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Effect of nano titanium dioxide spraying on essential oil yield and traits of cumin (*Cuminum cyminum* L.)

Elham Morteza[1], Tayebeh Morteza[2*], Hadi Saemi[3], Ali Joorabloo[4]

[1]*Department of Agronomy and Plant Breeding, Aburaihan Campus, University of Tehran, Iran*

[2]*Department of Plant Protection, Islamic Azad University of Damghan, Damghan, Iran*

[3]*Department of Horticulture, Guilan University, Rasht, Iran*

[4]*Department of Agronomy, Agriculture Jahad Office of Garmsar, Garmsar, Iran*

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Key words: Nano titanium dioxide, Cumin, 1000 grain weight, Essential oil yield.

Abstract

An experiment was conducted to study the effects of nano titanium dioxide on essential oil yield and other traits of cumin (*Cuminum cyminum* L.). Factorial experiment based on a complete randomized block design with four replications was done, in the years of 2013 and 2014, in Garmsar- Iran. Treatments of various concentrations of titanium dioxide nanoparticle (0, 0.02%, 0.04% and 0.06%) and times of spraying of this nano particle (vegetative and reproductive stages) were applied. Traits of height, 1000 grain weight, grain yield, essential oil percentage and essential oil yield, were analysed using the SAS software. Results showed that the effect of concentrations of titanium dioxide nanoparticle was significant on all traits with the exception of essential oil percentage trati. But the effect of times of spraying of this nano particle, was significant only on trait of height. So that the highest amount of height, was obtained at vegetative stage. Also according to the results, concentration of 0.06% nano-TiO_2, in comparison with the other examined concentrations of this nanoparticle and control or no application of it, was the most positive and effective treatment. Therefore, in this experiment, application of nano-TiO2, especially at concentration of 0.06%, was effective for gain the highest essential oil yield of cumin.

***Corresponding Author:** Tayebeh Morteza ✉ tayebehmorteza@yahoo.com

Introduction

Medicinal plants are used to cure many ailments that are either non curable or seldomly cured through modern systems of medicine. Approximately 80% of the world population depends on medicinal plants for their health and healing (Aliyu, 2003). Cumin (*Cuminum cyminum* L.) is an aromatic plant included in the Apiaceae family and is used to flavor foods, added to fragrances, and for medicinal preparations (Iacobellis *et al.*, 2005). Historically, Iran has been the principal supplier of cumin, but currently the major sources are India, Sri Lanka, Syria, Pakistan, and Turkey (Farzami Sepehret *et al.*, 2012). Nanotechnology as the latest innovation has the potential to bring about changes as big as the European industrial revolution in the late 18^{th} and early 19^{th} century (Miller and Senjan, 2006; Hosseini*et al.*, 2011). Titania (TiO_2) nanoparticles (TNs) have become one of the most important substances in nanotechnology. Many studies have been conducted into the properties of various TNs in solutions to estimate their behaviour. The effect of size, pH, ionic strength, crystal phase and presence of proteins and other biomolecules on agglomeration, aggregation, hydrodynamic size and the behaviour of nanoparticles in solution have been thoroughly investigated. Lately interests and ideas have emerged to apply TNs in agricultural practices from pesticide formulations to growth stimulators (Palmqvist *et al.*, 2015).

According to the finding of Hong *et al.* (2005a,b) and Yang *et al.* (2006), nano-anatase TiO_2 have a photocatalyzed characteristic and improves the light absorbance and the transformation from light energy to electrical and chemical energy, and also induces carbon dioxide assimilation. TiO_2NPs protect chloroplast from aging for long time illumination. The nano-TiO_2 treatments also have obvious effects on the improvement of growth and development in spinach. Moreover, nano-anatase TiO_2 enhances the photosynthetic carbon assimilation by activating rubisco (complex of rubisco and rubiscoactivase) that could promote rubisco carboxylation, thereby increasing growth of plants (Gao *et al.*, 2006). Previous studies showed that the activities of nitrate reductase, glutamate dehydrogenase, glutamine synthase, and glutamic-pyruvic transaminase were obviously increased by nano-TiO_2 treatment. In addition, it should be noted that, nano-TiO_2 treatment could promote plant to absorb nitrate, accelerate inorganic nitrogen to be transformed into organic nitrogen (such as protein and chlorophyll), and enhance plant yield)Yang *et al.*, 2007(. Based on research finding, the nitrogen content of plant leaves affects their photosynthetic capacity, so that it has a generally positive correlation with plant photosynthesis. Nanoparticles can transport DNA and chemicals into plant cells after interance into plants cells and leaves. Therefore, it is important in plant biotechnology to explore of target specific gene's manipulation and expression in the specific cells of the plants (Galbraith, 2007; Torney *et al.*, 2007; Manzer *et al.*, 2015). The researches on improving photosynthesis of spinach suggested that, nanoanatase TiO_2 could increase light absorbance, accelerate transport and transformation of the light energy, protect chloroplasts (Chl) from aging, and prolong the photosynthetic time of Chl (Lei *et al.*, 2007).

Many researchers showed that efficacy of NPs depends on their concentration and varies from plants to plants. So that Zhang *et al.* (2005) reported that, the positive effects of nano-TiO_2 on the germination and growth of naturally aged seeds of *Spinacia oleracea* at 0.25-4% nano-TiO_2 treatments in comparison with the control treatment. Also finding of Castiglione and Cremonini (2009) experiment showed an increasing in the plant dry weight, the chlorophyll formation, the ribulose bisphosphate carboxylase/oxygenase activity and the photosynthetic rate during the growth stage. As well nano-TiO_2 can increases antioxidant stress by decreasing the accumulation of superoxide radicals, hydrogen peroxide, MDA and increasing antioxidant enzyme activity, which increases the evolution oxygen rate in spinach chloroplasts under stress (Lei *et al.*, 2008). Giraldo *et al.* (2014) proposed that nanoparticles can harvest more light energy into chloroplast beacause they allow chloroplast to capture wavelengths of light which is not in their normal range (such as ultraviolet, green, and near-infrared). They emphasized, anatase nano-TiO_2, has a large specific surface area, high thermal conductivity, and high photocatalytic ability that can inhibit the growth of bacteria, fungi, and algae. Significantly reduction leaf lesion areas and lower incidence rates, as well as promotion of chlorophyll and carotene syntheses by spraying of cucumber leaves with a 0.35% nano-TiO_2 semi-conductor sol was shown in one study. Also spraying with nano-anatase TiO_2 increased photosynthesis in cucumber, which in turn increases root growth (Zhang *et al.*, 2008a,b). Owolade *et al.*, (2008) as well stated that effects of nano TiO_2 spraying on evaluations for traits of grain number per pod, 1000 grain weight, grain yield, leaf area, pod number per plant and pod length of *Vina unguiculata*, were significant and evaluations of these traits significantly increased compared to the control. Thus, according to the positive effects of nano-TiO_2 on acceleration of plants growth and importance of cumin as one medicinal plant, the present study examined effects of nano-TiO_2 foliar spraying on the cumin.

Materials and method

Preparation of materials and cultivation of cumin

In order to study the effect of nano titanium dioxide on essential oil yield and other traits of cumin (*Cuminumcyminum* L.), this study was carried out at one farm in Garmsar, Iran during the winter and spring seasons of 2013-2014. Soil in the test was sandy loam. A field experiment was conducted as a factorial experiment based on a completely randomized block design with four replications. Treatments used in this experiment, consisted of various concentrations of titanium dioxide nanoparticle (0, 0.02%, 0.04% and 0.06%) and times of spraying of this nanoparticle (vegetative and reproductive stages). Nano-TiO_2 with average particle size of of 44.17 nm was supplied by chemical synthesis (Plasma Chem, Germany). The size of the TiO_2 nanoparticles were determined by scanning electron microscopy (SEM) at the Rezaei laboratory at Tehran, Iran (Fig. 1).

Seeds were planted on 15 November 2013 in 20 cm row distance, 1.5 cm sowing depth in 2 ×2 m^2 plots. All agricultural practices were performed in the same manner, as it is usually done in the cumin production areas. Titanium dioxide were sprayed by a portable spray machine on the cumin plants according to the treatments of nano-TiO_2 concentrations, at vegetative and reproductive stages of plant. In this test characteristics of height, 1000 grain weight, grain yield, essential oil percentage and essential oil yield, was evaluated. Plant height was measured at time of 50% flowering stage. Plants of 1 m^2 in each plot were harvested on 15 May 2014 at the end of growth season and after harvesting, branches were dried in the shade and 1000 grain weight and grain yield was measured using a carriage scale using standard moisture at 14%. Cumin grain s were ground in a laboratory dry grinder and 50 g of powder was subjected to hydro-distillation. Essential oil of seed was prepared and extracted by hydrodistillation of ground cumin using a Clevenger distillation. The plant materials were steam-distilled for 90 min in the full glass apparatus. The extraction was carried out for 2 h after 4 h maceration in 620 ml of water. The essential oils were stored in dark glass bottles in a freezer (Sowbhagya *et al.*, 2008). Finally, evaluation for the essential oil yield trait was determined by the following formula;

Essential oil yield= Essential oil percentage × Seed yield.

Statistics analysis

After normalization test, data were subjected to analysis of variance (ANOVA) using Statistical Analysis System (SAS Institute) and followed by Duncan's multiple range tests. Terms were considered significant at $P \leq 0.05$.

Results

Height

The simple effects of spraying times and concentrations of nano TiO_2 treatments on the height amount, were significant at $p \leq 0.05$ (Table 1).

Table 1. Analysis of variance results of the cumin traits under different concentrations of nano-TiO_2 spraying in two stages of plant growth.

		Mean squares				
Sources of variation	df	Height	1000 grain weight	Essential oil percentage	Grain yield	Essential oil yield
Replication	3	37.22**	0.680**	0.1714**	16464.46**	11.30**
Times of spraying (Stage of plant)	1	24.97*	0.008ns	0.0008ns	1548.18ns	0.76ns
Concentrations of nano-TiO_2	3	11.13*	0.335**	0.0053ns	13965.73**	7.60**
Times×Concentrations	3	1.52ns	0.032ns	0.0091ns	342.69ns	0.22ns
Error	21	3.14	0.034	0.0077	645.85	0.45

* and **: Significant at 5 and 1% levels respectively.

Moreover, the interaction effects of this nanoparticle concentrations and spraying times, were not significant on this trait.

According to the results of the Duncan'means comparison (Fig. 2), the highest amount of height (23.4cm) was achieved by the spraying of nano-TiO_2 at vegetative stage while the minimum amount of this trait (21.63 cm) was achieved by spraying of this nanoparticle at reproductive stage.

The results of Fig. 3 showed that the maximum measure of this attribute (23.75 and 23.18 cm respectively) was related to the application of 0.06% and 0.04% of nano-TiO2 respectively, and the minimum amount of this trait 21.10 cm) was achieved by the no-application of this nanoparticle. While the use of concentration of 0.02% with the height amount of 21.1 cm, was placed between maximum and minimum treatments.

1000 grain weight

The results of analysis of variance (Table 1) showed that, the simple effect of nano-TiO_2 concentrations treatment on the weight of 1000 grain, was significant at the level of one percent ($p \leq 0.01$), while the effects of spraying times' treatment and interaction of these treatments, were not significant.

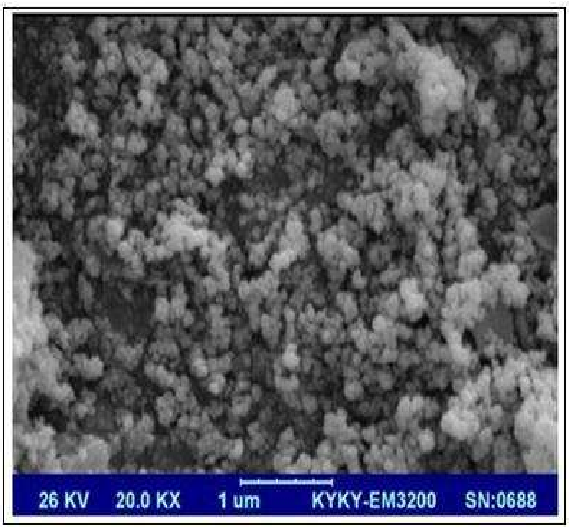

Fig. 1. Image of nano-TiO_2 by scanning electron microscopy (SEM).

As the results of the Duncan' means comparison (Fig. 4) shows, clearly that treatment of 0.06% nano titanium with the amount of 2.52 g, had the highest weight of 1000 grain and this treatment had no significant difference with treatment of 0.04% nano-TiO_2 spraying with the amount of 2.36 g and were placed in the superior group. While treatments of no-application of nano-TiO_2 with the amount of 2.05 g, had the least weight of 1000 grain and whereas this treatment, had no significant difference with the concentration of 0.02% nano titanium with the 1000 grain weight amount of 2.2 g.

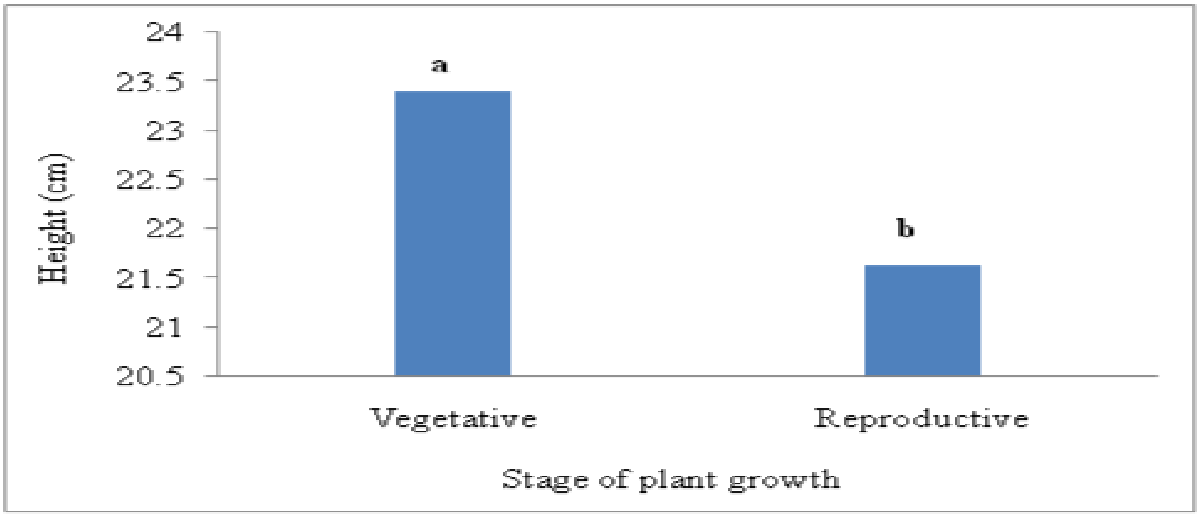

Fig. 2. Effect of times of nano-TiO_2 spraying on height of cumin.

Grain yield

According to the results of analysis of variance (Table 1), the main effect of nano-TiO_2 concentrations treatment on the grain yield trait, was significant at the level of $P \leq 0.01$.

The results of means comparison (Fig 5) showed that, use of 0.06% concentration of nano-TiO_2, had the highest grain yield (463.01 $kg.ha^{-1}$), while the treatment of no-use of this nanoparticle (control) had the minimum (364.17 $kg.ha^{-1}$) grain yield.

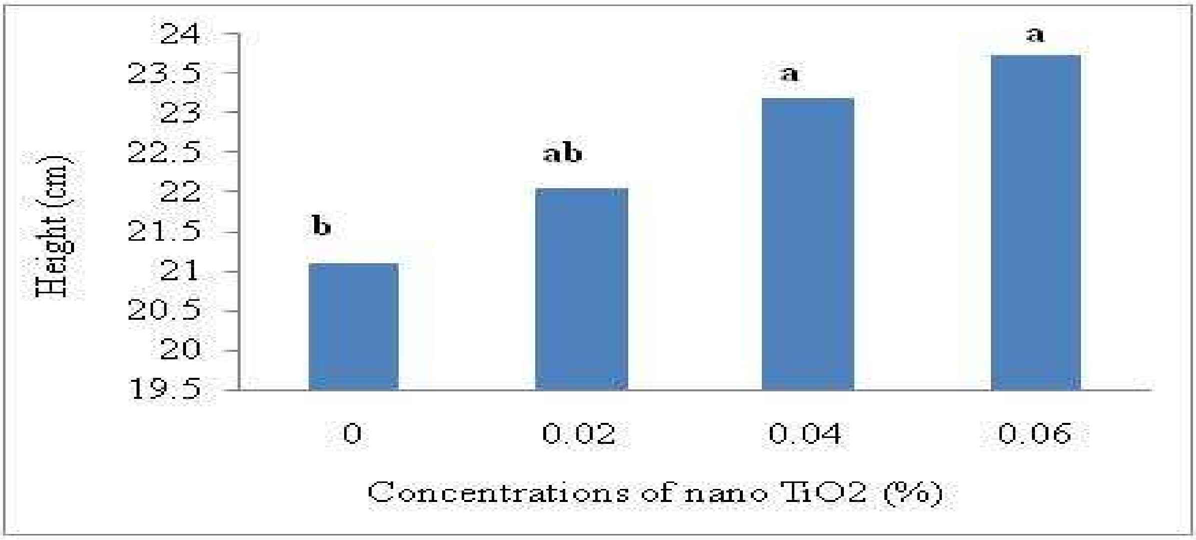

Fig. 3. Effect of nano-TiO_2 concentrations on height of cumin.

Essential oil yield

According to the results of analysis of variance (Table 1), the simple effect of nano-TiO_2 concentrations treatment on the essential oil yield, was significant ($p \leq 0.01$), whereas the effects of other treatments on this trait, were not significant.

The results for the means comparison of Duncan, (Fig. 6), demonstrated that the highest level of essential oil yield (9.84 $kg.ha^{-1}$) was obtained by the application of 0.06% of nano-TiO_2 that this treatment had no significant difference with the use of concentration of 0.04% with essential oil yield of 9.14 $kg.ha^{-1}$. While the minimum amount of this trait (7.51 $kg.ha^{-1}$) was achieved by no application of nano-TiO_2.

Discussion

In this experiment, spraying of cumin plant with nano-TiO_2, in comparison with the control treatment, caused an increasing in amounts of all traits (1000 grain weight, essential oil yield height, grain yield). Especially for the measured traits, a positive effect of this nanoparticle was very tangible at a concentration of 0.06%. Consistent with these results, the results of Castiglione and Cremonini (2009), showed that the application of this nanoparticle, increased growth and yield of plant. It should be emphasized, with increasing of nano-TiO_2 concentration, growth of cumin, increased. Therefore, stimulation of the plant growth by this nanoparticle because of nanometric size of nano-TiO_2, will be correlated with the concentration of nanoparticles (Lin and Xing, 2007).

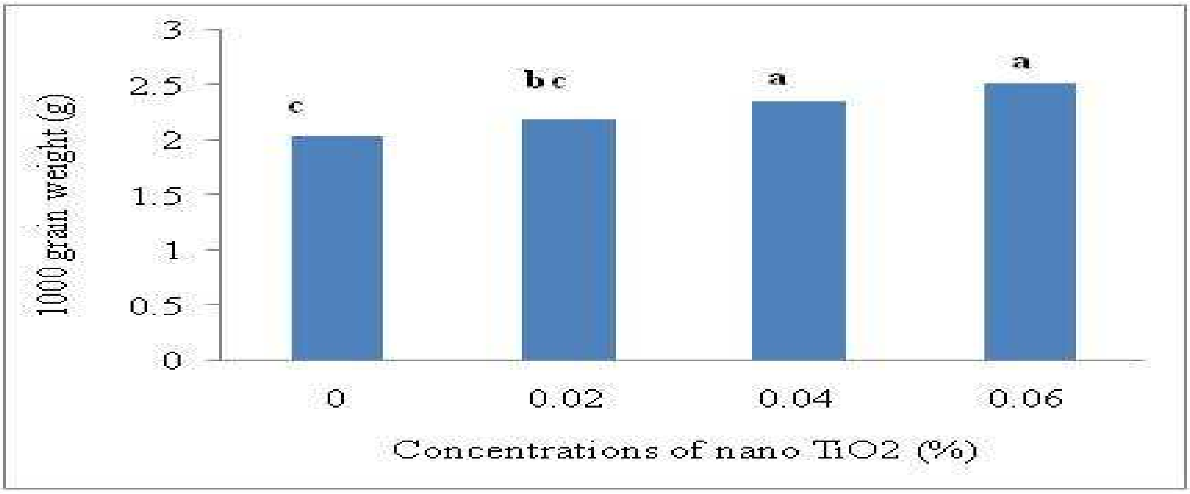

Fig. 4. Effect of nano-TiO_2 concentrations on 1000 grain weight of cumin.

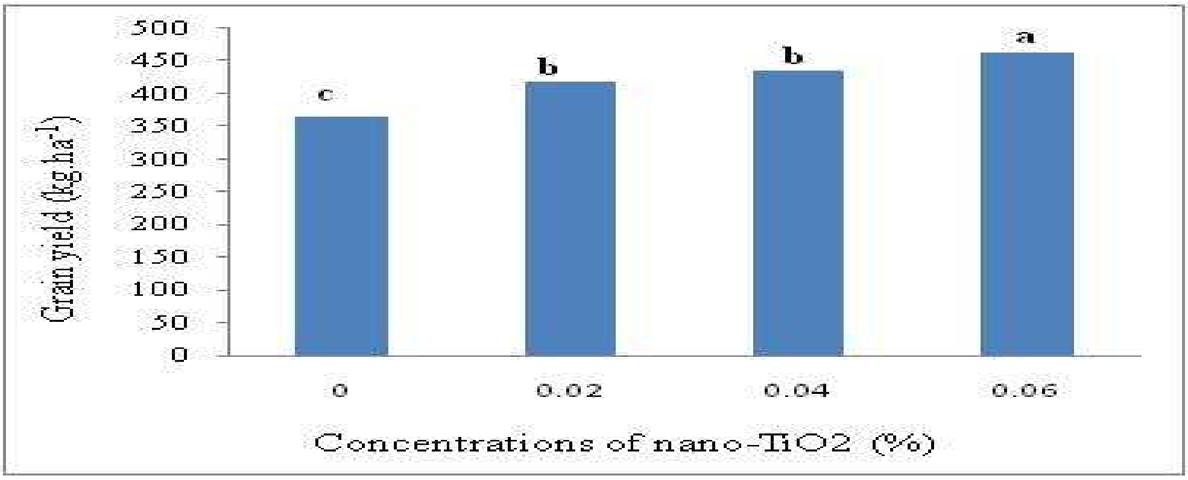

Fig. 5. Effect of nano-TiO_2 concentrations on grain yield of cumin.

According to the previous research, in this experiment, increasing of growth and yield of cumin by nano-TiO_2, are because of photocatalytic properties and improvement the light absorbance and the transformation from light energy to electrical and chemical energy, and also induction of carbon dioxide assimilation, protectection of chloroplast from aging for long time illumination of nano-TiO_2. Therefore, the nano-TiO_2 treatments have obvious effects on the improvement of growth and development (Hong *et al.*, 2005a; Yang *et al.*, 2006), as well, according to the results of Gao *et al.* (2006), nano-anatase-TiO_2 by enhancing the photosynthetic carbon assimilation by activation of rubisco (complex of rubisco and rubisco activase), could promote rubisco carboxylation, thereby increased growth of plants. Furthermore, based on Cossins (2014) and Giraldo *et al.* (2014) finding, anatase nano-TiO_2 has a large specific surface area, high thermal conductivity, and high photocatalytic ability that can inhibit the growth of bacteria, fungi, and algae. Significantly, reduction of leaf lesion areas and lower incidence rates, as well as promotion of chlorophyll and carotene syntheses are other the reasons of yield increasing by nano-TiO_2. In gneral, these results are in accordance with those reported by Owolade *et al.*, (2008).

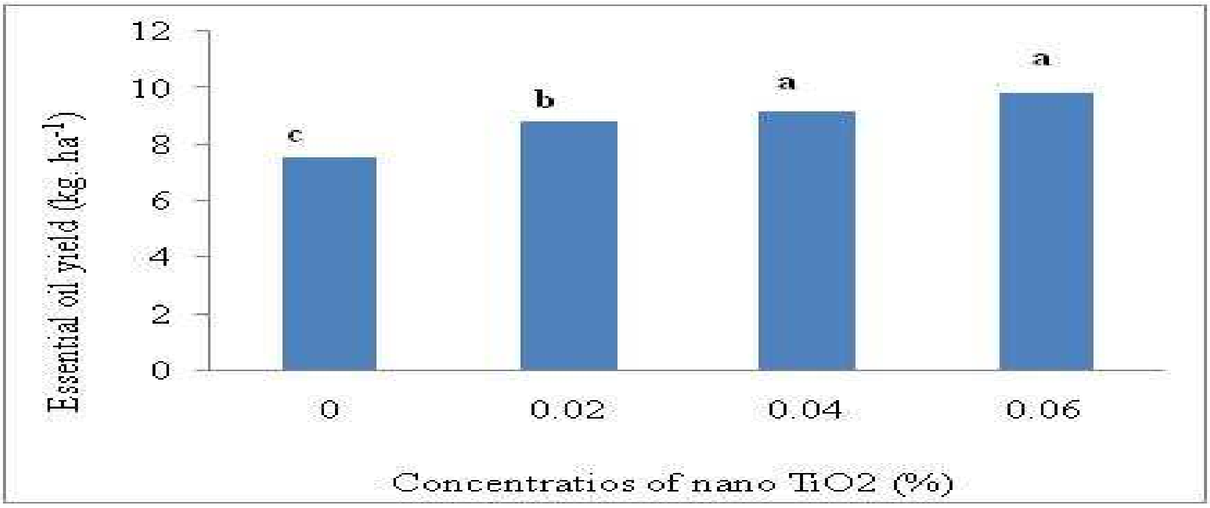

Fig. 6. Effect of nano-TiO_2 concentrations on essential oil yield of cumin.

Conclusion

Results for this study showed that nano-TiO_2 spraying of cumin, led to increasing of the 1000 grain weight, essential oil yield height, grain yield and essential oil percentage traits of this plant. Given that cumin is a very important medicinal plant that seed and its essential oil is widely used in the pharmaceutical industry and human food, therefore these findings perhaps aid commercial farmers of cumin plant and agricultural researchers in the application of nanoTiO_2.

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