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Complete Specification

CLAIMS:1. A nano-biofertilizer composition comprising a nanoparticle of a fertilizer material and a bacteria.

2. A nano-biofertilizer composition as in claim 1, wherein the nanoparticle of a fertilizer material is a nanoparticle of copper oxide.

3. A nano-biofertilizer composition as in claim 1, wherein the bacteria is at least one of a Nitrosomonas genus and a Nitrobacter genus.

4. A method of preparing a nano-biofertilizer composition comprising:

- Adding a predetermined quantity of a glacial acetic acid to an aqueous solution of CuCl₂ and heating to a predetermined temperature with stirring;
- Adding a predetermined quantity of NaOH to the aqueous solution of CuCl₂ till a desired pH is reached, wherein a precipitate is obtained;
- Centrifuging the aqueous solution of CuCl₂ to separate the precipitate;
- Washing the precipitate with deionised water a plurality of times;
- Drying the precipitate to get a nanoparticle of copper oxide; and
- Mixing a predetermined quantity of a bacteria to a predetermined quantity of the nanoparticle of copper oxide to get a nano-biofertilizer.

5. The method as in claim 4, wherein the bacteria is at least one of a Nitrosomonas genus and a Nitrobacter genus. ,TagSPECI:TECHNICAL FIELD

The present disclosure generally relates to the field of plant sciences. More particularly, the present disclosure relates to a nano-biofertilizer suitable for improved germination and growth of plants. .

BACKGROUND

Commercial chemical fertilizers are products that are synthetically created for providing additional nutrients that are essential for the growth of plants. These fertilisers render macronutrients like nitrogen, phosphorus, potassium etc. or micronutrients such as copper, iron, zinc etc. for optimum growth and development of the plants. They are either applied directly to the soil in which the plants grow or are applied on the plant surface. Though these fertilisers aid in the growth and development of plants there are negative environmental impacts that lessen the overall worth of these fertilizer applications. One of such environmental impact that is a cause of concern is contamination of water bodies due to fertilizer runoff from farms. Farmers cultivating large scale farms are facing various health hazards due to use of chemical fertilizers.

In the light of aforementioned discussion, there exists a need for minimising the use of such chemical fertilizers. The present invention discloses a novel nano-biofertilizer that provides nutrients essential for plant growth as nanoparticles along with biofertilisers. The disclosed nano-biofertilizers are eco-friendly and improve seed germination as well as plant growth and development.

BRIEF SUMMARY

The following presents a simplified summary of the disclosure in order to provide a basic understanding to the reader. This summary is not an extensive overview of the disclosure and it does not identify key/critical elements of the invention or delineate the scope of the invention. Its sole purpose is to present some concepts disclosed herein in a simplified form as a prelude to the more detailed description that is presented later.

Exemplary embodiments of the present disclosure are directed towards a nano-biofertilizer composition comprising copper oxide nanoparticles and bacteria of the genus Nitrobacter and Nitrosomonas.

Another exemplary aspect of the present subject matter is directed towards a method of preparing a nano-biofertilizer composition comprising: adding a predetermined quantity of a glacial acetic acid to an aqueous solution of CuCl₂ and heating to a predetermined temperature with stirring; adding a predetermined quantity of NaOH to the aqueous solution of CuCl₂ till a desired pH is reached, wherein a precipitate is obtained; centrifuging the aqueous solution of CuCl₂ to separate the precipitate; washing the precipitate with deionised water a plurality of times; drying the precipitate to get a nanoparticle of copper oxide; and mixing a predetermined quantity of a bacteria to a predetermined quantity of the nanoparticle of copper oxide to get a nano-biofertilizer.

It is an object of the present invention to disclose a nano-biofertilizer composition that is environment friendly.

It is another object of the present invention to disclose a nano-biofertilizer composition wherein the purity of the nutrient element is very high and hence a small amount of the nutrient would suffice to provide the nutrition.

It is another object of the present invention to disclose a nano-biofertilizer composition that would disperse nutrients to the plants in a homogenous manner.

It is another object of the present invention to increase the quantity and quality of the agricultural crops by

means of improving the germination rate of the seeds as well as the growth and development of the plants.

BRIEF DESCRIPTION OF DRAWINGS

Other objects and advantages of the present invention will become apparent to those skilled in the art upon reading the following detailed description of the preferred embodiments, in conjunction with the accompanying drawings, wherein like reference numerals have been used to designate like elements, and wherein:

FIG. 1A is an image showing the phenotypes of 21-day-old sunflower seedlings grown on medium having different concentrations of copper oxide nanoparticles along with control.

FIG. 1B is an image showing the effect of copper oxide nanoparticles on the growth and development of 21-day-old sunflower seedlings.

FIG. 2A is an image showing the phenotypes of 21-day-old sunflower seedlings grown on medium having Nitrobacter and Nitrosomonas bacteria along with control.

FIG. 2B is an image showing the effect of Nitrobacter and Nitrosomonas bacteria on the growth and development of 21-day-old sunflower seedlings.

FIG. 3A is an image showing the phenotypes of 21-day-old sunflower seedlings grown on medium having nano-biofertilizer along with control.

FIG. 3B is an image showing the effect of nano-biofertilizers on the germination of sunflower seeds grown in medium having nano-biofertilizers.

FIG. 3C is an image showing the effect of nano-biofertilizers on the growth and development of 21-day-old sunflower seedlings.

DETAILED DESCRIPTION

It is to be understood that the present disclosure is not limited in its application to the details of construction and the arrangement of components set forth in the following description or illustrated in the drawings. The present disclosure is capable of other embodiments and of being practiced or of being carried out in various ways. Also, it is to be understood that the phraseology and terminology used herein is for the purpose of description and should not be regarded as limiting.

The use of “including”, “comprising” or “having” and variations thereof herein is meant to encompass the items listed thereafter and equivalents thereof as well as additional items. The terms “a” and “an” herein do not denote a limitation of quantity, but rather denote the presence of at least one of the referenced item. Further, the use of terms “first”, “second”, and “third”, and the like, herein do not denote any order, quantity, or importance, but rather are used to distinguish one element from another.

According to a non limiting exemplary embodiment of the present disclosure, a nano-biofertilizer composition comprising a nanoparticle of a fertilizer material and a bacteria is disclosed.

In accordance with a non limiting exemplary embodiment of the present subject matter, a nano-biofertilizer composition comprising a copper oxide nanoparticle and bacteria comprising at least one of a Nitrobacter genus and a Nitrosomonas genus is disclosed.

According to a non limiting exemplary embodiment of the present disclosure, a method of preparing a nano-biofertilizer composition comprising: adding a predetermined quantity of a glacial acetic acid to an aqueous solution of CuCl₂ and heating to a predetermined temperature with stirring; adding a predetermined quantity of NaOH to the aqueous solution of CuCl₂ till a desired pH is reached, wherein a precipitate is obtained; centrifuging the aqueous solution of CuCl₂ to separate the precipitate; washing the precipitate with deionised water a plurality of times; drying the precipitate to get a nanoparticle of copper oxide; and mixing a predetermined quantity of a bacteria to the nanoparticle of copper oxide to get a nano-biofertilizer is disclosed.

Examples:

Synthesis of Copper Oxide Nanoparticles:

Copper oxide (CuO) nanoparticles were prepared by sol-gel method. 1 ml of glacial acetic acid was added to an aqueous solution of CuCl₂·6H₂O (0.2 M) and heated to 100°C with continuous stirring. 8 M of NaOH is added to the above heated solution till pH reached 7.0. Then the color of the solution turned from blue to black immediately and a large amount of black precipitate formed at the bottom of the flask. The precipitate was separated from the aqueous solution by centrifugation and washed several times with deionized water. The obtained precipitate was placed in hot air oven for drying to get the nanoparticles of copper oxide.

Collection of Seeds:

Sunflower (*Helianthus annuus*) seeds were procured from the market in and around Kakinada zone, Andhra Pradesh, India. It is the one of the most important oil seed crops containing high quality edible oil.

Bacteria:

The bacteria of genus Nitrobacter and Nitrosomonas are available in aqua fishery feed shop. They are readily accessible and nominal in price. These bacteria are generally used for purification of water and soil in aqua culture ponds.

Germination of sunflower seeds:

Seeds of sunflower (*Helianthus annuus*) were sterilized by 10 min treatment with 50% chlorox solution and then rinsed several times with sterile water. Sterile sunflower seeds were placed on Murashige and skoog medium (MS) media (finer nagasawa modifications) for germination with different concentrations of copper oxide nanoparticles and bacteria. Sterile phyta jar square container boxes were used for all germination experiments.

Effect of copper oxide nanoparticles on sunflower seeds and seedlings:

Referring to FIG. 1A, it is an image showing the phenotypes of 21-day-old sunflower seedlings grown on medium having different concentrations of copper oxide nanoparticles along with control.

Referring to FIG. 1B, it is an image showing the effect of copper oxide nanoparticles on the growth and development of 21-day-old sunflower seedlings.

To test whether the synthesized Copper oxide nanoparticles could affect germination and growth of crop seedling, sterile sunflower seeds were placed in standard MS media correspondingly (finer nagasawa modifications) adding agar medium, supplemented with different concentrations of copper oxide nanoparticles [100 μ l (b), 200 μ l (c), 300 μ l (d), 400 μ l (e)]. The MS medium without copper oxide nanoparticles was used as a control (a).

Addition of copper oxide nanoparticles to agar medium was found to accelerate the process of seed germination and significantly shortened the germination time. Sunflower seeds placed on medium with different concentrations of copper oxide nanoparticles [100 μ l (b), 200 μ l (c), 300 μ l (d), 400 μ l (e)] germinated rapidly from the third day, while the sunflower seeds placed on regular MS media (a) did not germinate at that time. The germination rates during the following days were dramatically higher for seeds that were treated with nanoparticles. The germination percentage for seeds that were placed on regular medium averaged 28% in 12 days and 69% in 21 days, while germination percentage of seeds placed in medium supplemented with nanoparticles averaged 76-84% in 12 days.

Effect of Nitrobacter and Nitrosomonas on sunflower seeds and seedlings:

Referring to FIG. 2A, it is an image showing the phenotypes of 21-day-old sunflower seedlings grown on medium having Nitrobacter and Nitrosomonas bacteria along with control.

Referring to FIG. 2B, it is an image showing the effect of Nitrobacter and Nitrosomonas bacteria on the growth and development of 21-day-old sunflower 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 μ L (b) of Nitrobacter and Nitrosomonas. The MS medium without Nitrobacter and Nitrosomonas was used as a control (a).

Addition of Nitrobacter and Nitrosomonas to agar medium was found to accelerate the process of seed germination and significantly reduced the germination time. Sunflower seeds placed on medium with Nitrobacter and nitrosomonas [400 μ L (b)] germinated rapidly from the third day, while the sunflower seeds placed on regular MS media (a) did not germinate at that time. The germination rates during the following days were dramatically higher for seeds that were grown in media supplemented with bacteria. The germination percentage for seeds that were placed on regular medium averaged 28% in 12 days and 69% in 21 days, while germination percentage of seeds placed on medium supplemented with Nitrobacter and Nitrosomonas averaged 72-78% in 12 days and 86% in 21 days.

Effect of nano-biofertilizer on sunflower seeds and seedlings:

Referring to FIG. 3A, it is an image showing the phenotypes of 21-day-old sunflower seedlings grown on medium having nano-biofertilizer along with control.

Referring to FIG. 3B, it is an image showing the effect of nano-biofertilizers on the germination of sunflower seeds grown in medium having nano-biofertilizers.

Referring to FIG. 3C, it is an image showing the effect of nano-biofertilizers on the growth and development of 21-day-old sunflower seedlings.

Copper oxide Nanoparticles along with Bacteria from Nitrobacter and Nitrosomonas genus comprise the Nano-biofertilizer. To test whether the Nano-biofertilizer 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 nano-biofertilizer (b), wherein the concentrations of copper oxide nanoparticle was 400 μ L and the concentration of bacteria was 400 μ L. The MS medium without nano-biofertilizer was used as control (a).

Addition of nano-biofertilizers to the standard medium was found to accelerate the process of seed germination and significantly shortened the germination time. Sunflower seeds placed on medium with nano-biofertilizers (b), germinated rapidly from the third day while the sunflower seeds placed on regular MS media (a) did not germinate at that time. The germination percentage rates during the next days were dramatically higher for seeds that were supplemented with nano-biofertilizers. The germination percentage for seeds that were placed on regular medium averaged 28% in 12 days and 69% in 21 days, while germination percentage of seeds placed on medium supplemented with copper oxide nanoparticles and Nitrobacter and Nitrosomonas averaged 78-86% in 12 days and 96% in 21 days.

Our results demonstrated for the first time that Nano-biofertilizer can improve seed germination and plant development. The nano-biofertilizer was found to accelerate the growth significantly, shortened the germination time and crop yield duration. Plants that are exposed to nano-biofertilizers exhibited a dramatic increase in vegetative biomass. Therefore, an observed positive effect of Nano-biofertilizers on plant germination and growth could have significant economic importance for Agriculture.

Although the present disclosure has been described in terms of certain preferred embodiments and illustrations thereof, other embodiments and modifications to preferred embodiments may be possible that are within the principles and spirit of the invention. The above descriptions and figures are therefore to be regarded as illustrative and not restrictive.

Thus the scope of the present disclosure is defined by the appended claims and includes both combinations and sub combinations of the various features described herein above as well as variations and modifications thereof, which would occur to persons skilled in the art upon reading the foregoing description.

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FORM 5
THE PATENTS ACT, 1970
(39 of 1970)
&
The Patents Rules, 2003
DECLARATION AS TO INVENTORSHIP
[See section 10(6) and rule 13(6)]

1. NAME OF APPLICANT

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hereby declare that the true and first inventor of the invention disclosed in the complete specification is

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Dated this January 21, 2015

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