production in medicinal plants.

Materials and Methods

Planting, growth conditions, and treatments

The research was conducted in 2021 as a factorial trial based on a completely randomized design with three replications in the open field of the Faculty of Agriculture at Lorestan University/Iran. The first variable was the foliar application of fullerene nanoparticles at five concentrations (0, 125, 250, 500, and 1000 mg L^{-1}), while the second variable was the harvest stage at two intervals (121 and 243 d post-germination). The pots used in the experiment were 20 cm in diameter and 19 cm in height, each filled with 8 kg of a growth medium composed of field soil, sand, and manure in a 1:1:1 ratio. Physical and chemical properties of the soil were assessed prior to the experiment (Table 1).

Table 1. Analysis of soil physical and chemical testing.

Parameters (Units)	Soil Values
pH	7.13
ECe (dS m^{-1})	2.51
Organic matter content (%)	1.74
Organic carbon content (%)	1.04
Total nitrogen (%)	0.15
Potassium (mg kg^{-1})	331
Phosphorus (mg kg^{-1})	12.4
Textural class	Sandy Clay Loam

The study was conducted under open field conditions (natural light) at Lorestan University (longitude 48.35°E, latitude 32.29°N, altitude 1147.8 m) from April to November. No fertilizers were used in the experiment. Prior to beginning the experiment, all seeds were disinfected by soaking in a 1% sodium hypochlorite solution for 3 min, followed by four rinses with sterile distilled water. Initially, five seeds were planted in each pot on the fifth of March. However, once they had all germinated, only one seedling was left in each pot. Before spraying, the fullerene solution was sonicated using an ultrasonic device (Powersonic, UB-405, 45 kHz for 30 min) to obtain a relatively homogeneous solution. The first foliar application of different fullerene concentrations was conducted 28 d after seed germination, and the second application was performed two weeks later (Ahmadi et al., 2020). Feverfew seeds were provided by the Medicinal Plants Research Institute at Shahid Beheshti University. The spherical carbon nanoparticles used in this experiment were purchased from Pishgaman Nano Materials Co. in Mashhad, and the characteristics of the nanomaterial are presented in Table 2.

Table 2. Characteristics of carbon nanoparticles in this study.

Morphology	Color	Decoloration Rate	Purity	Sterilization	APS	H2O	Ash	pH	True density	Bulk density
spherical	Black	99%	>95%	Cobalt-60 Radiation	20-40nm	<5%	<2%	7-10	0.44 g mL^{-1}	0.32 g mL^{-1}

Morphological traits

Before harvesting, measurements were taken for plant height (cm), the number of main stems, side stems, and flowers. The plants were then cut 4 cm above the pot surface and weighed. The diameter of the flower was measured with digital calipers. After drying the plants in the shade, the dry weight (g) of the whole plant, as well as the dry weight of leaves and flowers, were measured with the same scale. The

harvest index was calculated using the formula provided by Ntanos and Koutroubas (2002).

$$Harvest\ Index = \frac{Economic\ Yield}{Biological\ Yield} \times 100$$

$$Biological\ Yield = Dry\ weight\ of\ the\ entire\ plant\ (roots + all\ aerial\ parts)$$

Economic yield = dry weight of leaves and flowers

Chlorophyll index (SPAD)

The chlorophyll index indicates the greenness of a leaf resulting from its chlorophyll content, which is measured using a SPAD device (Model: KONICA MINOLTA 502, Japan). Leaf greenness was randomly measured from three different leaf sections at the beginning of the plant's reproductive stage, and the average of these three measurements was recorded. For each plant, the SPAD readings of three specified leaves were averaged to determine the chlorophyll index.

Parthenolide extraction and quantification

To prepare the extract for parthenolide assessment, 100 mg of powdered leaves and flowers were mixed with 10 mL of acetonitrile diluted with distilled water in a 1:9 ratio. The mixture was then placed in an ultrasonic bath for 15 min and subsequently centrifuged at 3000 rpm for 12 min. A 1.5 mL aliquot of the supernatant was transferred into a special HPLC vial for parthenolide quantification using high-performance liquid chromatography (HPLC) (Chaves and Da Costa, 2008).

Specifications and conditions for high-performance liquid chromatography (HPLC)

The HPLC analysis was conducted using a Waters liquid chromatography system, which included a Waters 2695 separation unit and a Waters 2487 dual absorbance detector. The sample injector syringe was fitted with a 100 µL loop. Data acquisition and integration were managed using Millennium 32 software. Chromatographic separation was accomplished on a 25 cm × 4.6 mm column, with a pre-column Eurospher 100-5 C18 and an analytical column supplied by Knauer (Berlin, Germany). The reversed-phase matrix (5 µm) Waters phase and system rinse were performed using acetonitrile as the organic phase (solvent A) and distilled water (solvent B) at a flow rate of 1 mL min^{-1}. Peaks were monitored at a wavelength of 220 nm (Fig. 1). The injection volume was 20 µL, and the temperature was maintained at 25 °C. All injections were repeated three times. Calibration curves were generated based on linear regression analysis of peak areas for concentrations of 1, 10, 25, 50, 80, 120, 150, and 200 mgL^{-1} (Fig. 2) (Chaves and Da Costa, 2008).

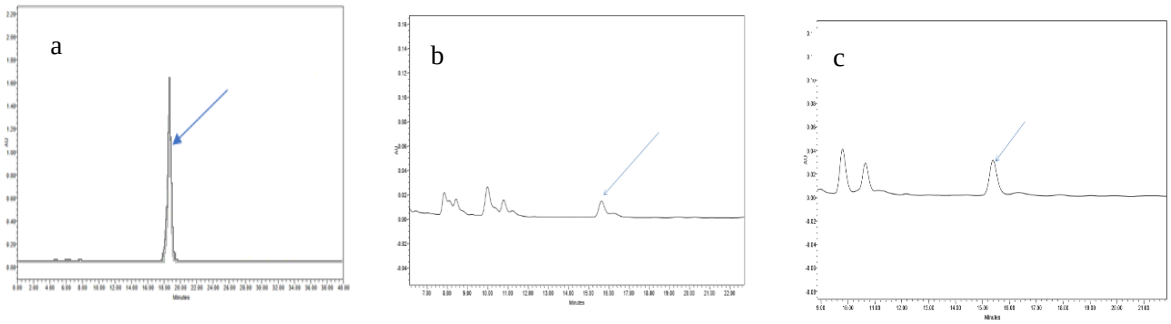

Fig. 1. Chromatogram sample of parthenolide measurement in the studied plant samples at a wavelength of 220 nm. (**a**) standard sample (pure parthenolide); (**b**) sample from the first harvest; (**c**) sample from the second harvest.

Essential oil extraction

Ten grams of dried aerial parts of feverfew were weighed and essential oil was extracted using steam distillation with a Clevenger apparatus for 3 h (Akpulat et al., 2005). The excess moisture in the essential oil was removed using anhydrous sodium sulfate (Na_2SO_4). The yield of the essential oil was reported based on a volumetric-weight method.

Scanning electron microscopy (SEM)

Following the protocol outlined by Rao and Shkhawat (2014), fully developed leaf samples of the plant were preserved in a solution containing glutaraldehyde (2.5% v/v) and potassium phosphate buffer (0.05 M, pH 7.1) for 8 h. Subsequently, the samples were dehydrated using series of ethanol concentrations (10, 20, 30, 50, and 70%) for 15 min each, followed by coating with a gold layer. SEM imaging was conducted using a JEOL model SEM at the central laboratory of Shahid Beheshti University.

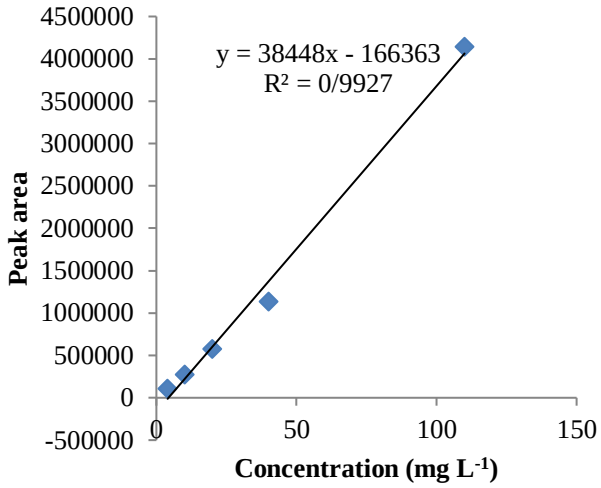

Fig. 2. The parthenolide standard curve was constructed using linear regression analysis of peak areas for pure parthenolide concentrations at 1, 10, 25, 50, 80, 120, 150, and 200 mg L^{-1}.

Data analysis and statistical calculations

The data was analyzed using analysis of variance (ANOVA) with SAS software (version 9.1). Duncan's multiple range test was used for comparing mean values. All data were presented as mean ± standard deviation. Graphs were created using Microsoft Excel 2007.

Results

The analysis of variance (Tables 3 and 4) revealed that harvest time had a significant effect ($p \leq 0.01$) on plant height, flower number, number of main and side stems, greenness index, essential oil content, and parthenolide concentration. Different concentrations of nano-fullerene also exerted significant effects ($p \leq 0.01$) on flower number, number of side stems, essential oil content, and parthenolide concentration, as well as a significant effect at the 5% level ($p \leq 0.05$) on fresh weight. Furthermore, the interaction between harvest time and nano-fullerene concentration was significant ($p \leq 0.01$) for fresh and dry weights, dry weights of flowers and leaves, essential oil content, and parthenolide concentration, and significant at the 5% level ($p \leq 0.05$) for flower count.

Table 3. Analysis of variance of morphological traits under the influence of fullerene at different concentrations and harvest times of feverfew.

S.O	d.f	Mean squares								
		BH	NF	NSS	NMS	FD	FW	DW	DWFL	HI
Harvest (H)	1	820.1**	3151.87**	282.1**	216.01**	0.04^{ns}	43.14^{ns}	21.35^{ns}	0.84^{ns}	360.86^{ns}
Fullerene (F)	4	64.8^{ns}	755.1**	23.39**	5.7^{ns}	0.03^{ns}	312.55*	9.99^{ns}	4.85^{ns}	254.03^{ns}
H×F	4	64.4^{ns}	172.3*	1.99^{ns}	5.5^{ns}	0.05*	961.59**	53.39**	27.68**	28.96^{ns}
Error	20	92.8	46.98	3.9	3.82	0.014	106.3	6.02	5.77	133.4
C.V(%)		27.66	18.8	24.2	25.99	7.94	28.4	22.1	28.1	14.85

**Significant at 1% level, *Significant at 5%, ^{ns} not statistically significant. BH; Bush height, NF; Number of flowers, NSS; Number of side stems, NMS; Number of main stem, FD; Flower diameter, FW; Fresh weight, DW; Dry weight, DWFL; Dry weight Flowers and leaves, HI; Harvest index.

Table 4. Analysis of variance of physiological and metabolic traits under the influence of Fullerene different concentrations and harvest time of medicinal plant of feverfew.

S.O	d.f	Mean square		
		EOP	PC	GI
Harvest(H)	1	0.001**	909.53**	259.31**
Fullerene (F)	4	0.002**	14997.9**	33.7 ns
H×F	4	0.0006**	343.14**	47.7ns
Error	20	0.00009	0.0000	27.94
C.V(%)		13.23	0.0000	13.9

**Significant at 1% level, *Significant at 5%, ns not statistically significant. EOP; Essential oil Percentage, PC; Parthenolide content, GI; Greenness Index.

When analyzing the impact of the harvest stage on plant height, number of main and side stems, and greenness index (Table 5), it was found that the highest values for these traits were observed in the first harvest stage. Specifically, they were 35, 112.5, 121, and 17% higher than the second harvest stage, respectively. Furthermore, comparing the mean effect of different concentrations of nano fullerene on the number of side stems revealed that the greatest number of stems occurred with the application of 1000 mgL^{-1} nano fullerene, resulting in a 46% increase compared to the control.

Table 5. Comparison of the mean effect of harvest treatments and carbon nanoparticle foliar spraying alone on the morphophysiological characteristics of feverfew.

treatments	Treatment levels	BH (cm)	NMS	NSS	GI (%)
Harvest	First	40.06^{a}	10.2^{a}	11.2^{a}	40.88^{a}
	Second	29.6^{b}	4.8^{b}	5.07^{b}	35.00^{b}
treatments	Treatment levels			NSS	
carbon nanoparticle foliar spraying	Control			7.83^{b}	
	fullerene (125 mg L^{-1})			6.92^{b}	
	fullerene (250 mg L^{-1})			8.17^{b}	
	fullerene (500mg L^{-1})			6.33^{b}	
	fullerene (1000mg L^{-1})			11.42^{a}	

Means with the same letters in Hurston do not have a significant difference at the 1% probability level based on the Duncan test. BH; Bush height, NMS; Number of main stem, NSS; Number of side stems, GI; Greenness Index.

Number of flowers

According to Figure 3, a significant difference was observed between the two harvest stages. In the first harvest stage, the number of flowers was 76% higher than in the second harvest stage. The number of flowers increased in both harvest stages with the application of various concentrations of nano fullerene. Specifically, the 1000 mg L^{-1} concentration in the first harvest stage showed a 43.2% increase compared to the control, while the 250 mg L^{-1} concentration in the second harvest stage showed an 82.8% increase compared to the control.

Fresh and dry weight

The interaction effect of harvest stage and nano fullerene concentrations on the fresh weight of aerial parts (Fig. 4) showed that the highest fresh weight was observed in the second harvest stage with the application of 500 mg L^{-1} spherical nanocarbon, reaching 60.70 g. This result was not significantly different from the treatment of the first harvest stage with the application of 250 mg L^{-1} spherical nanocarbon. The application of 500 mg L^{-1} nano fullerene in the second harvest stage increased the fresh weight of aerial parts by 157.5% compared to the absence of nano application in the same stage. Similarly, the application of 250 mg L^{-1} nano fullerene in the first harvest stage resulted in a 68% increase in fresh weight compared to the absence of nano application in the same stage.

Regarding the interaction effect of harvest stage and spherical nanocarbon concentrations on the dry weight of aerial parts (Fig. 5), the highest dry weight was recorded in the first harvest stage with the

application of 250 mgL^{-1} spherical nanocarbon, reaching 17.6 g, which represents a 63% increase compared to the absence of spherical nanocarbon application in the same stage.

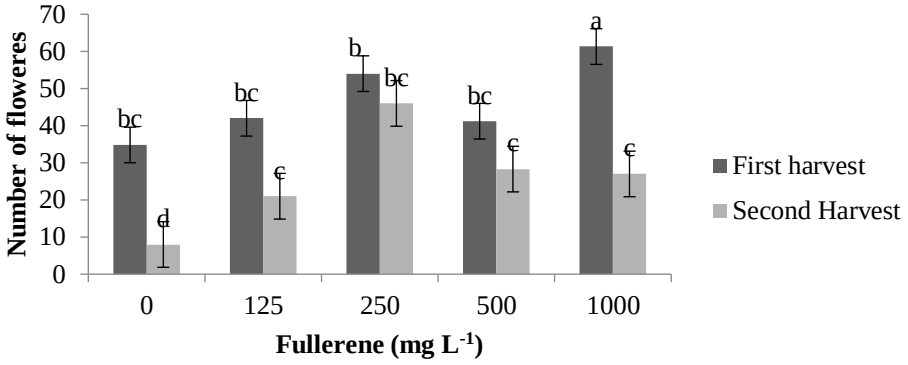

Fig. 3. Changes in flower count in feverfew plants exposed to various concentrations of nano fullerene (0, 125, 250, 500, and 1000 mg L^{-1}) at two harvest stages (121 and 243 d after germination). The values reported are means ±SD (n = 3) and bars with different letters indicate significant differences among treatments at a probability level of $P < 0.05$ using Duncan's test.

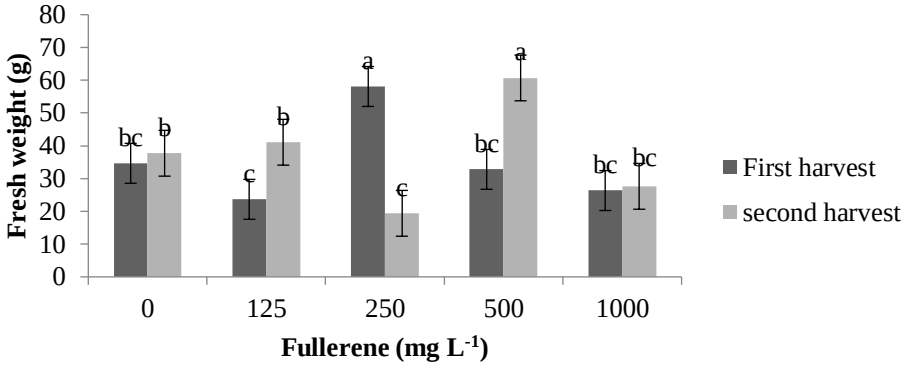

Fig. 4. Changes in fresh weight of feverfew plants exposed to various concentrations of nano fullerene (0, 125, 250, 500, and 1000 mg L^{-1}) at two harvest stages (121 and 243 d after germination). The values reported are means ± SD (n = 3) and bars with different letters indicate significant differences among treatments at $P < 0.05$ using Duncan's test.

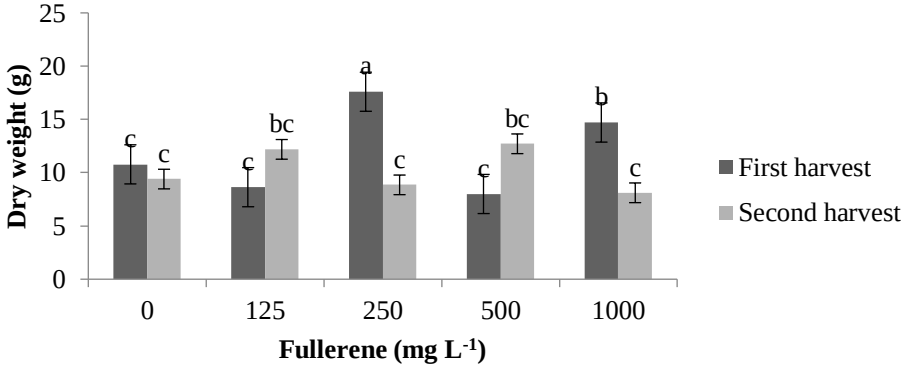

Fig. 5. Changes in dry weight of feverfew plants exposed to various concentrations of nano fullerene (0, 125, 250, 500, and 1000 mg L^{-1}) at two harvest stages (121 and 243 d after germination). The values reported are means ± SD (n = 3) and bars with different letters indicate significant differences among treatments at a probability level of P <0.05 using Duncan's test.

Dry weight of flowers and leaves

The interaction effect of harvest stage and nano fullerene concentrations on the dry weight of flowers and leaves per plant (Fig. 6) showed that the highest dry weight was recorded in the first harvest stage with the application of 250 mg L^{-1} nano fullerene, reaching 12.99 g. This represented a 53% increase compared to the absence of nano fullerene application in the same harvest stage.

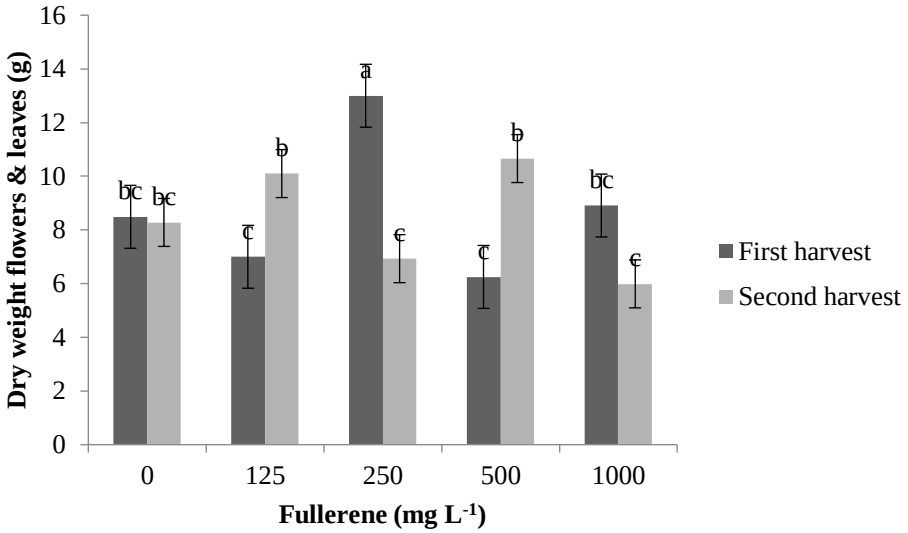

Fig. 6. Changes in dry weight of flowers and leaves of feverfew plants exposed to various concentrations of nano fullerene (0, 125, 250, 500, and 1000 mg L^{-1}) at two harvest stages (121 and 243 d after germination). The values reported are means ± SD (n = 3) and bars with different letters indicate significant differences among treatments at $P < 0.05$ using Duncan's test.

Essential oil percentage and parthenolide content

In comparing the mean interaction effect of harvest stage and nano fullerene concentrations on the essential oil percentage per plant (Fig. 7), the highest essential oil content was observed in the first harvest stage without the application of nano fullerene, reaching 1.1%.

Regarding the interaction effect of harvest stage and nano fullerene concentrations on parthenolide content in flowers and leaves per plant (Fig. 8), the highest parthenolide content was recorded in the second harvest stage with the application of 500 mg L^{-1} nano fullerene, reaching 127.48 µg per 0.1 g. This represented a 127.5% increase compared to the absence of nano fullerene application in the same harvest stage.

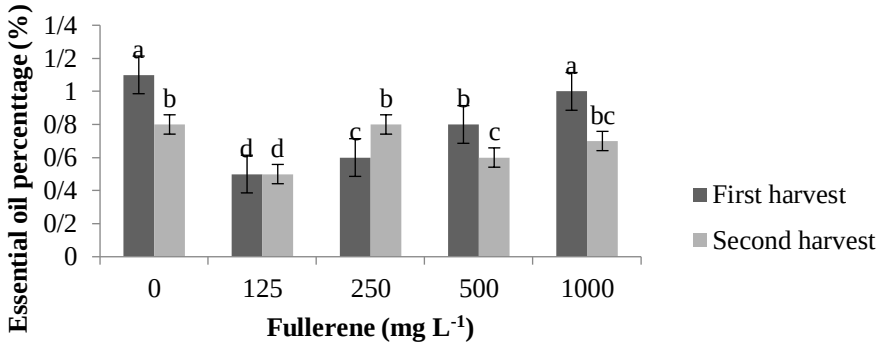

Fig. 7. Changes in the percentage of essential oil in feverfew plants exposed to various concentrations of nano fullerene (0, 125, 250, 500, and 1000 mgL^{-1}) at two harvest stages (121 and 243 d after germination). The values reported are means ± SD (n = 3) and bars with different letters indicate significant differences among treatments at $P < 0.05$ using Duncan's test.

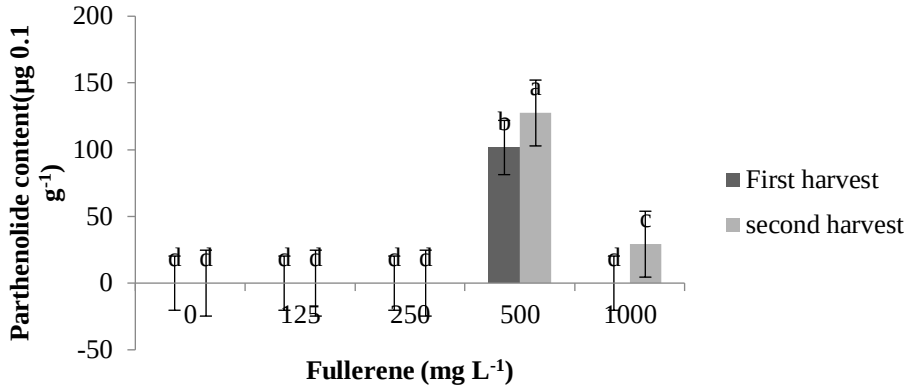

Fig. 8. Changes in parthenolide content of feverfew plants exposed to various concentrations of nano fullerene (0, 125, 250, 500, and 1000 mg L^{-1}) at two harvest stages (121 and 243 d after germination). The values reported are means ± SD (n = 3) and bars with different letters indicate significant differences among treatments at $P < 0.05$ using Duncan's test.

Analysis of variance (ANOVA) for the regression equation used in predicting parthenolide levels

By applying multiple regression analysis to the experimental data, the response variable (parthenolide) and the independent variables were related through the following polynomial equation:

$$Parthenolide = 22.4 + 1.64(Fresh\ weight) - 5.05(Dry\ weight)$$

Table 6 presents the results of the ANOVA for this regression equation. The linear coefficients were significant ($P < 0.05$), indicating a significant linear relationship between the response variable (parthenolide) and the independent variables. To assess the adequacy of the model, a Lack of Fit test was performed, showing non-significance ($P < 0.05$), suggesting that the model fits the experimental data well. Mallows' Cp index was also used as a criterion for measuring the model's accuracy. A lower value of this index indicates higher accuracy and lower error of the model. Mallows' Cp value for the proposed model was 2.44, indicating a good model fit. Based on the data presented in the table, the fresh weight of the aerial part had the greatest influence on predicting parthenolide levels.

Table 6. Analysis of variance (ANOVA)was conducted for the regression equation used to predict the levels of parthenolide.

S.O.V	d.f	Mean Square	F-Value	*P*-Value
Regression	2	7721	4.45	0.021
Fresh weight	1	14555	8.39	0.007
Dry weight	1	7515	4.33	0.047
Error	27	1734		
Total	29			
Adj R^2 = 19.23%		R^2 = 24.80%	Pred R^2 = 9.68%	S = 41.6472	Mallows' Cp = -2.44
Parthenolide = 22.4 + 1.64(Fresh weight) – 5.05(Dry weight)				

SEM observations

The uptake and translocation of nano fullerene by the leaf system of feverfew were confirmed using Scanning Electron Microscopy (SEM) (Fig. 9). The SEM analysis of plants treated with C60 fullerene at a concentration of 1000 mgL^{-1} compared to the control group is shown (Fig. 9). The leaves of the control group plants had longer trichomes compared to the treated plants (Fig. 9A). After exposure to 1000 mg L^{-1} of C60 fullerene, lower trichome density

was observed compared to the control (Fig. 9C). The leaf surfaces of the control plants appeared healthy with no morphological alterations observed (Fig. 9B). However, plants exposed to nano-fullerene exhibited tearing and damage. Additionally, the SEM images showed spherical carbon nanoparticles larger than the applied size (Fig. 9D), which could be attributed to protein binding to the nanomaterial surface (Hatami et al., 2016). Ahmadi et al. (2024) reported that SEM images confirmed nanoparticle absorption through foliar application and that carbon nanoparticles induced changes in the stomatal cells of bell pepper, with these changes being more pronounced with nanotube application. Numerous published scientific reports, often contradictory, exist regarding the absorption, penetration, translocation, accumulation, biotransformation, and role of engineered nanoparticles in various plant species and genotypes (Ma et al., 2010; Hatami et al., 2016).

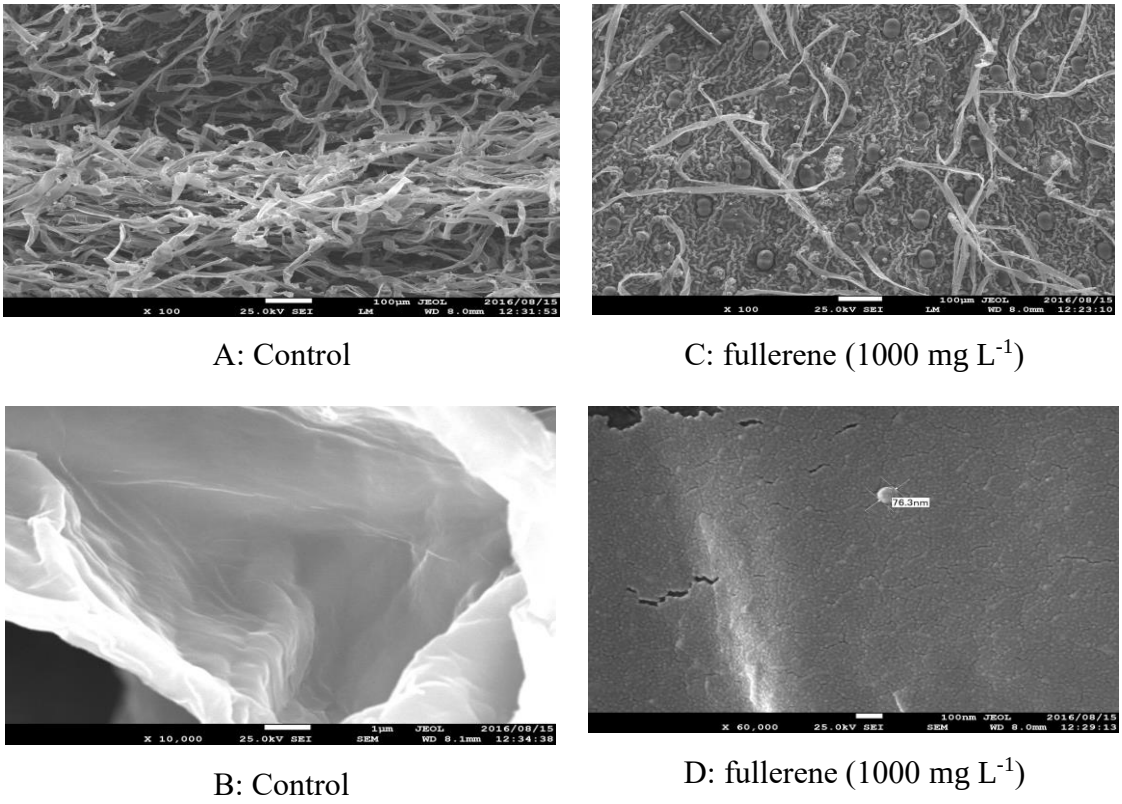

Fig. 9. Scanning electron microscopy (SEM) images of the untreated (**A** and **B**) control leaf sample; (**C** and **D**) and the leaf sample treated with nano fullerene (1000 mg L^{-1}).

Discussion

The results of the morphological trait analysis in two harvest stages indicated that the first harvest stage exhibited better morphological characteristics, likely due to the long photoperiod and favorable ecological conditions during that period. Different concentrations of nano fullerene significantly influenced several evaluated morphological traits of feverfew in both harvest stages. Notably, the concentration of 1000 mg L^{-1} of nano fullerene increased the number of flowers per plant by up to 76%. Additionally, the application of 250 mg L^{-1} of nanocarbon increased the dry weight of the aerial parts by 63% and the dry weight of flowers and leaves by 53% compared to the control. Furthermore, the concentration of 500 mg L^{-1} of nano fullerene increased the fresh weight of the aerial parts by 157.5%.

These results can be attributed to the bio-stimulatory properties of this nanocarbon concentration, which may induce the expression of genes related to flowering and shoot production, ultimately enhancing plant growth. Carbon nanoparticles of unspecified size have been reported to influence all three major photoreceptor signaling pathways, leading to increased tissue size in plants (Kumar et al., 2018). Studies on mung bean morphology and physiology showed that moderate concentrations (100-150 µM) of carbon nanoparticles improved total chlorophyll content (1.9 times), protein content (1.14 times), and plant biomass (fresh weight: 1.2 times, dry weight: 1.14 times) (Shekhawat et al., 2021). Similarly, foliar application of fluorol increased biomass and fruit yield in bitter melon (Kole et al., 2013).

In the present study, the highest essential oil production was observed during the first harvest stage. The plant directed its energy toward flowering and seed production during the second growth phase, leading to reduced essential oil content in the second harvest. However, parthenolide content increased in the later growth stages, explaining the higher parthenolide levels observed in the second harvest. The spherical nanocarbon concentration of 500 mg L^{-1} increased parthenolide production by 127.5% compared to the control.

Nanomaterials, as bio-stimulants, have significant potential in modulating both quantitative and qualitative aspects of secondary metabolites (Hatami et al., 2016). The binding of elicitors to plasma membrane receptors can trigger a hypersensitive response in plants, leading to the production of jasmonates and salicylic acid as secondary messengers, which subsequently activate defense enzymes and secondary metabolite production (Ghorbanpour and Hadian, 2015). Ahmadi et al. (2024) reported that the application of carbon nanoparticles increased total phenol, total flavonoid content, and antioxidant activity in bell pepper. Furthermore, Kole et al. (2013), and Ghorbanpour and Hadian (2015) indicated that carbon-based nanomaterials can enhance the production of active compounds in medicinal plants by influencing the expression of key genes. The results of the present study are consistent with these previous findings.

The results of the analysis of variance (ANOVA) for the regression equation predicting parthenolide levels indicate that, although the proposed model accounts for some of the observed variation in parthenolide concentration based on the fresh and dry weight of the shoot, the relatively low coefficient of determination (R^2 = 24.8%) suggests that a substantial proportion of the variability remains unexplained. Specifically, more than 70% of the variation in parthenolide content is likely influenced by factors not included in the current model. These may include additional physiological and biochemical parameters such as plant developmental stage, photosynthetic efficiency, chlorophyll content, soil composition and texture, nutritional status, and environmental factors like light intensity and temperature, as well as molecular mechanisms involved in secondary metabolite biosynthesis. To construct a more accurate and comprehensive predictive model for parthenolide accumulation, future studies should incorporate a broader range of variables and consider applying advanced statistical approaches, including nonlinear or multivariate modeling techniques.

Conclusion

The application of fullerene nanoparticles, especially at concentrations between 250 and 1000 mg L^{-1}, led to a significant improvement in growth and secondary metabolite production in feverfew. The use of these nanoparticles resulted in increased fresh and dry weight of aerial parts, flower count, essential oil percentage, and parthenolide content. Furthermore, the harvest stage had an impact on morphological characteristics, with the most favorable results seen during the initial harvest. This study illustrates that fullerene nanoparticles can serve as effective biostimulants, playing a crucial role in enhancing the performance of medicinal plants and encouraging the production of secondary metabolites.

Acknowledgments

The author is deeply grateful to Lorestan University for their support of this research.

Author Contributions

Developed the theoretical framework, conducted the analytical calculations, and designed the experiments, ZA.

Funding

This research received no external funding.

Conflict of Interest

The authors indicate no conflict of interest in this work.

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