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Full Length Research Paper

TiO_2 and TiO_2 Nanoparticles' Impact on Mentha Piperita's Photosynthetic Pigments, Root and Shoot Length, and Germination

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Aims: The application of nanoparticles has being researched in several scientific domains nowadays. The physiology of medicinal plants is arguably one of the areas that receives less attention. Investigating the phytotoxicity or advantageous effects of titanium dioxide (TiO_2) and titanium dioxide nanoparticle (NP-TiO_2) treatments on Mentha piperita's root and shoot length, photosynthetic pigments, seed germination, and potential inhibitory effects is the goal of this study. This study was evaluated in 2012-2013 in the Islamic Azad University of Falavarjan, Isfahan, Iran, research Laboratory.

Methodology: TiO_2 and NP-TiO_2 treatments were applied to the seeds. Four replicates were used for each treatment, and the mean ±SE (standard error of the mean) was used to display the results. The SPSS 18 was used to analyze photosynthetic pigments, root and shoot length, and germination %.

Results: The germination percentage and shoot length were significantly impacted negatively by the varying concentrations of NP-TiO_2 and TiO_2. However, NP-TiO_2 concentrations of 100 mg L-1 had a greater effect on root length than nonNP-TiO_2 concentrations. There was a noticeable impact on photosynthetic pigments (carotenoids and chlorophyll a and b) at 200 mg L-1 of TiO_2 and 100 mg L-1 of NP-TiO_2.

Conclusion: The experiment's findings demonstrated that while NP-TiO2 at lower concentrations considerably boosted root length, TiO2 at higher concentrations had noticeable effects on photosynthetic pigments.

Key words: Mentha piperita; TiO_2; NP-TiO_2; Germination percentage; Root length; Shoot length;

Photosynthetic pigments; Medicinal plant.

INTRODUCTION

The Particles that range in size from 1 to 1000 nm are called nanoparticles. Applications of nanotechnology are numerous in a variety of scientific fields, including medicine and agriculture. NP-TiO2 is being widely utilized in industry [1]. Research and industry changes were brought about by NP-TiO2. There are noncrystalline forms of this nanoparticle in addition to the three crystal forms known as rutile, anatase, and brukite. NP-TiO2 is used in athletic goods, optical modules, dipole electron tubes, coated surfaces, and disinfectant sprays, among other products. It has an impact on plant physiologic parameters and biological systems. When exposed to UV light, NP-TiO2 affects redox systems oxygen (ROS) in a number of ways [2]. NP-TiO2 has a greater impact on fennel (Foeniculum vulgare Mill) seed germination [3]. In Glycine max, NP-TiO2 can accelerate germination and growth, boost the antioxidant system, and improve the plant's capacity to absorb and use water [4].

In the earth's crust, titanium is the ninth most abundant element [3]. The metal titanium's oxide, TiO2, is a member of the transition metal oxide family [5]. It is thermally stable and insoluble in water [6]. The study found that TiO2 increases the amount of chlorophyll in green algae (Chlorella pyrenoidosa) and paprika (Capsicum annuum L.) [7].

The herbaceous, perennial, glabrous, and highly fragrant plant Mentha piperita is a member of the Lamiaceae (Labiatae) family. Its leaves cross over a smooth, typically reddish-purple, square stem. They are serrated, oblong-ovate, and short (2.5–5 cm) [8,9].Mentha aquatic and Mentha spicata were crossed to create the sterile hybrid mint known as M. piperita, which is marketed as a transplant and cultivated species. It is native to the US and Canada, found in the wild in central and southern Europe, and has become naturalized in several regions of India [8,10].

Higher plant assimilatory tissues require the pigments chlorophyll (Chl) and carotenoids (Car), which give them their dark-green to yellow hues. The primary pigments found in green leaves are Car and Chl. Chl may convert solar light energy into chemical energy using the carbon organic molecules found in cells [11]. The amount of chlorophyll in leaves varies greatly. Chl is a component in organogenesis, and plants have their highest concentration of Chl at the beginning of the flowering phase [12].

A class of naturally occurring fat-soluble pigments, carotenoids (Car) are mostly found in plants, algae, and photosynthetic bacteria, where they are also essential to the photosynthetic process. Carotenoids are typically represented by two carotenes (α and β) and xanthophylls (lutein, zeaxanthin, and violaxanthin). They are distributed unevenly in photosystems and individual pigment-protein complexes of chloroplasts and show strong light absorption in the blue region of the spectrum [13]. They contribute to seed dissemination and pollinator attraction [11].

According to certain findings, TiO2 and NP-TiO2 can cause phytotoxicity and have a detrimental effect on seed germination and growth. Fennel seed germination may be inhibited by the use of TiO2 [14]. By promoting water absorption in spinach seeds and preventing photosynthesis in Chlamydomonas reinhardtii, NP-TiO2 may promote and quicken seed germination [15,16]. Radish, rape, corn, lettuce, and cucumber all had shorter shoots and roots when exposed to increased concentrations of NP-TiO2 [17]. According to the authors, root length and seed germination were inhibited by nanoparticles [18]. Several studies link the uptake of nanoparticles by plant roots to the transmission of nanoparticles to the shoots. The accumulation and translocation of NP-TiO2 to Arabidopsis thaliana shoots was observed. NP-TiO2 enhances spinach growth and encourages photosynthesis [18,19]. Flax seed soaking in anatase-nanoparticle solutions has been shown to enhance root development and seed germination [20]. Mentha piperita is one of the most significant medicinal plants in the world, however some research has been done on the effects of NP-TiO2 and TiO2 on other plants, including Triticum aestivum, Zea mays, and Salvia officinalis [3,17,21]. Regretfully, it has received less research attention despite being utilized to treat a wide range of illnesses, including emphysema, nausea, and stomach stimulations. The purpose of this study was to determine the phytotoxicity or advantageous effects of varying NP-TiO2 and TiO2 concentrations on Mentha piperita photosynthetic pigments, root and shoot length, and seed germination. Because its seed is hard to grow and if germination is hastened [22], we can help this plant grow, that is why we chose it above other portions. Furthermore, their impact on the length of the roots and shoots as well as the quantity of photosynthetic pigments is physiologically significant since improved root growth would increase the plant's access to food and water resources in its surroundings. When competing with other plants, increased stem development will also result in improved light absorption. As previously said, pigments are the most crucial component for absorbing the light required for photosynthesis, which is regarded as an essential function for all plants.

2. MATERIALS AND METHODS

In this work, we looked into how TiO2 and NP-TiO2 affected Mentha piperita's photosynthetic pigments, root and shoot length, and seed germination. In the Laboratory Research of the Islamic Azad University of Falavarjan in Isfahan, Iran, an experiment was conducted.

2.1 Chemicals

Different concentrations of Titanium dioxide (TiO2, 99.99%, Merck) and Titanium dioxide nanoparticle (NP-TiO2, 99.5%, 10-10 A, Merck) were prepared by suspending 0, 100, 200 and 300 mg L-1 in double distilled water through ultrasonication (100W, 60 KHz) for 180 minutes.

2.2 Seeds

Seeds were collected from the Pakan Bazr institute, Isfahan, Iran, and were used in this study.

2.3 Seeds Germination

For 15 minutes, mentha seeds were submerged in a 2.5% sodium hypochlorite solution to sterilize them. Ten healthy, evenly sized seeds were chosen after two rounds of rinsing with double-distilled water. They were then planted at equal intervals in a 90 x 15 mm petri dish lined with filter paper (Whatman No.42, Ashless, England). Next, two milliliters of double-distilled water containing suspensions of TiO2 and NP-TiO2 was added. Control seeds were also collected for comparison with the treated seeds in each experiment. Additionally, the petri dish was filled with solely double-distilled water as a control. Since light inhibits germination, the petri dish was kept in a dark atmosphere within the culture chamber. As a result, we gave our seeds a dark environment, similar to that found beneath soil, and, once the germination process was complete, we placed them in light conditions and soil to continue growing normally.

The experiment involved irrigating the seeds with TiO_2 and NP-TiO_2 suspensions and counting the number of seeds that germinated each day (Fig.1). The associated formula was used to determine the germination percentage after 10 days of treatment. A ruler was also used to measure the length of the roots and shoots.

2.4 Measure of Pigments

At room temperature, 0.2g of newly tested leaves were ground in 80% acetone to extract the pigments. Using absorbents recorded at 647 nm, 663 nm, and 470 nm for maximal absorption of carotenoid, chlorophyll-a, and chlorophyll-b, respectively, the levels of carotenoids and chlorophyll were quantified. The Unico-UV2100 UV-Vis spectrophotometer was used to calculate the extinction coefficients. Characteristics of carotenoids (Car) and chlorophyll (Chl) were evaluated. Chl (a, b) and Car concentrations were determined using the procedure described in Lichtenthaler [23].

2.5 Statistical Analysis

This study was conducted using a complete randomized design with four replications as a factorial experiment. Standard error of the mean, or mean± SE, was used to display the data.

The SPSS18 program was used to analyze the data. All measured attributes' significant levels of difference were determined, and Duncan's multiple range tests were used to compare the means at the 5% level.

3. RESULTS AND DISCUSSION

Table 1 shows the effect of TiO_2 and NP-TiO_2 concentrations on seed germination, root length, shoot length and photosynthetic pigments.

3.1 Germination

Different concentrations of TiO_2 and NP-TiO_2 had no statistically significant influence on germination when compared to the control group, and the proportion of seeds that germinated dropped when exposed to these concentrations. We only displayed TiO_2 and NP-TiO_2 in Figure 2 and ignored them in the other figures since their concentration of 300 mg L^{-1} impeded germination. Refer to Figure 2. According to other research, NP-TiO_2's germination index steadily decreased as concentration rose. The detrimental impact on seed germination implies that the presence of TiO_2 and NP-TiO_2 probably caused stress to the seeds [24].

3.2 Root Length

The root length was reduced at all TiO_2 concentrations. Compared to the control, the impact of a 100 mg L^{-1} concentration of NP-TiO_2 on root length was substantially greater (Fig. 3). At appropriate quantities, NP-TiO_2 had a promotory influence on root length, but at excessive concentrations, it had an inhibitory effect [17]. Particle aggregation and root pore blockage caused by rising TiO_2 and NP-TiO_2 concentrations prevented seeds from absorbing water [14]. The antibacterial qualities of the anatase crystalline structure of TiO_2, which boost plant resilience to stress, may be the cause of the beneficial effect on root length [20].

3.3 Shoot Length

The graphs shown in Fig. 4 show the shoot length of Mentha piperita. The effect of 200 mg L^{-1} of TiO_2 on shoot length was identical to that of the control group. Shoot length was less affected by NP-TiO_2. The application of TiO_2 and NP-TiO_2, which has significantly reduced shoot biomass, may be the cause [3]. Chouychai et al. demonstrated the same findings [25].

3.4 Photosynthetic Pigments

3.4.1 Chlorophylls

The graphs in Figures 5 and 6 show the results of measuring the amount of chlorophyll in Mentha piperita leaves. The concentration of chlorophyll a and chlorophyll b was considerably elevated by the TiO_2 at the 200 mg L^{-1} and 100 mg L^{-1} NP-TiO_2 concentrations. According to other research, at an ideal concentration, NP-TiO_2 and TiO_2 can increase the rate of photosynthesis, chlorophyll production, and nitrogen metabolism. The findings indicated that the physiological effects were correlated with particle size [15], and that the complimentary effects of other natural nutrients, such as iron (Fe), magnesium (Mg), and sulfur (S), may be the cause of increased chlorophyll accumulation [17]. Improved chlorophyll structure, increased light absorbance, easier pigment formation, improved sunlight absorption, improved light energy transfer to active electrons, chemical activity, and an impact on photosynthesis are all possible with NP-TiO_2 [21]. [16,17,26] established the same findings.

3.4.2 Carotenoids

TiO_2 and NP-TiO_2 concentrations in 200 mg L^{-1} and 100 mg L-

1 had a significant stimulant effect on the amount of carotenoids in comparison with the control group Fig. 7.

TiO2 first reduced the effect on Chlamydomonas reinhardtii carotenoids, but later increased them greatly, according to other research that found NP-TiO2 significantly boosted Zea mays pigments such carotenoids [16,21]. When heavy metals quench ROS, the synthesis of carotenoid rises. The most crucial internal components are photosynthetic pigments. The presence of an oxidant may be the cause of decreased levels of carotenoids and chlorophyll [27]. Increased concentrations of harmful metals and metalloids can sometimes cause pigment loss [28].

4. CONCLUSION

In addition to being utilized as food, Mentha piperita L. is a medicinal plant. Analyzing the penetration and transport of nanoparticles in plants is crucial to comprehending the potential benefits of using them in the nutritional and pharmaceutical industries. The applied NP-TiO2 and TiO2 were evidently harmful to seed germination, according to the results of our investigations. Experimental treatments also had a detrimental effect on seed germination, while other research found that they had a greater effect [15,29]. Due to their ability to reduce shoot biomass, TiO2 and NP-TiO2 shortened the shoots [3]. These findings are significant for the relationship between material size and concentration and its effects on germination and other parameters, and they will aid in the understanding of the inhibitory effects of TiO2 and NP-TiO2.

Since plants are vital to human life, it appears necessary to investigate the effects of phytotoxicity mechanisms, such as the size distribution of nanoparticles and potential benefits of uptake and translocation of engineered nanoparticles by plants, even though this study shows the effects of TiO2 and NP-TiO2 in plant physiology.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Fig. 1. Germination of seeds (control)

Table 1. Calculated mean square values from the statistical analysis corresponding to data collected between TiO_2 and NP-TiO_2 of Mentha piperita

Mean square							
Sources of Variation		**%Germination**	**Root length**	**Shoot length**	**Chl a**	**Chl b**	**Carotenoid**
TiO_2 and Np-TiO_2(A)	1	22.781^{ns}	0.845**	0.217^{ns}	1.014E-02**	0.175**	4.623E-02**
Treatment(B)	3	1032.365*	8.850*	9.510*	8.005*	2.644*	0.356*
A*B	3	8.615^{ns}	1.549**	1.089**	3.062*	0.458*	0.152*
Error		97.365	0.189	0.349	1.675E-02	1.222E-02	3.099E-03

*Note: Ns: non-significant, * P < 0.01;*
*** P < 0.05*