INTRODUCTION

Nitrogen is naturally present in the soil but it cannot be completely utilized by the plants. So, it is supplemented to the plants artificially with the help of fertilizers. Nitrogen is an important source which is essential for the growth of plant. A urea is the most widely used water soluble plant nitrogen source. Due to leaching the nitrogen content in the soil gets decreased due to leaching. So NUE (Nitrogen utilization efficiency) is low. Urea modified hydroxyapatite particles have been employed to agriculture, because of their higher NUE and slow release of the nitrogen to the soil. These maximize the NUE utilization by plants and minimize the adverse effects to the environment. Hydroxyapatite was prepared using chemical combination of ortho phosphoric acid and calcium hydroxide. Then prepared colloidal hydroxyapatite was kept in refrigerator for overnight. Powdered hydroxyapatite molecule was prepared in different concentration (10%, 20%, 30% and 40%) and was mixed with nanourea molecule using ultrasonicator. Urea modified composite molecule was applied to the field green gram (Vigna radiata) as both seed treated and directly applied to soil. Urea modified HA particle has the highest pH of 7.81 compared to that of normal urea. Seed germination was observed in all the pot. HA particle treated seed shows the good germination of 100% towards the yield whereas urea alone gives only 70% of seed germination. Height of the plant clone of HA treated as 11.8cm for 5days time interval in contrast Urea alone treated has only 10cm and density also low compared to HA treat. Nitrogen release was estimated by using nitrate estimation. Nitrate estimation of HA particle was calculated as 100µg/ml whereas urea treated was calculated as 50µg/ml. In this context, we concluded that Hydroxyapatite molecule shows the good utilization of nitrogen source. So it has the better yield than other conventional fertilizer.

Key words: Nitrogen Utilization Efficiency, Hydroxyapatite, Vigna radiata, Fertilizer.

ABSTRACT

Fertilizers are chemical compounds applied to promote plant growth. It is applied either through the soil or by foliar feeding. Artificial fertilizers are inorganic fertilizers formulated in appropriate concentration to supply the nutrients. Nitrogen is an important source which is essential for the growth of plant. Nitrogen is naturally present in the soil but it cannot be completely utilized by the plants. So, it is supplemented to the plants artificially with the help of fertilizers. Basically the nitrogen applied using conventional fertilizers, with particle size dimensions greater than 100mm, is lost to the soil due to leaching, so nitrogen utilization efficiency (NUE) by plants is low[10]. This leaching occurs due to water-soluble nitrates, emission as ammonia and nitrogen oxides and soil microorganisms-mediated incorporation into soil organic matter [11]. To reduce the leaching and to improve the NUE the surface area of the fertilizer to nanosize. And this strategy involves the slow-release of fertilizer also [11].

Controlled-release fertilizers

Controlled-release fertilizers provide an attractive alternative to granular fertilizers. These are fertilizer 'cocktails' that slowly release nutrients to the substrate [12]. The release depends on water availability or soil temperature. Controlled-release fertilizers are more expensive than the more common water soluble fertilizers, but they have several advantages:

• The danger of over-fertilizing is reduced as the release of fertilizers occurs gradually.
• Fertilizing is necessary only occasionally, sometimes only once in a season
• A balanced fertilizer mixture is provided at all times as the plants get what they need at different growth stages.

R.Subbaiya1, M.Priyanka1, M.Masilamani Selvam2

1Department of Biotechnology, K.S.Rangasamy College of Technology, Tiruchengode, Namakkal-637 215, Tamil Nadu, India.
2Department of Biotechnology, Sathyabama University, Jeppiaar Nagar, Chennai-600 119, Tamil Nadu, India.

Received on:12-06-2012; Revised on: 17-07-2012; Accepted on:26-08-2012

MATERIALS AND METHODS

Collection of Rhodococcus Culture
Rhodococcus spp-2891 was obtained from National Collection of Industrial Microorganism, Pune, India. The obtained species was inoculated in MGYP media which acts as growth media for its growth and proliferation.

Preparation of Hydroxypatite Nanoparticles
HA nanoparticles are to be synthesized by using aqueous solution of Ca(OH)_2 and H_3PO_4. First take 19.29gm of Ca(OH)_2 to be dissolved in 250ml of distilled water then prepare 0.6M H_3PO_4(250ml). Add H_3PO_4 to the Ca(OH)_2 suspension in dropwise, while stirring it vigorously under mechanical agitation (1000 rpm) \[ \text{6H}_3\text{PO}_4 + 10\text{Ca(OH)}_2 \rightarrow \text{Ca}_3(\text{PO}_4)_2 + 18\text{H}_2\text{O} \]
The synthesized HA nanoparticles are to be refrigerated overnight to form precipitate.

Biological Synthesis
Urea solution were prepared at different concentrations like 0.2mM, 0.4mM, 0.6mM, 0.8mM & 1mM. The synthesized HA nanoparticles are to be refrigerated overnight to form precipitate.

Preparation of Hydroxypatite Nanoparticles
HA nanoparticles are to be synthesized by using aqueous solution of Ca(OH)_2 and H_3PO_4. First take 19.29gm of Ca(OH)_2 to be dissolved in 250ml of distilled water then prepare 0.6M H_3PO_4(250ml). Add H_3PO_4 to the Ca(OH)_2 suspension in dropwise, while stirring it vigorously under mechanical agitation (1000 rpm) \[ \text{6H}_3\text{PO}_4 + 10\text{Ca(OH)}_2 \rightarrow \text{Ca}_3(\text{PO}_4)_2 + 18\text{H}_2\text{O} \]
The synthesized HA nanoparticles are to be refrigerated overnight to form precipitate.

Synthesis of Nanourea
0.005 mol of urea molecule are to be mixed with 5% of 10ml Trisodium citrate.
- Mixture is heated to 70°C for 30minutes
- Color gets changed into ash color solution
- It indicates the presence of Nanourea molecule
- It is further confirmed by using UV spectrophotometer (580nm)

Composite Preparation
About 0.05gm of nanourea is to be added to the 100ml of HA nanoparticles and this mixture is subjected to ultrasonic mixing (30 KHz for 1hr). The resulting mixture is then allowed to settle and the excess liquid is drained off. Now the nanourea modified HA particles are subjected to lyophilization to remove the moisture content present in it. The nanourea modified HA nanoparticles are characterized using UV spectrophotometer, FTIR and SEM.

Characterization Studies
The characterization studies were performed through UV-spectrophotometer, FTIR & SEM analysis.

Encapsulation
The nanocomposite mixture was then encapsulated into the hydroxyapatite using ultrasonicator to enhance the slow release of nitrogen to the plants.

Field Studies
The soil is collected from K.S.Rangasamy College of Technology and the green gram are to be treated with chemically synthesized and biologically synthesized nanourea in different concentrations and they are to be potted in a small pot.

Estimation of nitrogen (AOAC, 1990)
Measure one gram of sample in 100 ml conical flask and add 3 ml of salicylic acid (3.2% in concentrated sulphuric acid) and a pinch of sodium thiosulphate. Digestion is done on a hot plate after adding 5 ml of hydrogen peroxide till the digest become colourless. Simultaneously, a blank is also run. The contents are neutralized with sodium hydroxide and transferred to a 100 ml volumetric flask, then add one ml of 10% sodium potassium tartarate and one ml of Nessler’s reagent. The solution is made up to the mark with distilled water and the absorbance is measured at 420 nm using a spectrophotometer. Ammoniacal nitrogen (ammonium sulphate) is used as the standard.

Estimation of Urea by DAM method
0.2 to 1ml of standard was taken in the test tubes marked as S1-S5. The given unknown solution was made up to 100ml with distilled water and take 0.5 and 1ml in test tubes marked as T1 and T2. Simultaneously the blank is also prepared which is marked as B. All the test tubes were made up to 1ml with distilled water. 3ml of orthophosphoric acid solution. The content of the test tubes followed by 3ml of orthophosphoric acid solution. The content of the test tubes was mixed well and kept in boiling water bath for 30°C for colour development and cooled. Then the colour developed was read calorimetrically at 540nm. Draw a graph by taking the concentration in X-axis and the optical density on Y-axis. From which calculate the amount of urea present in the whole of the given unknown solution.

Peroxidase Assay
Pipette out 3ml of already prepared Phosphate buffer (pH 7.0) solution in the series of test tubes marked as T1, T2, T4 and T3. 0.05ml of guaiacol solution was added to each test tube. Add 0.03ml of H_2O_2 solution into each test tube. As soon as the solution is transferred into the cuvette, 0.1ml of respective enzyme extract was added, mixed well and the absorbance was measured at 436nm. The presence of enzyme activity was detected by reading the absorbance for every 10min.

The enzyme activity was calculated using the formula,
\[
\text{Enzyme Activity (U/ml) = } \frac{\text{A}_436 \times 4 \times \text{V}_t \times \text{Dilution factor}}{\text{Min} \times \text{Molecular Extension Coefficient} \times \text{Vs}}
\]

RESULTS
The study on chemical and biological synthesis of urea nanoparticles by the Rhodococcus spp., and its field studies were carried out in this work using Vigna radiata. For chemical synthesis the appearance of ash colour formation during heating suggested the formation of urea nanoparticles. The synthesized nanourea particles were encapsulated inside the hydroxyapatite particles by performing ultra sonication. UV-spectrophotometer reading was taken for the conformation of the nanourea particles. The biologically synthesized nanourea have shown the turbidity and for further conformation UV-spectrophotometer reading was taken, then SEM & FTIR reading was taken and then finally the nanourea was observed in reduced size. The FTIR spectrum of the encapsulated material clearly indicated that the structural integrity of nanourea-modified HA nanoparticles was maintained. Then prepared composite was lyophilized, then the particle size and morphology of the synthesized samples were studied using a Scanning Electron Microscopy(SEM) \[ \text{Siemens, Germany} \]. The chemical nature and molecular bonding of the synthesized samples were studied using Fourier Transform Infra Red Spectroscopy(FTIR) in a range from 600 to 4000 cm\(^{-1}\) using attenuated total reflectance (ATR) technique. The field studies were performed using green gram (Vigna radiata) as a plant model.

Inoculation
The Rhodococcus spp., was inoculated into the SCN media with different concentration of urea solution and kept for incubation (3-4days). The 0.4mM shows the maximum growth and high absorbance at 324nm. So, it was then mass cultured, filtered using membrane filtration and the sample was lyophilized. The lyophilized sample was characterized using FTIR, SEM. Then the field studies was performed using the lyophilized sample.

The pellet at the concentration of 200 mg showed the maximum growth and high absorbance at 206nm. Then the culture was lyophilized and the char-
Characterization studies were performed using FTIR, SEM and the field studies were carried out using this composite.

**Fig. 1. Supernatant solution.**

**0.4mM Supernatant Solution**
The supernatant solution does not show any colour difference in the tubes this indicates the absence of nanourea synthesis by the *Rhodococcus* spp, extracellularly.

**Fig. 2. Pellet Solution.**

**0.4mM Pellet solution**
The 0.4mM pellet solution shows turbidity in its test tubes this indicates the reduction of urea size (i.e.,) nanourea synthesis by the *Rhodococcus* spp., intracellularly.

**Table 1. Peroxidase assay.**

<table>
<thead>
<tr>
<th>Test sample</th>
<th>OD at 436nm</th>
<th>Enzyme activity (U/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
<td>After 10min</td>
</tr>
<tr>
<td>Sample 1</td>
<td>0.084</td>
<td>0.101</td>
</tr>
</tbody>
</table>

**Calculation for Peroxidase Assay**

Enzyme Activity (U/ml) = \( \frac{\Delta A_{436} \times 4 \times V_t \times \text{Dilution factor}}{\text{Min} \times \text{Molecular Extension Coefficient} \times V_s} \)

Where,

- \( V_t \) - Volume of total sample
- \( V_s \) - Volume of test sample

Enzyme Activity (U/ml) = \( \frac{\Delta A_{436} \times 4 \times V_t \times \text{Dilution factor}}{\text{Min} \times \text{Molecular Extension Coefficient} \times V_s} \)

**Characterization Studies**

**UV-Spec reading for the 0.4mM *Rhodococcus* solution**
The 0.4mM solution where the *Rhodococcus* was inoculated has shown the maximum growth and production of nanourea at a 324nm.

**UV-Spectrophotometer reading for pellet solution**
The 0.4mM pellet solution has shown the maximum growth and production of nanourea at a 295nm.

**FTIR studies**
The chemical nature and molecular bonding of the synthesized samples were studied using Fourier Transform Infra Red Spectroscopy (FTIR).
Different forms of composites:
The composites were prepared in different forms (i.e.,) chemically synthesised and encapsulated into the hydroxyapatite particle, biologically synthesised nanourea are given below:

POT Studies
The pot studies were carried out using Vigna radiata as a plant model. Chemically and biologically synthesised nanourea was used and the results obtained are given below:
Table 2. Measurement of growth of Vigna radiata by applying different forms of prepared Nanourea.

<table>
<thead>
<tr>
<th>S.No</th>
<th>Days</th>
<th>Biologically synthesized nanourea using 0.4mM HAp filterate (Cm)</th>
<th>Chemically synthesized nanourea using 0.4mM HAp (Cm)</th>
<th>Biologically synthesized nanourea using 200mg of pellet solution (Cm)</th>
<th>Normal urea (Cm)</th>
<th>Control (Cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>4th day</td>
<td>3.6±0.212</td>
<td>3.9±0.188</td>
<td>3.06±0.144</td>
<td>2.9±0.124</td>
<td>2.8±0.169</td>
</tr>
<tr>
<td>2.</td>
<td>5th day</td>
<td>3.80±190</td>
<td>4.1±0.190</td>
<td>3.3±0.169</td>
<td>3.13±0.151</td>
<td>2.9±0.178</td>
</tr>
<tr>
<td>3.</td>
<td>6th day</td>
<td>4.1±0.222</td>
<td>4.5±0.216</td>
<td>3.5±0.190</td>
<td>3.3±0.151</td>
<td>3.1±0.178</td>
</tr>
<tr>
<td>4.</td>
<td>7th day</td>
<td>4.4±0.216</td>
<td>4.7±0.241</td>
<td>3.8±0.196</td>
<td>3.6±0.169</td>
<td>3.1±0.205</td>
</tr>
<tr>
<td>5.</td>
<td>8th day</td>
<td>4.6±0.241</td>
<td>5.1±0.249</td>
<td>4.06±0.222</td>
<td>3.8±0.169</td>
<td>3.4±0.196</td>
</tr>
<tr>
<td>6.</td>
<td>9th day</td>
<td>4.9±0.242</td>
<td>5.3±0.268</td>
<td>4.3±0.276</td>
<td>4.03±0.196</td>
<td>3.6±0.196</td>
</tr>
<tr>
<td>7.</td>
<td>10th day</td>
<td>5.2±0.222</td>
<td>5.6±0.294</td>
<td>4.5±0.268</td>
<td>4.3±0.222</td>
<td>3.7±0.190</td>
</tr>
<tr>
<td>8.</td>
<td>11th day</td>
<td>5.5±0.223</td>
<td>5.8±0.284</td>
<td>4.8±0.294</td>
<td>4.5±0.237</td>
<td>3.9±0.216</td>
</tr>
<tr>
<td>9.</td>
<td>12th day</td>
<td>5.8±0.241</td>
<td>6.2±0.262</td>
<td>5.0±0.329</td>
<td>4.73±0.259</td>
<td>4.06±0.237</td>
</tr>
<tr>
<td>10.</td>
<td>13th day</td>
<td>6.1±0.241</td>
<td>6.5±0.241</td>
<td>5.26±0.347</td>
<td>5.03±0.259</td>
<td>4.23±0.259</td>
</tr>
<tr>
<td>11.</td>
<td>14th day</td>
<td>6.4±0.243</td>
<td>6.8±0.212</td>
<td>5.46±0.349</td>
<td>5.26±0.284</td>
<td>4.36±0.237</td>
</tr>
<tr>
<td>12.</td>
<td>15th day</td>
<td>6.8±0.243</td>
<td>7.2±0.188</td>
<td>5.66±0.351</td>
<td>5.46±0.284</td>
<td>4.53±0.259</td>
</tr>
</tbody>
</table>

DISCUSSION

- The major advantage of the above slow release fertilizer is to improve the crop efficiency and higher crop yield by providing the nutrients through slow release to the plants.
- Due to its slow release property it results in reduced environmental damage from leaching of nitrogen, compared to conventional water soluble fertilizers.
- This fertilizer composition may maximize the NUE while minimizing the adverse effects to the environments due to use of large quantities of fertilizer in agriculture.

ACKNOWLEDGEMENT

Authors are thankful to the management of KSR College of Technology and Head of the Department Dr. P. Ponmurugan for providing the facilities to carry out the research work.
REFERENCES


Source of support: Nil, Conflict of interest: None Declared