Seed germination and growth responses of Macrotyloma uniflorum (Lam.) Verdc. exposed to Zinc and Zinc nanoparticles
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ABSTRACT

The increasing application and use of nanoparticles are directly related to their release in the environment. The interactions of nanoparticles with plants and other organisms have been poorly studied. The studies on investigation of possible positive and phytotoxic effects of nanoparticles on crop plants are rare. In the present investigation an attempt has been made to assess the effect of Zn and nZn on the seed germination and seedling growth of Macrotyloma uniflorum (Lam.) Verdc. The treatment included four concentrations of both Zn and nZn (2, 10, 50 and 100 ppm) and Control (Without Zn and nZn). Zn and nZn didn’t show any effect on the germination percentage but delay in the germination was observed, the differences in other parameter such as Mean Germination Time (MGT), Seedling vigor, dry and wet biomass of seedling, root and shoot were also observed. The lowest and the highest MGT were observed at the concentration 50 ppm of Zn and 100 ppm of nZn respectively. The lower concentration of Zn (2 ppm) showed the highest MGT, the same was observed in the concentration (10 ppm) of nZn. The effect of Zn and nZn treatment had no significant impact over the root and shoot elongation. Root length decreased in all treatments indicating the negative impact of treatment over the root elongation however the shoot length increased 50 ppm Zn, 2 and 50 ppm nZn.

Keywords: Seed Germination, Germination rate, nZn (Nano Zinc), Macrotyloma uniflorum and seed vigor.

1. Introduction

Nanoparticles show unique physical and chemical properties and have attracted much attention for their distinct characteristics. Their uniqueness arises specifically from higher surface to volume ratio. They represent an increasingly important material in the development of nanotechnology which can be used in numerous biological, physical, biomedical and pharmaceutical applications. Nanoparticulates are produced by various natural processes such as volcano, fire, and soil erosion and are also synthesized by chemical and physical processes for their important applications in many fields. Such manufactured nanoparticles can enter the environment unintentionally through atmospheric emissions, domestic wastewater, agriculture, and accidental release during manufacture, transport; or through intentional releases such as remediation efforts (Zhang et al., 2006). The use of nanomaterials poses many toxicological risks as it is difficult to evaluate the possible toxic effects of such pollutants as toxicity is dependent on both the nanoparticulate form and the toxic metal ions which can be released by the NPs (Franklin et al., 2007, Navarro et al., 2008). Zn NPs are one of the most commonly used and widely applied types of nanomaterials. These compounds have been shown to have high anticorrosion properties (Heinlaan et al. 2008). In addition, they are known to have antibiotic properties, which have encouraged the
pharmacological preparation of these compounds as well as assessment of their potential use in pharmaceuticals and cosmetology (Nel, et al. 2006). Moreover, ZnO NPs are now used in personal care products such as sunscreens, as well as in coatings and paints due to their high levels of UV absorption efficiency and transparency (Lin and Xing 2007).

The mechanism through which nanomaterials alter biological systems is dependent on their size, shape, composition, and surface properties (Dhawan et al., 2009). In terms of ecotoxicity, there is significantly greater focus on aquatic species rather than terrestrial. An increasing amount of research on nanoparticles toxicity and ecotoxicity has been conducted due to their recent widespread use. Several studies have been undertaken to evaluate the effect of nanoparticles on seed germination. However, some reports have shown that NPs have adverse effects on seed germination and plant growth (Yang and Watts, 2005; Lin and Xing, 2007, 2008). A study by Zhu et al, (2008) showed that nanoparticles can be absorbed, translocated, and accumulated within tissues of pumpkin plant. Khodakovskaya et al, (2009) demonstrated that the exposure of tomato seeds to carbon nanotubes enhanced germination and root growth of radish, rape, ryegrass, lettuce, corn and cucumber. They found that only zinc and zinc oxide nanoparticles had significant inhibitory effect on seed germination and root growth. Barrena et al, (2009) observed a similar germination index with Au, Ag and Fe3O4 nanoparticles; however, their presence induced root growth. In view of these above observations the present investigation has been undertaken to study the negative and positive effects of Zn and nZn on seed germination and seedling growth of Macrotyloma uniflorum (Lam.) Verdc. as it is an important agricultural crop and no such work has been undertaken on this crop plant.

2. Materials and methods

2.1 Description of the materials

Seeds of Macrotyloma uniflorum (Lam.) Verdc. (Horse gram) were obtained from Dept. of Seed Technology, University of Agriculture Sciences, Dharwad. ZnSo4 obtained from HiMedia Laboratories Ltd. Mumbai and Zn nano powder having the particle size less than 50 nm was obtained from Sigma Alderich Co. Belgium. HR-SEM imaging and EDAX analysis were preformed to confirm size, shape and purity of the nano sample (Figure 1 &2).

2.2 Experimental design and data observation

2.2.1 Seed treatment and germination experiments
Uniform sized seeds were selected and surface sterilized with 0.2% Mercurous chloride. Twenty five seeds were transferred to each petridish containing filter paper moistened with 10 ml distilled water or nanoparticles solution as the case may be. Five different concentrations of Zn and nZn viz. 2, 10, 50 and 100 ppm were used in the experiment. The seeds were allowed to germinate under laboratory conditions for 8 days. Germination rate was recorded at every 24 hrs following the International rules for Seed Testing Association (ISTA) 2009. Seeds were considered to be germinated when the radical attained a length of 1mm and plumule has just unfolded.

2.2.2 Assessment of developmental responses

Root and shoot lengths of the seedlings were measured daily for eight days. The fresh weight of seedling, root, shoot and dry weight of seedling, root and shoot were determined after oven drying 10 seedlings of each treatment at 70°C for two days. MGT was calculated based on Mathews and Khajeh-Hosseini Equation (Matthews and Khajeh-Hosseini, 2007).

\[
MGT = \frac{F}{X}
\]

Where ‘F’ is the number of seeds newly germinated at the time of X and X is the number of days from sowing.

Germination rate was calculated based on Maguire Equation (Maguire, 1982)

\[
Germination \ rate = \left(\frac{a}{1}\right) + \left(\frac{b-a}{2}\right) + \left(\frac{c-b}{3}\right) + \ldots + \left(\frac{n-n-1}{N}\right)
\]

Where a, b, c ………n are number of germinated seeds after 1, 2, 3…………..N days from the day of imbibitions.

Seedling vigor was computed by using Vashisht and Nagarajan Equations (Vashisth and Nagarajan, 2010).

Vigor Index I = Germination % × Seedling length

Vigor Index II = Germination % × Seedling weight

2.2.3 Data analysis
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All the experiments were conducted in triplicate; the values are expressed as Mean±SE. Duncan’s post hoc test and one way analysis of variance were used to test for differences using SPSS ver.17.0 software at 5% probability level.

3. Results and discussion

3.1 Effect of Zinc and Zinc nanoparticles on seed germination

The germination experiment conducted revealed the insignificant impact of the treatments over the seed germination. The seed germination percentage increased in 10 ppm, 50 ppm of nZn and in 100 ppm Zn, whereas decreased in all the remaining treatments. The hundred percent seed germination was observed in 2 ppm Zn treatment. This enhanced germination may be attributed to the photo-sterilization and photo-generation of active oxygen like superoxide and hydroxide anions that enhance seed stress resistance and encouraged capsule penetration for intake of water and oxygen needed for quick germination (Khot et al. 2012). The lowest and the highest MGT of 1.2 and 1.8 days were observed in the treatments 50 ppm and 2 ppm Zn respectively (Table 1) however the remaining treatments have shown the positive impact over the MGT. Hassan et al., 2011 in their work, impact of bulk and nano sized TiO2 on wheat seed germination and seedling growth with four different concentrations (2, 10, 100 & 500 ppm) reported that among the wheat germination indices only mean germination time was affected by treatment. In an investigation by the Lin and Xing in 2007 among the five metallic nanoparticles tested for phytotoxicity only Zinc and Zinc Oxide showed inhibition of seed germination in Lolium multiform and Zea mays respectively similarly in the present investigation the seed germination was inhibited in lower (2 ppm) of nZn and optimal concentrations (10 and 50 ppm) Zn.

![Figure3: Effect of Zn and nZn on seed germination](image)

3.2 Effect of Zinc and Zinc nanoparticles on seedling growth

Root length, shoot length, seedling length, root dry matter, shoot dry matter and seedling dry matter were affected by both nZn and Zn treatments (Table 2) but the treatments enhanced the wet biomass. Similarly the presence of ZnO nanoparticles in rye grass significantly reduced the biomass, root tips shrank and the root epidermal & cortical cells highly vacuolated and collapsed (Lin and Xing, 2008). The root fresh weight increased in all the treatments of Zn except 50 ppm however it is decreased in all nZn treatments except 100 ppm. The shoot biomass increased in response to all the treatments of Zn and nZn. The dry biomass of root, shoot and seedlings decreased in all treatments of Zn and nZn. The

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treatments exhibited the significant impact on the development of root hairs (Absorption machinery of the plant system). The number of root hairs increased in higher concentrations (50 and 100ppm) of Zn and lower concentration (2 and 10ppm) of nZn indicating the positive role of treatments on absorption machinery. Whereas the remaining treatments had the negative role in the development of absorption machinery. Opposite results were reported by Ghodake et al., 2010 where the multiwalled carbon nanotubes (MCNT’s) in concentrations of 10, 20, 40µg/ml did not show any effect on the seed germination but the higher concentration of CNTs 40µg/ml showed reduction in the hairy roots thus reducing the absorption mechanism.

Table 1: Effect of Zn and nZn on Germination and elongation of M. uniflorum Seedlings

<table>
<thead>
<tr>
<th>Concentration (ppm)</th>
<th>Germination rate</th>
<th>Mean Germination Time (cm)</th>
<th>Root length (cm)</th>
<th>Shoot length (cm)</th>
<th>Seedling length (cm)</th>
<th>No. of root hairs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6.5±0.286 ab</td>
<td>1.3±0.0577 ab</td>
<td>11.93±1.023 a</td>
<td>6.45±0.533 ab</td>
<td>18.38±1.313 ab</td>
<td>7±0.73 ab</td>
</tr>
<tr>
<td>Zn</td>
<td>2 6.66±0.003 ab</td>
<td>1.8±0.577 c</td>
<td>9.8±0.482 ab</td>
<td>5.75±0.416 c</td>
<td>15.6±0.637 ab</td>
<td>6.57±0.557 ab</td>
</tr>
<tr>
<td></td>
<td>10 7.83±0.166 a</td>
<td>1.4±0.5777 ab</td>
<td>11.41±0.397 ab</td>
<td>6.2±0.228 ab</td>
<td>17.61±0.426 ab</td>
<td>6.83±0.6 ab</td>
</tr>
<tr>
<td></td>
<td>50 5.83±0.166 a</td>
<td>1.2±0.577 a</td>
<td>10.46±0.519 a</td>
<td>6.66±0.095 a</td>
<td>17.13±0.476 ab</td>
<td>9.5±0.763 c</td>
</tr>
<tr>
<td></td>
<td>100 6.5±0.763 ab</td>
<td>1.3±0.333 ab</td>
<td>10.83±0.678 ab</td>
<td>6.33±0.364 ab</td>
<td>17.16±0.714 ab</td>
<td>7.3±0.421 ab</td>
</tr>
<tr>
<td>nZn</td>
<td>2 6.66±0.166 a</td>
<td>1.47±0.577 a</td>
<td>10.93±0.587 a</td>
<td>7.01±0.36 a</td>
<td>17.95±0.9 ab</td>
<td>10.33±0.918 a</td>
</tr>
<tr>
<td></td>
<td>10 5.83±0.44 ab</td>
<td>1.73±0.145 b</td>
<td>10.55±0.435 ab</td>
<td>6.68±0.366 b</td>
<td>16.63±0.547 ab</td>
<td>8.66±0.666 bc</td>
</tr>
<tr>
<td></td>
<td>50 7.16±0.166 a</td>
<td>1.43±0.033 c</td>
<td>11.1±0.614 ab</td>
<td>6.96±0.213 ab</td>
<td>18.06±0.677 a</td>
<td>5.6±0.421 a</td>
</tr>
<tr>
<td></td>
<td>100 6.39±0.11 ab</td>
<td>1.3±0.057 c</td>
<td>11.43±0.475 a</td>
<td>6.18±0.230 a</td>
<td>17.61±0.632 ab</td>
<td>8.66±0.494 bc</td>
</tr>
</tbody>
</table>

Conclusion

The effect of treatments on seed germination was not significant. The germination percentage increased in 10 ppm and 50 ppm of nZn and 100 ppm of Zn indicating the positive impact of optimal concentrations of nZn and higher concentration of Zn over the seed germination. The seed germination rate and mean germination time increased in lower concentration of Zn and optimal concentration of nZn. No significant impact was observed on root, shoot elongation, the enhancement in the absorption machinery in higher concentration of Zn and the lower
concentration of nZn was observed. It can be concluded that the action of nZn in lower concentration is similar to the action of Zn at higher concentration.

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Figure 5: Vigor Index II

Table 2: Effect of Zn and nZn on wet and dry biomass of M. uniflorum seedlings

<table>
<thead>
<tr>
<th>Concentration (ppm)</th>
<th>Wet biomass</th>
<th></th>
<th>Dry biomass</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Root</td>
<td>Shoot</td>
<td>Root</td>
<td>Shoot</td>
<td>Root</td>
</tr>
<tr>
<td>Control</td>
<td>0</td>
<td>9.73d</td>
<td>5.63a</td>
<td>15.36b</td>
<td>7.9d</td>
</tr>
<tr>
<td>Zn</td>
<td>2</td>
<td>11.9b</td>
<td>11.13g</td>
<td>23.03h</td>
<td>7.33d</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>11.63g</td>
<td>10.8f</td>
<td>22.43g</td>
<td>6.0b</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>9.16c</td>
<td>7.16b</td>
<td>16.33c</td>
<td>4.86a</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>10.06c</td>
<td>10.03c</td>
<td>20.1f</td>
<td>6.96cd</td>
</tr>
<tr>
<td>nZn</td>
<td>2</td>
<td>7.63a</td>
<td>5.73a</td>
<td>13.36a</td>
<td>6.0b</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>9.3c</td>
<td>7.76c</td>
<td>17.06d</td>
<td>6.9cd</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>7.96b</td>
<td>7.56c</td>
<td>15.53b</td>
<td>6.76c</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>10.83f</td>
<td>7.23b</td>
<td>18.06c</td>
<td>6.76c</td>
</tr>
</tbody>
</table>

Means in each column followed by similar letters are not significantly different at the 5% probability level using Duncan’s test.
6. References


15. Yang, L., and Watts, D. J., (2005), Particle surface characteristics may play an important role in phytotoxicity of alumina nanoparticles, Toxicological Letters, 158, pp 122–132.


