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Effects of nano-particle TiO_2 solution spray on some biochemical enzymes and flower number of medicinal plant of marigold (*Calendula arvensis* L.)

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Abstract
Marigold (calendula arvensis L.) as a medicinal herb with many health benefits, has always been a human concern. The effectiveness of medicinal herbs is a result of secondary metabolism, and any stress or outside factors could affect their metabolism process. On the other hand, dioxide titanium (Tio_2) is an effective nanoparticle for photosynthesis. In the current study, the potential effects of spraying Tio_2 nanoparticles on effective ingredients of marigold have been investigated. The effect of Tio_2 nanoparticles on the plant qualitative characteristics is investigated in five different levels (i.e. zero, bulk, 0.01, 0.02, 0.03 percent) and two growth stages (i.e. four leaves and flowering stages). The experiment was performed based on a factorial experiment in a completely randomized design with four repeats. The various concentrations of Tio_2 nanoparticles in two growth stages of four leaves and flowering had a significant effect on qualitative characters such as superoxide dismutase (SOD) enzyme, malondialdehyde (MDA) biomarkers, and quantitative character of the number of flowers in various concentrations of solution spraying with Tio_2. Generally, utilization of a higher number of nanoparticles improved the antioxidant enzyme and destroying biomarkers, and decreased the number of flowers in the plant. This increased level of enzymes is one of the beneficial results of spraying plants with higher levels of nanoparticles.

Keywords: marigold, Tio_2 nanoparticle, enzyme, antioxidant, destroying biomarker, flower number

Introduction
Considering the general approach of the world people to the use of natural and herbal medicines in recent years, and in addition to the fact that nanoscience is a new science in the world, an attempt was made to study two global concerns that have been considered. Titanium dioxide (Tio_2) nanoparticles are of special importance in plants due to their special properties at the nanoscale, including their photocatalytic nature. Barley *et al* reported that the consumption of Tio_2 in food at a rate of 1% by weight of final products is harmless [1]. Changmei *et al* investigated the effects of titanium dioxide nanoparticles on germination and seed growth of spinach [2].
An increase of 0.25 to 4% was observed in the treatment impregnated with Tio_2 nanoparticles and this increase was in the following cases: 1. the increase in dry weight, 2. the increase in chlorophyll structure, 3. the increase in ribulose phosphate carboxylase, 4. the increase in oxygenase activity, and 5. the increase in the rate of photosynthesis. This study proved that nano-sized particles have physiological effects on the plant condition. The results of study by Wang *et al* were similar to Changmei *et al*'s results. Also, Changmei *et al* had shown that activity Nitrate reductase, superoxide dismutase (SOD), catalase (CAT), peroxidase (POD) significantly increased the germination of soybean seeds mixed with Sio_2 – Nano and Tio_2 – Nano [2]. In another study, Wang *et al* emphasized that Nano Sio_2 can increase the strength and resistance of rice plants to diseases and thus increase the yield of that plant [3]. Nano anatase of Tio_2 can change the electron transfer reaction under light. The study of Tio_2 nanoparticles in spinach chloroplasts under UV-β radiation showed that Tio_2 nanoparticles can adsorb Significantly reduce radical superoxide and hydrogen peroxide and malondialdehyde (MDA). Wang *et al*. showed that Tio_2 nanoparticles in spinach increased the activity of superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), glutathione peroxidase (GPX) and also accelerated the conversion of total oxygen in Chloroplasts. Other investigators are focused on these parameters based on simulations [4-21].
According to the results of the mentioned studies, determining the best time of nanoparticle consumption and the effect of nanoparticles on the biochemical traits of marigold was one of the objectives of the present study to answer some questions such as Tio_2 nanoparticles with which concentration leads to improvement of qualitative traits of the plants.

Materials and Methods
This study was conducted to investigate the effect of solution application of Tio_2 nanoparticles on some physiological and biochemical aspects of the low-filling marigold plant in 2019 crop in a research farm located in the west of Tehran with longitude 4º21´ west and latitude 27º38´ north was implemented. Also, the height of the experimental farm was 1417 meters above sea level. The average rainfall in the region was about 235 mm, the minimum temperature was -20°C and the maximum temperature was 38°C. In this research, factorial experiment was performed in a completely randomized design with four replications per lysimeter, due to the ability to measure and control the factors affecting the culture medium. So that five levels of Tio_2 nanoparticles (Zero (control), Bulk (non-nano), 0.01% 0.02%, 0.03% by weight 20% colloid) sprayed in two stages of four leaves, and flowering during the growth period sampling was done for quality measurement. Duncan's mean comparison for main levels and statistical analysis were performed using SPSS software.

Measurement of superoxide enzyme performed by Mira and Fridovich method, and measurement of malondialdehyde (MDA) carried out by Steven method. The flowers were collected from the beginning of flowering to the end of it every morning and dried and packed at room temperature and light.

Results and discussion

The analysis results of the variance of the effect of different concentrations of Tio_2 nanoparticles in two stages of four-leaf growth and flowering on superoxide dismutase (SOD) and malondialdehyde (MDA) destructive biomarker and the number of flowers are presented in Figure 1. The highest level of enzyme and the lowest level of biomarker were in the flowering stage. The different concentrations of Tio_2 nanoparticles and different stages of growth did not have a significant effect on enzyme and biomarker ($P<0.01$). While the number of flowers only in different concentrations of Tio_2 nanoparticles, and the interaction effects of different concentrations of nanoparticles with different growth stages had a significant effect ($P <0.05$). It should be noted that the highest number of flowers was in the control sample (Table 1).

Examination of the interaction effects of different concentrations of Tio_2 in different growth stages showed that the number of flowers in the four-leaf stage for the control sample had the highest value. In the four-leaf stage, the concentration of three-hundredths had the lowest value. Also, the interaction effects of different concentrations of Tio_2 at different growth stages showed that superoxide dismutase had the highest value for the flowering stage at a concentration of 0.02 nanoparticles and the biomarker malondialdehyde had the lowest value in the flowering stage at a concentration of 0.03 nanoparticles (Figure 1).

According to the results, the level of destructive biomarkers decreases, and the number of antioxidant enzymes increases in the concentration of 0.03% and in the flowering stage due to the high association of nanoparticles with this biomarker and enzyme. The use of compounds or substances containing Tio_2 nanoparticles can significantly reduce the adsorption of superoxide radicals and hydrogen peroxide and the amount of malondialdehyde (MDA). Increasing the amount of oxidative enzymes and decreasing oxygen-free radicals H_2O_2 may reduce the destructive biomarkers and their destructive effects on marigold. Wang *et al* confirmed these results [22].

Table 1: Values of the degree of freedom (Dof), mean squares (Ms) and the level of significancy (F) of the desired traits in the analysis of variance.

Sources of changes	Number of flowers		MDA Biomarker (Micromoles per fresh weight)		SOD enzyme (Units per mg of protein)		Dof
	F	Ms	F	Ms	F	Ms	
Different densities Titanium dioxide nanoparticles (Tio_2) (C)	3.198*	37850.313	198.393**	2203.282	228.366**	607748.413	4
Different stages of development (G)	0.004^{ns}	44.100	**617/183	2039.184	325.546**	686178.025	1
C × G interactions	3.198*	37734.412	6.534**	72.563	46.989**	99041.462	4
Trial error	---	11834.500	---	11.106	---	2107.771	30

Explanation: ** is significant at 1% level, * is significant at 5% level and ns is not significant at 1% and 5% levels.

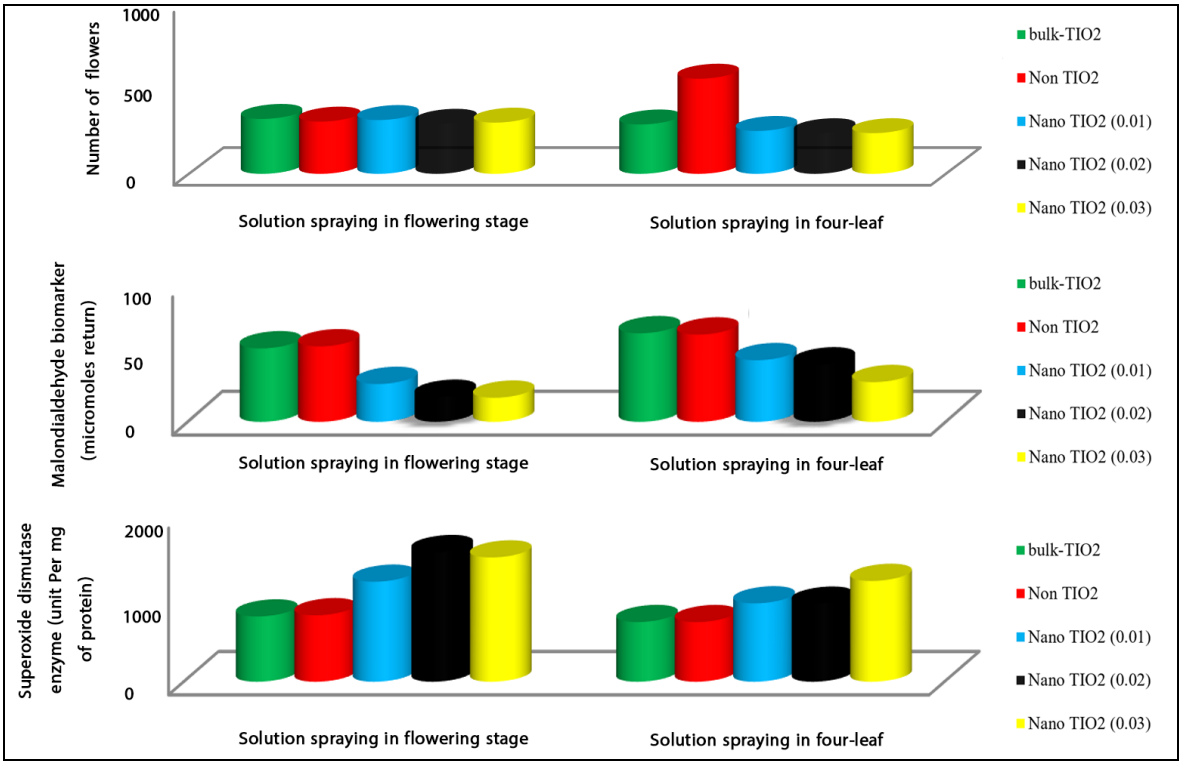

Fig 1: Comparison of the mean interactions of different growth stages and different concentrations of titanium dioxide nanoparticles on superoxide dismutase enzyme and malondialdehyde biomarker by Duncan method.

Therefore, solution application of Tio_2 nanoparticles in marigold can be used as a suitable solution to increase resistance to environmental stresses, especially oxidative stresses. This indicates the success of the application of this particle in the plant which reduces oxidative stress. Solution application of Tio_2 nanoparticles in different stages and concentrations reduced the number of flowers compared to non-solution application. Surfaces of Tio_2 nanoparticles were according to studies by Wang *et al* [22] and Zheng *et al* [23]. By affecting photosynthetic mechanisms such as participating in the activity of Rubis coenzyme and increasing its activity level and interfering with photosystem reactions and increasing the rate of electron transfer, it is likely to increase dry matter production or biological function. There is an inverse relationship between vegetative and reproductive phases. This can be justified that Tio_2 nanoparticles may increase dry matter production by storage in photosynthetic mechanisms.

Conclusion

The results of the present study showed that utilization of a higher number of nanoparticles improved the antioxidant enzyme and destroying biomarkers, and decreased the number of flowers in the plant. This increased level of enzymes is one of the beneficial results of spraying plants with higher levels of nanoparticles.

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Conflicts of interest

No potential conflict of interest was reported by the author.

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