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EFFECTS OF BULK & NANO-TITANIUM DIOXIDE AND ZINC OXIDE ON PHYSIO-MORPHOLOGICAL CHANGES IN *TRITICUM AESTIVUM* LINN

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Abstract

Comparative toxic effects of bulk and nano-Titanium dioxide (TiO_2) and Zinc oxide (ZnO) on seed germination, shoot - root growth, mitotic cell division, photosynthetic pigments and total protein content in *Triticum aestivum* (Wheat) were investigated. Both bulk and nano-TiO_2 & ZnO have no adverse effects on seed germination, shoot-root growth and cell division. But significant increases of chlorophyll and protein content were observed in nano-ZnO treated sample and no changes were observed in bulk-ZnO and bulk & nano-TiO_2 treated samples.

Key words: Phytotoxicity, Nanoparticle, Titanium dioxide, Zinc oxide, *Triticum aestivum*, Nanotoxicology.

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INTRODUCTION

The development of nanotechnology has introduced significant amounts of manufactured nanoparticles (NPs) into the environment, including those in the ambient air and water. In order to protect human health and wildlife from the potential adverse effects of a broad range of NPs, an increasing number of studies have focused on the assessment of the toxicity of the NPs [1].

NPs are atomic or molecular aggregates with at least one dimension between 1 to 100 nm [2, 3], that can drastically modify their physico-chemical properties compared to the bulk material [4]. It is worth noting that NPs can be made from a fully variety of bulk materials and that they can explicate their actions depending on both the chemical composition and on the size and or shape of the particles [5].

There is now an extensive debate about the risks and benefits of the many NPs into the environment [6] and in order to evaluate their potential adverse effects on the ecosystems and on human health, the scientific community is working with increasing attention to this topic. The literatures on the ecotoxicity of NPs or nano-materials as well as the chemistry of both manufactured and natural are summarized in recent studies [7,8]. Because of their widespread use in consumer products it is expected that NPs will find their way into aquatic, terrestrial and atmosphere environments, where their fate and behavior are largely unknown. Therefore organisms and especially those that interact strongly with their immediate environments are expected to be affected as a result to their exposition to NPs. Navarro et al. [9] underlined three topics 1) sources, transformation and fate of NPs; 2) biotransformation that engineered NPs can experience in contact with algae, fungi and plants and then the enhance and fate of these organisms; 3) the mechanism of engineered NPs toxicity and their effect on organism and how these toxic effects might be transferred through food chains, thus affecting communities and whole ecosystems. Even as there has been an increasing amount of research on the toxicity of NPs to animal kingdom and bacteria, limited studies are available in higher plants.

Therefore a necessity arises to study the phytotoxicity that is the degree of toxic effect by a compound on higher plants. Hence the present study has been carried out to evaluate the comparative effect of both bulk and nano-TiO_2 and ZnO on physio-morphological changes in common wheat (*Triticum aestivum* L.).

MATERIALS AND METHODS

TEST CHEMICAL

Nano-TiO_2 and nano-ZnO were purchased from Nanoshel, Intelligent Materials Pvt. Ltd. Haryana, India. Bulk TiO_2 was purchased from Ranbaxy Laboratories Ltd., India and the Bulk ZnO oxide were purchased from RFCL, Ltd.

PROPERTIES OF THE NANO- TIO_2 (NS1102>) AND NANO-ZNO (ZN01)

NANO-TIO_2 (NS1102>)

Weight – min 93 %, Alumina – Yes, Amorphous silica – Yes, Specific gravity – 4.0, Bulking valueL/Kg (gal/lb) – 0.25 (0.03), Organic treatment – Yes, Color CIE L* - 99.6, Median particle size – 40-60 mm, Oil absorption – 16.2, pH – 7.9, Resistant at 30°C (86°F) (1,000 ohm) – 8.1, Carbon black undertone – 11.7

NANO-ZNO (NANO-ZN01)

Appearance – white or pale yellow powder, Purity – 99.7%, Grain size – 20-50nm, Specific surface area (m2/g) - >90, Loss on drying - <0.3%, Loss on burning - <0.2%, Pb - ≤0.037%, Mn - ≤0.0001%, Cu - ≤0.0002%.

PREPARATION OF TEST SOLUTION

The bulk and NPs were suspended directly in distilled water and dispersed by ultrasonic vibration. For the present study four concentrations viz. 250, 500, 1000 and 2000 mg/L of both bulk and NPs were used and for all experiments freshly prepared solutions were used.

TEST SYSTEM

Commercially available seeds of common wheat -*Triticum aestivum* Linn. (syn; *Triticum vulgare* Vill.) [$2n = 6x = 42$] were used as the test system.

SEED GERMINATION TEST

The seed germination bioassay was evaluated according to the procedure of EPA. For each treatment triplicate of 50 healthy and uniform size seeds were used. Seeds were surface sterilized with 10% sodium hypochlorite for ten minutes then washed with sterile distilled water and placed on sterile filter paper in the Petri dishes. Fresh test solutions were added to the Petri dishes and the plates were placed in a B.O.D incubator in the dark for 120 h at 25 ± 1°C to facilitate linear growth. The numbers of seeds that germinated were counted until at least 65 percent of the control seeds germinated. Radicle length 5 mm was considered as germinated and relative seed germinations were calculated.

MEASUREMENT OF SHOOT AND ROOT LENGTH, CHLOROPHYLL AND PROTEIN CONTENT

For each experiment triplicate of each 50 healthy and uniform size seeds were used. The seeds were sowed in the plastic cups (10 x 15 cm) containing sand. Fresh test solutions were added in the cups and kept in the natural environmental for 7 days to grow and subsequently on the 7^{th} day the root and shoot lengths (EPA guidelines), Chlorophyll content [10] and total protein content [11] were measured.

CYTOGENETIC ASSAY

Healthy seeds were surface sterilized with 10% sodium hypochlorite for ten minutes, washed with distilled water and allowed to germinate on filter paper in Petri dishes containing four concentrations viz. 250, 500, 1000 and 2000 mg/L of bulk and nanoTiO_2 & ZnO separately. Control was treated with distilled water. The plates were placed in a BOD incubator in the dark at 25 ± 1°C. Roots were excised when the root length reached about 1 – 1.5 cm and fixed in freshly prepared acetic acid: ethanol (1:3). Cytological studies were prepared by adopting haematoxylin squash techniques [12]. A minimum of 5000 cells from 10 root tips were scored for determining the frequency of mitotic index (MI), chromosomal aberrations (CA), such as metaphase and anaphasic abnormalities.

STATISTICAL ANALYSIS

All the experimental values are expressed as mean ± SD. Comparisons between the control and treated groups were evaluated by oneway ANOVA using SPSS software package and $P<0.05$ was considered as the level of significance.

RESULTS

EFFECT OF BULK & NANO-TIO_2 AND ZNO ON SEED GERMINATION AND SHOOT - ROOT GROWTH

The experimental results on the effects of bulk and nano-TiO_2 and ZnO on seed germination, shoot and root growth are presented in figure 1 - 3. No significant changes were observed in the seed germination, shoot and root growth treated with various concentrations of both bulk and nano-TiO_2 and ZnO when compared to control sample.

EFFECT OF BULK AND NANO-TIO_2 & ZNO ON MITOTIC DIVISION AND CHROMOSOMAL ABERRATION

Results on the frequencies of mitotic index and chromosomal aberrations observed in the root tip cells of *Triticum aestivum* treated with bulk and nano-TiO_2 & ZnO are presented in figure 4 & 5. The result shows that the seeds treated with both bulk and nano-TiO_2 & ZnO shows no significant changes in the frequency of mitotic index and chromosomal aberrations when compared to control samples, which reveals that these chemicals has no effects on the mitotic division and chromosomes.

EFFECT OF BULK AND NANO-TIO_2 & ZNO ON CHLOROPHYLL & PROTEIN CONTENT

The results on the chlorophyll (chlorophyll – a, b & total chlorophyll) & total protein content following treatment with bulk and nano-TiO_2 & ZnO are presented in Figure - 6 & 7. Chlorophyll – a, b and total chlorophyll content and total protein content were significantly ($P<0.05$) increased in the nano-ZnO treated samples. But no changes were observed in the other groups.

DISCUSSION AND CONCLUSION

The field of nanotechnology has shown tremendous growth in recent times leading to the development of applications in different areas of research which involves the manufacture and use of engineered NPs. The use of these NPs leads to the discharge and accumulation of the NPs in the environment affecting both plant and animals systems, therefore leading to the study on nanotoxicology. Developments in nanotechnology are leading to a rapid proliferation of new materials that are likely to become a source of engineered NPs to the environment, where their possible ecotoxicological impacts remain unknown [9]. There is an increase in concern about the safety of various types of nanomaterials, of which some (such as those in computer processors) are considered benign, whilst others (detachable or free nanostructures) are likely to cause adverse health effects. Some NPs are even beneficial to human health [13].

Bulk and NPs of TiO_2 & ZnO are being used in various products such as medicines, personal care, coating and paints, on account of their UV absorption and transparency to visible light. The main concern is whether the unknown risks of engineered NPs, in particular their impact on health and environment, outweighs their established benefits for society. Therefore the present study has been carried out to evaluate the various toxicity of both bulk and NPs of TiO_2 & ZnO comparatively on *Triticum aestivum* L.

The experimental results from the present study shows that these tested chemicals have no adverse effects on seed germination, shoot and root growth. This may be due to the plant is not sensitive to these chemicals. Lin and Xing [14] also reported that the seeds treated with five types of multi-walled NPs such as carbon nanotube, aluminum, alumina, zinc, and zinc oxide have no effects on the germination in *Raphanus sativus*, *Brassica napus*, *Lactuca sativa* and *Cucumis sativus*. However, they observed that the seed germination was inhibited by nanoscale Zn in *Lolium multiflorum* and nanoscale ZnO in *Lolium multiflorum.* Also they observed that the inhibition of root growth varied greatly among NPs and plants and it is partially correlated to NPs concentration. Avinash et al. [15] observed increases in germination and growth rate in the seeds of *Cicer arietinum* treated with nano-ZnO.

Zheng et al. [16] studied that the effects of nano-TiO2 and non-nano-TiO2 on the germination and growth of naturally aged seeds of *Spinacia oleracea* by measuring the germination rate and vigor indexes. An increase of these indexes was observed at 0.25-4‰ nano-TiO2 treatments.
Another study shows that ZnO NPs significantly reduced the biomass of ryegrass, root tip shrank and root epidermal and cortical highly vacuolated or collapsed cells. ZnO NPs greatly adhered onto the root surface and individual NPs were found in the apoplast and protoplast of the root endodermis and stele. Translocation factor of Zn from root to shoot remained very low under ZnO NPs treatments and the author evidenced that the phytotoxicity of ZnO NPs was not directly correlated with their limited dissolution in the bulk nutrient solution or rizosphere [17].
Limited reports are only available on the effects of nanoparticles on cell division and chromosomal aberration. Babu et al. [18] reported that the root tips treated with nano-silver at various concentrations shows decrease in mitotic index and increase in the chromosomal aberrations. Hu et al. [19] evaluated the NPs of TiO_2 and ZnO toxicities to the earthworm *Eisenia fetida* in soil, artificial soil systems and the results shows that nano-TiO2 and ZnO could induce significant damage to earthworms when doses were greater than 1.0 g $kg^{-}1$. They found that Ti and Zn, especially Zn, were bioaccumulated, and that mitochondria were damaged at the highest dose in soil (5.0 g $kg^{-}1$). The activity of cellulase was significantly inhibited when organisms were exposed to 5.0 g $kg^{-}1$ of nano-ZnO and concluded that both nano-TiO2 and ZnO exert harmful effects to *E. fetida* when their levels are higher than 1.0 g $kg^{-}1$ in soil and that toxicity of nano-ZnO was higher than TiO2.
Hong et al. [20,21] reported that the nano-TiO2 treatments induced an increase of the Hill reaction and the activity of chloroplasts in *Spinacia oleracea*. Moreover non-cyclic photophosphorylation activity was higher than cyclic photophosphorylation activity. In our study, we observed the increases of chlorophyll and protein content only in nano-ZnO and not in TiO_2.
The toxicity of NPs depends on their property, test organism species, and surrounding solution conditions. If a test organism is very susceptible to a metal ion, the toxicity of metal-based NPs could be overwhelmed by the dissolved metal ions. Therefore, more research is needed to clarify the contribution of dissolution to the toxicity of metal-based NPs [22].
From the present study, it can be concluded that TiO_2 and ZnO NPs does not exhibit significant phytotoxicity, but increases the chlorophyll and protein content in nano-ZnO only. The reason for this can be attributed to the fact that nano TiO_2 and ZnO are insoluble in water and the particles are rapidly lost from solution, probably due to sedimentation as a result of aggregation or sensitivity of the present test organism. Thus clearly shows that the toxic effects of NPs depend on their properties and may vary species to species. Further studies may be carried out to understand the size distribution of NPs in solution and its effect on phytotoxicity, possible uptake and translocation of NPs by plants, and physical and chemical properties of NPs in rhizosphere and on root surfaces.

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Figure – 1. Effect of bulk & nano-TiO_2 & ZnO on relative seed germination

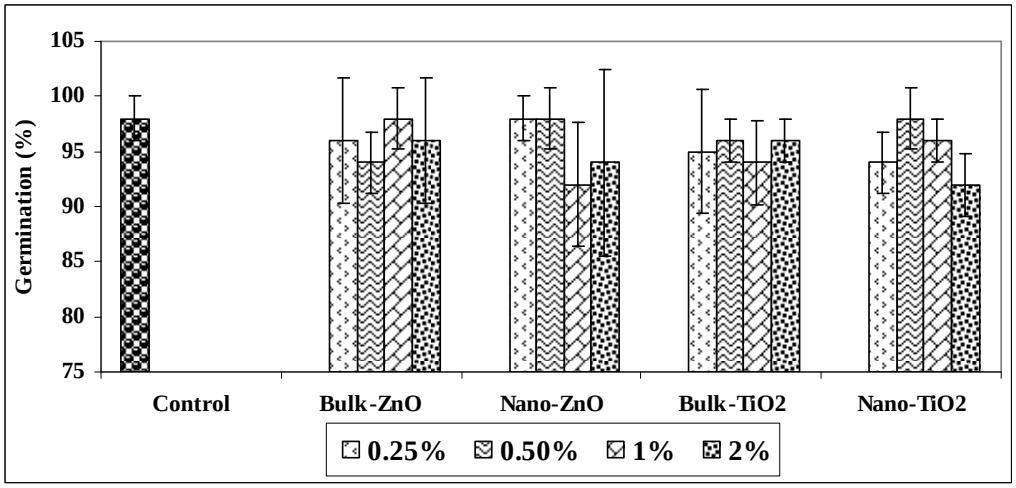


Figure – 2. Effect of bulk & nano-TiO_2 & ZnO on shoot growth

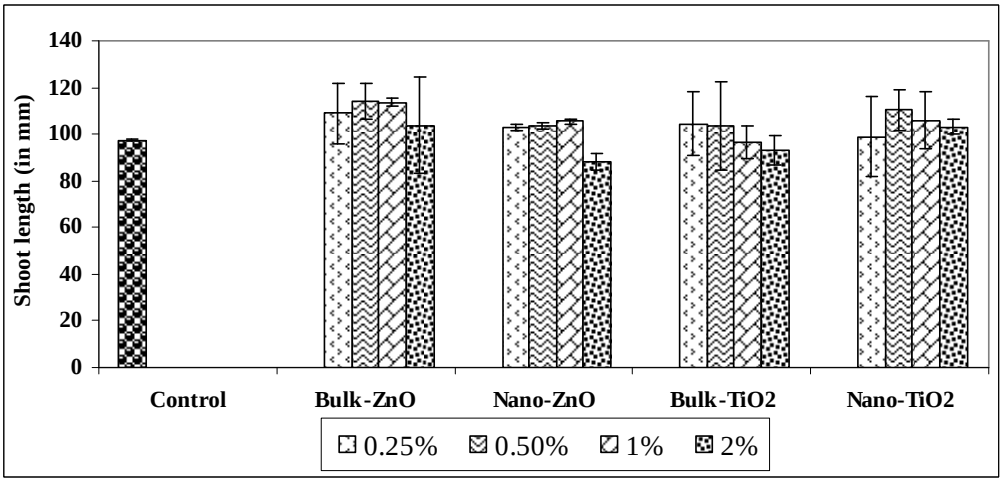


Figure – 3. Effect of bulk & nano-TiO_2 & ZnO on root growth

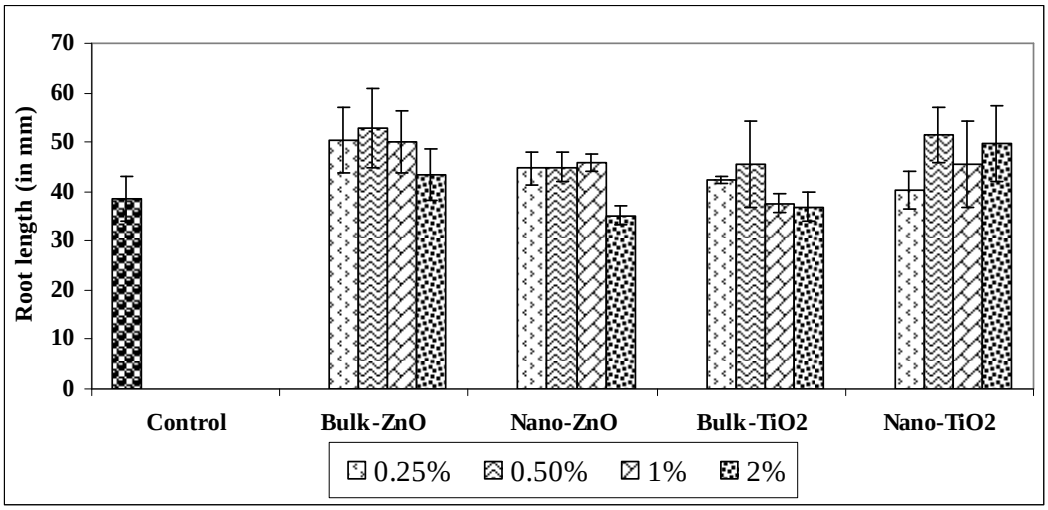


Figure – 4. Effect of bulk & nano-TiO_2 & ZnO on Mitotic index

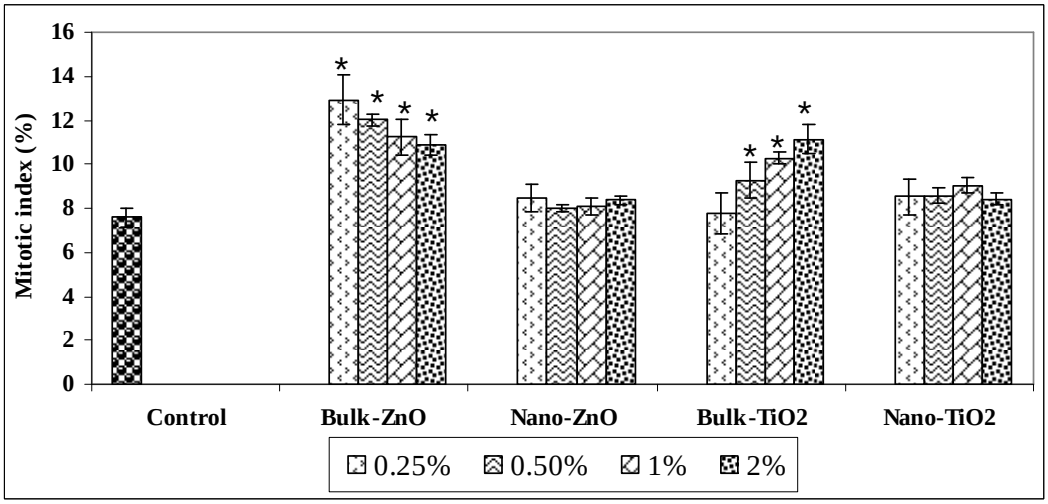


Figure – 5. Effect of bulk & nano-TiO_2 & ZnO on chromosomal aberration

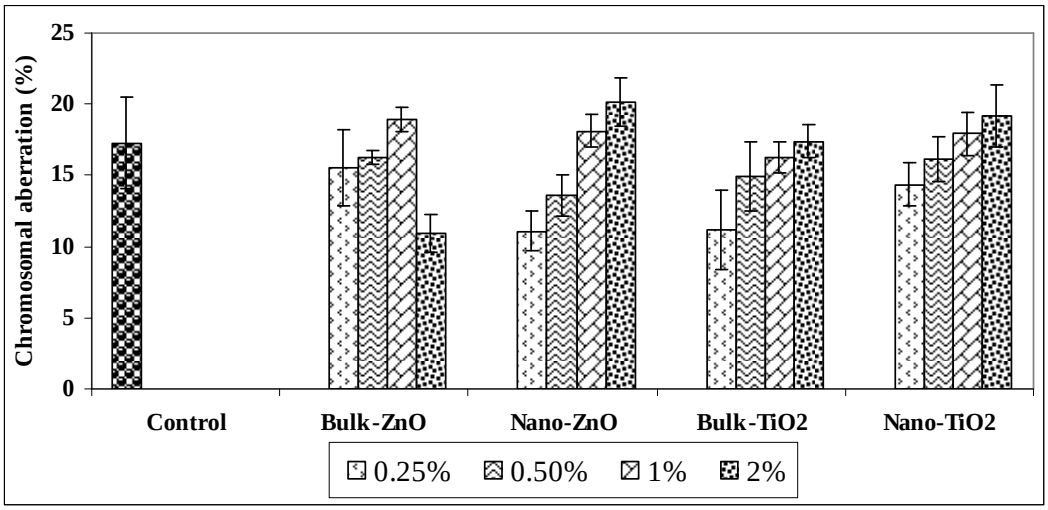


Figure – 6. Effect of bulk & nano-TiO_2 & ZnO on chlorophyll content

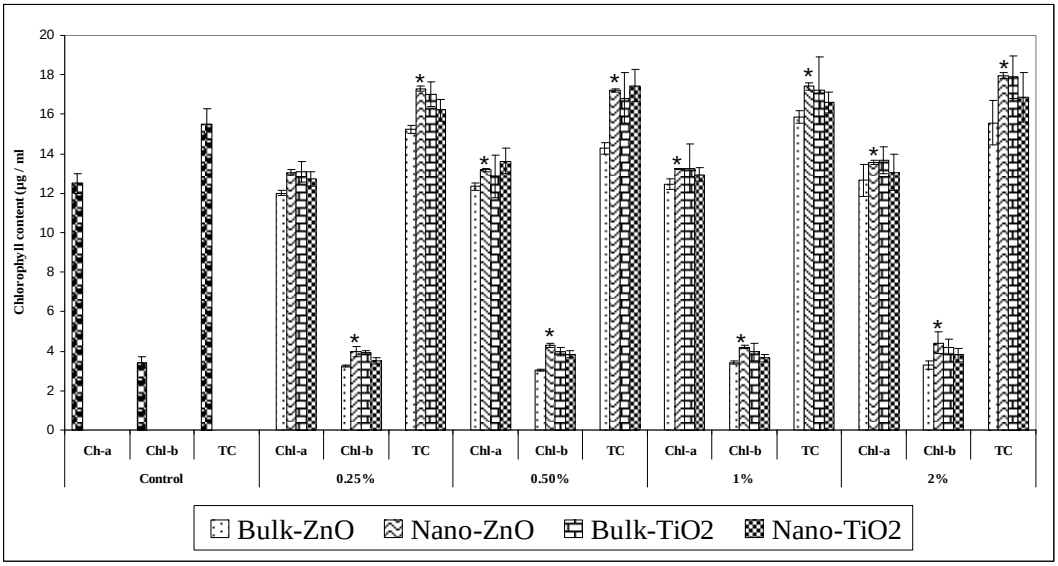
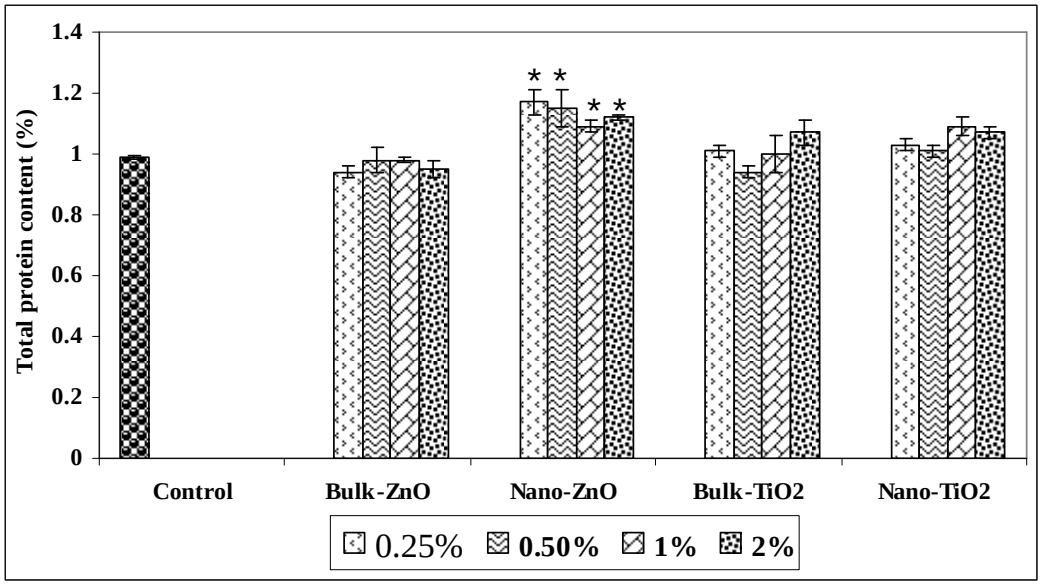


Figure – 7. Effect of bulk & nano-TiO_2 & ZnO on total protein content



* - P<0.05

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