
Nano copper oxide can penetrate as nutrient in seeds and plants in an economical nano-biofertiliser application

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Abstract: Increasing the use of chemical fertilisers leads to various health hazards, also contaminating the water bodies etc., it is essential for minimising the usage of such chemical fertilisers. The present invention discloses a novel nano-biofertiliser that provides essential nutrients for plant growth as nano-particles along with bio bacteria. The nano-biofertilisers develop seed enhancement in addition to plant growth and improvement. The exemplary personifications in the present concept disclosed towards a nano-biofertiliser composition comprising copper oxide nanoparticles and bacteria from *Nitrobacter* genus and *Nitrosomonas* genus. This composition improves the germination rate along with the growth and improvement on plants. This fertiliser is an innovative approach and environment-friendly playing a major part in soil fertility, besides reducing problems due to overuse of commercial chemical fertilisers. The nano-biofertiliser composition delivers the nutrients to plants in a homogenous manner.

Keywords: nano-biofertilisers; copper oxide nanoparticles; *Nitrobacter*; *Nitrosomonas*; *helianthus Annuus*.

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Biographical notes: Murali Krishna Tummalapalli is a PhD research scholar in Mechanical Engineering at the University of Saskatchewan, Canada and also a reviewer for Elsevier journal. His research extents are in the areas of nanotechnology, green synthesis and characterisation of gold nanoparticles using various plants extracts; a nano-biofertiliser for improving seed germination and plant development; computational nanotechnology modelling and applications. He holds one patent and five papers.

1 Introduction

An innovative aspect of the current subject focused towards a technique of preparing a nano-biofertiliser composition by accumulating the particular size and quantity of nanoparticles with bio bacteria. The purpose of the current invention was an environment-friendly, the composition wherein the nutrient element has high purity and

hence a slight quantity of the nutrient would suffice to provide the nutrition. This would disperse nutrients to the plants in a homogenous manner.

The enlargement of quantity and quality of the agricultural crops by means of improving the germination rate of the seeds along with the growing and improvement of the plants. The environment and health problems from chemical fertilisers usage, many attentions has been drawn to the application of nano-biofertilisers, which contains copper oxide (CuO) nanoparticles and bacteria's of *Nitrobacter* and *Nitrosomonas* prepared at economical and easily existing materials.

It promotes the growth by increasing the source and necessary nutrients to plants (Javahery and Rokhzadi, 2011; Vessey, 2003). Nanoscale of copper oxide and bacteria's (*Nitrobacter* and *Nitrosomonas*) are used as recycle of soil and positive effects on growth and productivity of plants through numerous mechanisms of hormones in plants creation and substantial nitrogen complex in soil (Kloepper and Schroth, 1981; Kloepper et al., 1989; Glick, 1995; Rodelas et al., 1999; Rodriguez and Fraga, 1999; Sindhu et al., 2002; Persello-Cartieaux et al., 2003; Rudresh et al., 2005; Rokhzadi and Toashih, 2011). The nanoscale materials are atomic or molecular combinations with at least one dimension in the size range of 1 to 100 nm (Ball, 2002). That can drastically alter their physicochemical properties related to the bulk materials (Battke et al., 2008).

Nanoparticles prepared from a full dissimilarity materials and that they can explicate their actions depending on both the chemical configuration and on the size and/or shape of the particles (Brunner et al., 2006; Monica and Cremonin, 2009). Straight, however, copper is toxic to life at levels, that actually depending on the organism. Human is required and not exceed 1–2 mg/L copper with their drinking water (OJEC, 1998; EPA, 2007b). While some freshwater animals and plants experience acute toxic effect at absorptions as small as 10 mg/L (EPA, 2007a). Since the human food sequence creates with plants it is critical to understand how repeatedly focused in soils as an outcome of pesticides applications, sewage sludge deposition, mining and industrial activities (Vassilev et al., 2004; Manceau et al., 2008).

At present exertion (CuO) and bacteria's used for nitrogen fixing in the soil, confirmed in the *helianthus Annuus*, and it is the important oil seeding crop having great quality edible oil it is comfortable cultivated compared to other oil crops and grown in dissimilar environments and soils (Kaya and Kolsarici, 2011; Lopez-Valdez et al., 2011). However, *helianthus Annuus* oil has outstanding nutrient properties and a comparatively high appeal of linoleic acid (Seiler, 2007). It is one of the leading crops producing the oil requirements for several countries and it had an important position in crop rotation system of many farmlands so achieving sustainable systems for sunflower cultivation would be of great value in agricultural management (Javahery and Rokhzadi, 2011). Therefore, this application aimed to impact the nano-biofertilisers for growth and phenology of sunflower, and also for the other crops.

2 Materials and methodology

2.1 Synthesis of copper oxide nanoparticles

Nano size copper oxide was prepared by the sol-gel procedure. The $\text{CuCl}_2 \cdot 6\text{H}_2\text{O}$ (0.2 m) solution was prepared in clean round bottom flask followed by glacial acetic of 1 ml was a supplement to above aqueous solution and therefore heated to 100°C with constant

stirring, 8 m NaOH was drop by drop supplement to the above heated solution and remain it until pH reached to 7.

Then the colour differs from blue to dark black instantaneously and the remained amount of dark black substance was obtained in the base of the bowl. The precipitous was centrifuged and washes away for many times with triple filtered deionised water. The obtained precipitate was placed in a hot air oven for drying, later the dried substance was a grind with mortar and pestle. Thus the nano-copper oxide powder was obtained and was used as the basis of nano-biofertilisers.

2.2 Seeds

Helianthus annuus (sunflower) seeds in use 250 normal medium in size. It is an essential oil seeding crop having high superiority in edible oil.

2.3 Bacteria

The bacteria genus of *Nitrobacter* and *Nitrosomonas* are collectible from aqua fishery feeds. They are easily accessible and normally available. These bacteria are mostly recycled in the purification of soil and water in the ponds of aquaculture.

2.4 Germination of sunflower seeds

Seeds of *helianthus Annuus* (sunflower) were sterilised by 10 min treatment with 50% Clorox solution and then rinsed several times with sterile water. Sterile sunflower seeds were placed on murashige and skoog medium MS media correspondingly (finer Nagasawa modifications) adding agar. With and without CuO nanoparticles and Bacteria's (100, 200, 300, and 400 µl) for germination. Sterile phyta jar square container boxes were arranged for all germination experiments.

3 Results

3.1 Effect of copper oxide nanoparticles on sunflower seeds and seedlings

To examine whether the synthesised copper oxide nanoparticles could affect germination and growth of seedling, we placed sterile sunflower seeds (*Helianthus annuus*) in standard MS media correspondingly (finer Nagasawa modifications) adding agar medium, supplemented with different concentrations of CuO nanoparticles (100, 200, 300, and 400 µl).

The MS media without copper oxide nanoparticles was used as a control, as presented in the Figure 1(a). The addition of copper oxide nanoparticles to MS media accelerated the procedure of seed sprouting and considerably fastens the germination time. Sunflower seeds placed on medium with CuO NP'S (100, 200, 300, and 400 µl) germinated rapidly from the third day, while the sunflower seeds placed on regular MS media did not sprout at that time. The growth percentage rates through the following days dramatically

developed for seeds that were preserved with nanoparticles. The seeds growth percentage were inoculated on regular medium with around 28% in 12 days whereas, germination part of seeds placed in medium enhanced with CuO NP'S averaged 76–84% in 12 days, presented in the Figure 1(a) and Figure 1(b).

Figure 1 Copper oxide nanoparticles in growth medium, (a) (A). phenotypes of 12-day-old sunflower seeds grown on medium without (0 μ l) and with CuO NP'S (100, 200, 300, 400 μ l) (b) effect of CuO NP'S on growth and improvement of 12-day-old sunflower seedling without (0 μ l) and with CuO NP'S (100, 200, 300, 400 μ l) (see online version for colours)

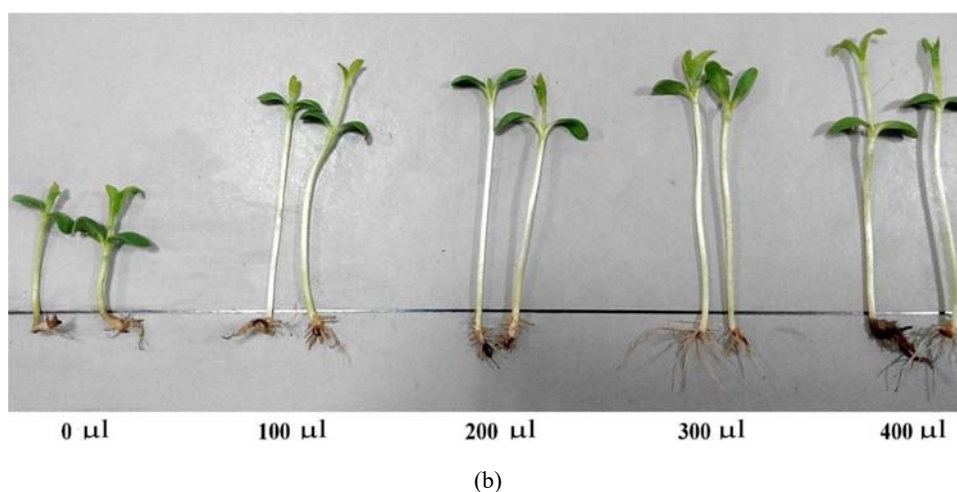
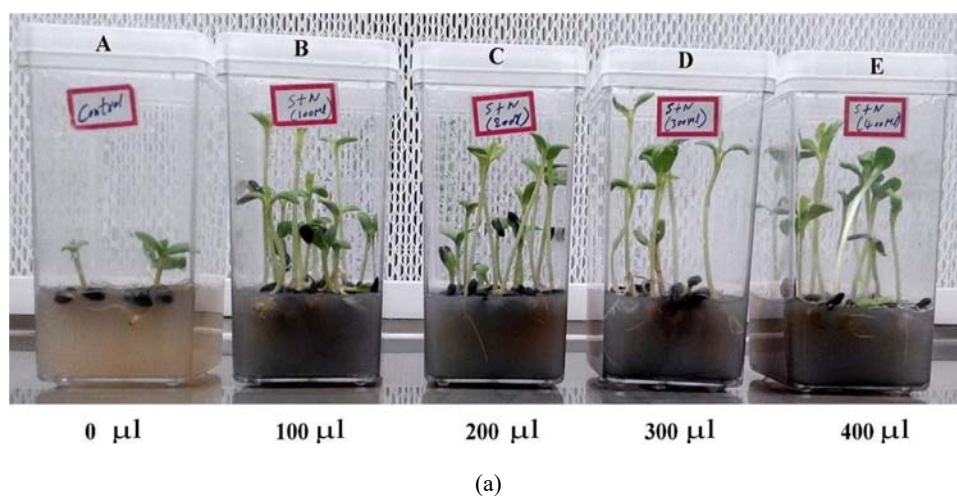
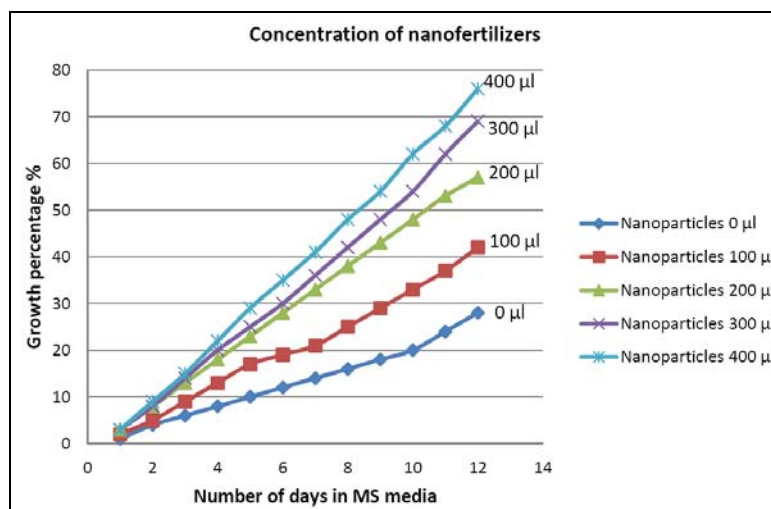


Figure 2 The growth percentage and concentration of nano-fertilisers in 12 days (see online version for colours)



The percentage of growth rate in MS media with control and different concentrations of CuO nanoparticles (100, 200, 300, and 400 µl) as taken, shown in the above graph.

3.2 Effect of *Nitrobacter* and *Nitrosomonas* on sunflower seeds and seedlings

To test whether the bacteria comprising *Nitrobacter* and *Nitrosomonas* could affect germination and development of crop seedling, we placed sterile sunflower seeds on standard MS media correspondingly (finer Nagasawa modifications) adding agar medium, supplemented with 400 µl, of *Nitrobacter* and *Nitrosomonas*. The MS medium without *Nitrobacter* and *Nitrosomonas* was used as a control, as presented in the Figure 3(a). The addition of *Nitrobacter* and *Nitrosomonas* to the agar medium to generate and accelerate the process of seed sprouting significantly reduced the germination time. Sunflower seeds placed on medium with *Nitrobacter* and *Nitrosomonas* 400 µl germinated quickly from the third day, while the sunflower seeds placed on regular MS media did not germinate at that time.

The germination rates during the following days were dramatically higher for seeds that were grown in media enhanced with bacteria.

The seeds growth percentage on regular medium with around 28% in 12 days whereas, growth percentage of seeds placed on medium supplemented with *Nitrobacter* and *Nitrosomonas* averaged 72–78% in 12 days as presented in the Figure 3(a) and Figure 3(b).

Figure 3 The concentration of bio-bacteria (*Nitrobacter* and *Nitrosomonas*) in the growth medium, (a) phenotypes of 12-day-old sunflower seed growth on medium with control and with *Nitrobacter* and *Nitrosomonas* 400 μ l (b) effect of *Nitrobacter* and *Nitrosomonas* on growth and improvement of 12-day-old sunflower seedling with control and with *Nitrobacter* and *Nitrosomonas* (see online version for colours)

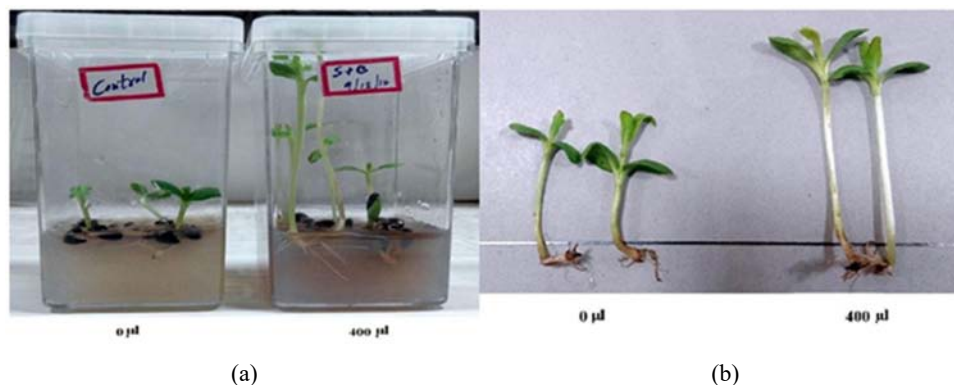
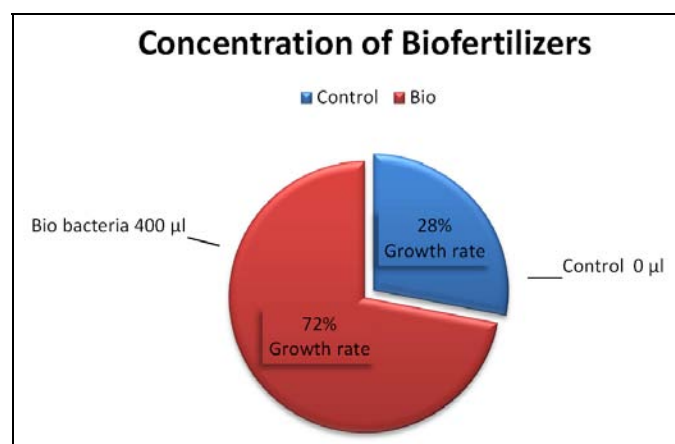


Figure 4 Effect of biofertilisers in the growth medium (see online version for colours)



The percentage of growth rate in MS media with control and concentrations of *Nitrobacter* and *Nitrosomonas* of 400 μ l as taken, shown in the above chart.

3.3 Effect of nano-biofertiliser on sunflower seeds and seedlings

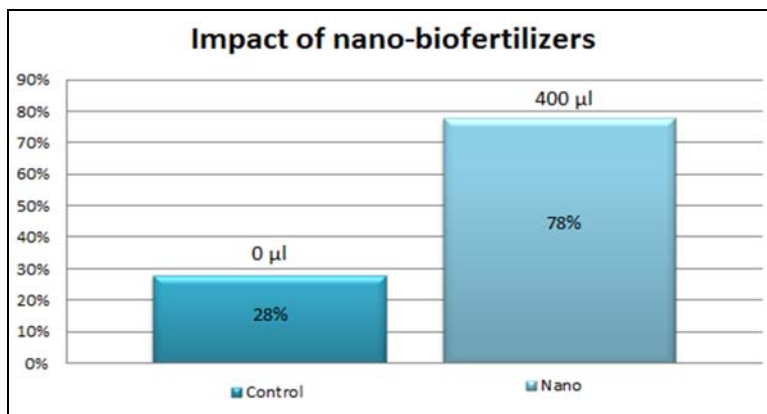
Copper oxide nanoparticles along with bacteria from *Nitrobacter* and *Nitrosomonas* genus combined to form the nano-biofertiliser. To test whether the nano-biofertiliser could affect germination and development of seedling, we placed sterile sunflower seeds on standard MS media correspondingly (finer Nagasawa modifications) adding agar medium supplemented with nano-biofertiliser wherein, the concentration of copper oxide nanoparticle was 200 μ l and the concentration of bacteria was 200 μ l. The MS medium without nano-biofertiliser utilised as control, presented in the Figure 5(a). The addition of nano-biofertilisers to the standard medium was found to accelerate the process of a seed

sprouting and significantly shortened the germination time shown in Figure 5(b). Sunflower seeds situated on the medium with nano-biofertilisers germinated rapidly from the third day whereas the sunflower seeds located on regular MS media (control) did not germinate at that time.

Figure 5 Effect of nano-biofertilisers in seeds and plants, (a) phenotypes of 12-day-old sunflower seed growth on medium without and with copper oxide nanoparticles and *Nitrobacter* and *Nitrosomonas* 200 μ l each (b) effect of CuO NP'S and bacteria's growth on the third day, sunflower seedling without and with nano-biofertilisers (c) the phenotype of 12-days-old sunflower seeds growing on medium without and with nano-biofertilisers (see online version for colours)



The growth percentage rates over the following days were intensely higher for seeds that were enhanced with nano-biofertilisers. The sprouting percentage for seeds that were situated on regular medium with around 28% in 12 days whereas, germination part of seeds placed on medium supplemented with copper oxide nanoparticles and *Nitrobacter* and *Nitrosomonas* averaged 78–86% in 12days, presented in Figure 5(c).

Figure 6 Effect of nano-biofertilisers on sunflower plants (see online version for colours)

The percentage of growth rate in MS media with control and concentrations of nano-biofertilisers (Nano + Bio) of 400 µl as taken, shown in the above chart.

4 Discussions

The nano-biofertiliser can improve seed sprouting and plant development. Nano-biofertiliser was found to accelerate the growth significantly. Shortened the germination time and crop yield duration. Plants that are exposed to nano-biofertilisers exhibited an intense growth in vegetative of the biomass. According to the previous research, there are few studies on nanofertilisers using expensive materials such as carbon nanotubes which shown the medium impact on tomato plants (Khodakovskaya et al., 2009) but, present research focused on economical nanoparticles such as CuO with adding bio-bacteria for environmental sustainability. Therefore, identified, the optimistic result of nano-biofertilisers on plant germination and growth could have essential economic importance for Agriculture.

5 Conclusions

The study demonstrates improvement and growth in an early stage by utilising nano-biofertilisers, enhanced with CuO nanoparticles for the reason the surface area is high while compared to bulk fertiliser materials. Although plants easily take-up very small particles that are in nanoscale, nitrogen-fixing for plants with the help of *Nitrobacter* and *Nitrosomonas*. This nano-biofertilisers is the new approach and helpful to small-scale farmers to development the crop and improvement, it maintains soil quality and ecological balance a pioneering way for an eco-friendly environment.

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