

بررسی اثرات غلظت‌های مختلف ذرات نانو و غیر نانو دی اکسید تیتانیوم بر جوانه زنی بذر و رشد گیاهچه رازیانه

حسن فیضی^{۱*}، مریم کمالی^۲، پرویز رضوانی مقدم^۳، سعید جاهدی پور^۴، حسین صحابی^۱
 1- عضو هیئت علمی دانشکده کشاورزی و منابع طبیعی، دانشگاه تربت حیدریه. 2- دانشجوی دکتری باغبانی، دانشکده کشاورزی دانشگاه فردوسی مشهد. 3- استاد گروه زراعت و اصلاح نباتات دانشکده کشاورزی، دانشگاه فردوسی مشهد. 4- دانشجوی دکتری بوم‌شناسی زراعی (اگرواکولوژی)، دانشکده کشاورزی دانشگاه فردوسی مشهد و مدرس دانشگاه پیام نور مشهد.

Email: hasanfeizi@yahoo.com

چکیده:

هدف از آزمایش حاضر بررسی غلظت‌های 0، 20، 40، 60 و 80 میلی گرم بر لیتر ذرات نانو و غیر نانو دی اکسید تیتانیوم بر جوانه زنی و رشد گیاهچه رازیانه بود. آزمایش به صورت طرح کاملاً تصادفی در آزمایشگاه دانشگاه فردوسی مشهد انجام شد. پس از 14 روز، درصد جوانه زنی بذرها به صورت معنی داری در تیمار کاربرد غلظت 60 میلی گرم بر لیتر ذرات نانو دی اکسید تیتانیوم (76 درصد) نسبت به سایر تیمارها و شاهد (54 درصد) افزایش یافت. این افزایش در وزن خشک ساقه و سرعت جوانه زنی بذرها نیز مشاهده شد. کاربرد ذرات غیر نانو وزن خشک ساقه را تا 50 درصد نسبت به شاهد کاهش داد. متوسط زمان جوانه زنی در اثر کاربرد نانو ذرات با غلظت 40 میلی گرم بر لیتر نسبت به شاهد حدود 31 درصد بهبود یافت در صورتی که ذرات غیر نانو متوسط زمان جوانه زنی را فقط 21 درصد بهبود داد. همچنین کاربرد ذرات نانو با غلظت کم و متوسط باعث بهبود شاخصه‌هایی نظیر ارزش جوانه زنی، شاخص بنيه و میانگین جوانه زنی روزانه شد. به طور کلی کاربرد نانو ذرات دی اکسید تیتانیوم به عنوان یک گزینه جهت بذرهایی که دارای مشکل جوانه زنی هستند و درصد جوانه زنی کمی دارند می‌تواند مورد استفاده قرار گیرد. کلمات کلیدی: نانو ذرات، گیاهان دارویی، جوانه زنی بذر.

**Effect of bulk and nano Titanium dioxide particle concentrations on fennel
(*Foeniculum vulgare* Mill)**

Hassan Feizi^{۱*}, Maryam Kamali^۲, Parviz Rezvani Moghaddam^۳, Saeed Jahedi pour^۴, Hossein Sahabi^۱

^۱Member of Scientific Board, Faculty of Agriculture and Natural Resources, University of Torbat-e- Heydarieh Torbat Heydarieh, IRAN.

^۲Ph.D. student, Horticulture Sciences Department, Faculty of Agriculture, Ferdowsi University of Mashhad, Mashhad, IRAN.

^۳Ph.D. Wales Professor, Faculty of Agriculture, Ferdowsi University of Mashhad, Mashhad, IRAN.

^۴Ph.D Student in Agroecology, Faculty of Agriculture, Ferdowsi University of Mashhad & Educator of Payame Noor University of Mashhad, Mashhad, IRAN.

E-mail: hasanfeizi@yahoo.com

Abstract:

The aim of this study was to compare concentrations of nanosized TiO₂ at 0, 5, 20, 40, 60 and 80 mg L⁻¹ with bulk TiO₂ for phytotoxic and stimulatory effects on fennel seed germination and early growth stage. After 14 days of seed incubation, germination percentage highly improved following exposure to 60 ppm nanosized TiO₂. Similar positive effects occurred in terms of shoot dry weight and germination rate. Application of bulk TiO₂ particles in 40 ppm concentration greatly decreased shoot biomass up to 50% compared to the control. Application of 40 ppm nanosized TiO₂ treatment improved mean germination time by 31.8% in comparison to the untreated control. In addition, low and intermediate concentrations of nanosized TiO₂ enhanced indices such as germination value, vigor index and mean daily germination. In general, there was a considerable response by fennel seed to nanosized TiO₂ presenting the possibility of a new approach to overcome problems with seed germination in some plant species, particularly medicinal plants.

Keywords: Nanosized TiO₂, Phytotoxicity, Medicinal plant, Seed improvement.

Introduction:

Nanotechnology has many applications in agricultural research, such as in reproductive science and technology, the transfer of agricultural and food waste to energy and other helpful by-products through enzymatic nanobioprocessing, disease prevention and various other plant treatments using nanocides (Carmen et al. 2003). An important effect of titanium compounds on plants used for various crops is improvement of yield (about 10-20%). Other effects of titanium on plants are increased contents of some essential elements in plant tissue; an

increase in enzyme activity such as peroxidase, catalase, and nitrate reductase activities in plant tissue and research has shown increased chlorophyll in paprika (*Capsicum anuum* L.) and green alga (*Chlorella pyrenoidosa*) (Hruby et al. ۲۰۰۲). Nanosized TiO₂ is a frequently used nanoparticle, consequently there has been an exponential increase in data collection on the effects of TiO₂ nanoparticles on different species but there is much less information on the effects of nanoparticles on plants compared to animals. Studies of effects of TiO₂ nanoparticles on plants provide information about the positive and stimulating effects as well as any negative impact (Klancnik et al. ۲۰۱۱).

Foeniculum vulgare commonly known as fennel is a well known and important medicinal and aromatic plant widely used to treat carminative, digestive, lactagogue, diuretic, respiratory and gastrointestinal disorders (Manzoor et al. ۲۰۱۲). Efficient seed germination and early seedling establishment are important processes in commercial agriculture. Rapid and uniform seedling emergence leads to successful plant establishment, as a deep root system is formed before the upper layers of the soil dry out, harden, or reach supra-optimal temperatures (Chen and Arora, ۲۰۱۲). This study was therefore done to investigate possible phytotoxicity and/or beneficial stimulatory effects of nanosized TiO₂ concentrations compared to bulk TiO₂ particles on fennel seed and seedling growth.

Materials and Methods:

Tests were done to assess the effect of different concentrations of bulk and nanosized TiO₂ on fennel seed germination in a completely randomized design with four replications. The treatments in the experiment were five concentrations (۰, ۲۰, ۴۰, ۶۰ and ۸۰ ppm) of bulk and five concentrations (۰, ۲۰, ۴۰, ۶۰ and ۸۰ ppm) of nanosized TiO₂ and an untreated control (without any TiO₂ types). The Experiment was performed in a germinator with an average temperature of ۲۰ ± ۱°C at the College of Agriculture, Ferdowsi University of Mashhad, Iran in ۲۰۱۲. Seeds of similar size were randomly selected and sterilized using ClONa (۰%) for ۳ minutes and then carefully washed with distilled water three times. In order to obtain properly dispersed and stable TiO₂ suspensions of each concentration, an ultra-sonication treatment was applied to bulk and nanoparticles TiO₂ powders dispersed in water for ۱۰ minutes. The seeds were placed on paper in four groups of ۲۰ seeds in Petri dishes, and after that ۳ ml of each concentration treatments was added to each. For the control, only distilled water was added to the Petri dishes. Germination tests were performed according to the rule issued by the International Seed Testing Association (ISTA, ۲۰۰۹). All concentrations of TiO₂ and the control were tested at the same time to ensure uniform conditions of light and temperature across all tests. Number of germinated seeds was noted daily for ۱۴ days. Seeds were considered germinated when the radicle showed at least ۲ mm in length (ISTA, ۲۰۰۹).

A one-way analysis of variance (ANOVA) was performed between treatment samples in a completely randomized design in four replications. Data were analyzed using MSTAT-C software. Significant levels of difference for all measured traits were calculated and means were compared by the multiple range Duncan test at ۰% level.

Results and Discussion:

Results demonstrated that treatments applied in this experiment had significant affects on most studied traits. Use of TiO₂ nanoparticles extraordinarily enhanced fennel seed germination, while seed germination percentages decreased from exposure to concentrations of bulk TiO₂ particles compared to the control group (Table ۱). Final seed germination percentage showed the most value (۷۶%) in ۶۰ ppm TiO₂ nanoparticles, whereas the lowest value (۴۱%) was in ۶۰ ppm bulk TiO₂ particles. However, fennel seeds exposed to TiO₂ nanoparticles improved germination percentage ۳۹.۰% compared to the unexposed control group and was ۸۳% more than those concentrations of bulk particles. The key reason for this increased growth rate could have been the photo-sterilization and photo-generation of “active oxygen like superoxide and hydroxide anions” by nano-TiO₂ that enhanced seed stress resistance and encouraged capsule penetration for intakes of water and oxygen needed for quick germination (Khot et al. ۲۰۱۲).

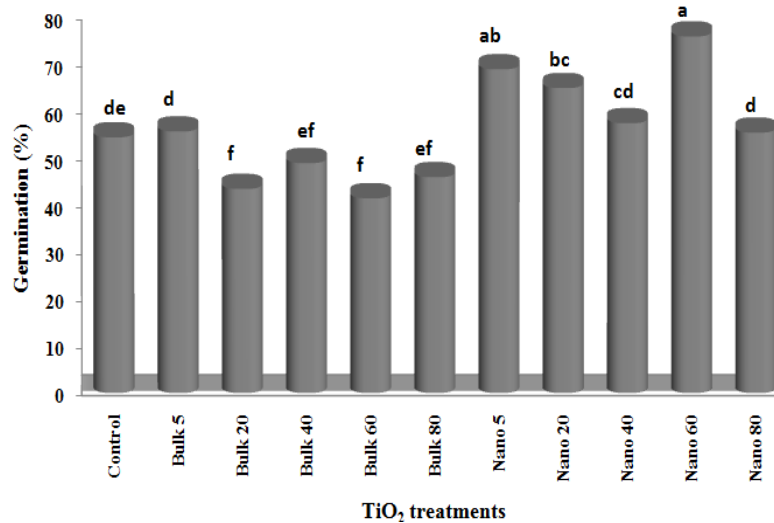


Figure 1. Effect of bulk and nano TiO₂ concentrations on germination percentage of fennel seed.

Shoot, root and seedling elongation were not significantly affected by bulk and nanosized TiO₂ concentrations. Application of bulk TiO₂ particles in 40 ppm concentration greatly decreased shoot biomass up to 50% compared to the control seeds but at the concentration of 40 ppm nano-TiO₂ did not demonstrate such reduction in shoot biomass (Table 1). The greatest shoot biomass was found in 20 ppm bulk particles (1.11 mg) and 40 ppm nanoparticles (1.16 mg) of titanium dioxide. The highest root biomass was achieved from concentrations of 20 ppm bulk-TiO₂, 20 and 40 ppm nano-TiO₂ but an increased concentration of bulk particles of 40 ppm significantly reduced root weight. It is probable that increasing the concentration of bulk-TiO₂ induced aggregation of particles and resulted in clogging of root pores that interrupted water uptake by seeds. In addition, application of 40 ppm concentration of bulk and nanosized TiO₂ decreased fennel seedling biomass. It seems that nano TiO₂ could stimulate process of germination similar to water and oxygen uptake by seed result in improves seed germination percentage but in later growth stages of seedling it might be has different effect on plant.

Table 1. Influence of bulk and nanosized TiO₂ concentrations on seed germination, elongation and biomass of fennel seedling.

TiO ₂ concentration (ppm)	Shoot length (cm)	Root length (cm)	Seedling length (cm)	Shoot dry weight (mg)	Root dry weight (mg)	Seedling weight (mg)	
Control	4.06a	5.81a	9.87a	1.07ab	0.38b	1.46ab	
Bulk TiO ₂	5	3.76a	5.25a	9.02a	1.18a	0.36bc	1.50a
	20	4.12a	6.21a	10.34a	1.12ab	0.54a	1.66a
	40	4.01a	5.51a	9.52a	0.53d	0.32bcd	0.85d
	60	3.89a	7.00a	10.89a	0.99ab	0.34bcd	1.33abc
	80	3.74a	6.25a	9.99a	0.95abc	0.2d	1.10bcd
	Nano TiO ₂	5	3.79a	5.55a	9.34a	1.01ab	0.4ab
20		4.38a	5.93a	10.31a	0.8bcd	0.4ab	1.21bc
40		4.05a	6.65a	10.71a	0.64cd	0.22cd	0.86d
60		4.11a	5.93a	10.05a	0.81bcd	0.27bcd	1.09cd
80		3.87a	7.39a	11.27a	1.16a	0.29bcd	1.45ab

*Means, in each column, followed by similar letter are not significantly different at the 5% probability level using Duncan's Multiple Range Test.

In general, lower mean germination time represents earlier germination. These results revealed that exposure of fennel seeds to 40 ppm nanosized TiO₂ obtained the lowest mean germination time (3.99 days) but higher concentrations did not improve mean germination time. Thus, 40 ppm concentration of nanosized TiO₂ treatment reduced mean germination time by 31.8% in comparison to the untreated control, whereas 40 ppm concentration of bulk TiO₂ contributed to a reduction of mean germination time of about 21% in comparison with the control (Table 2). Zheng et al. (2005) stated that the considerable effect of nanosized TiO₂ on spinach germination in tests was probably because of small particle size, which allowed nanoparticles to penetrate the seed during the treatment period, exerting its enhancing functions during growth.

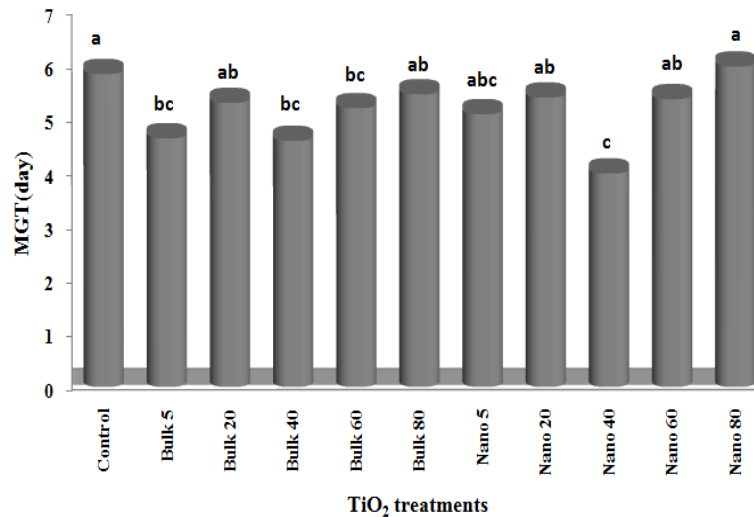


Figure 2. Effect of bulk and nano TiO₂ concentrations on mean germination time of fennel seed.

The highest germination rate was found in 0 ppm nano-TiO₂ particles (6.39 seed day⁻¹) and increasing concentration decreased the germination rate. 60 ppm bulk-TiO₂ treatment showed the lowest germination rate (2.49 seed day⁻¹). All of bulk TiO₂ particle treatments inhibited germination rates compared to the control.

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