FABRICATED NANO-ZEOUREA FERTILIZERS: BIOSAFETY STUDIES

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Abstract
Recent developments on nano-fertilizers gained importance in crop production. Nano-zeolite entrapped the urea through theirmeso, nanopores (<2nm) and voids. In this study fabricated nano-zeolite based nitrogen nano-fertilizers and referred as nano-zeourea. Its nitrogen use efficiency (NUE) and transformation pathway were determined with ^{15}N labelled using Irugur soil series light textured loamy sand soil. The 10% atom ^{15}N excess used for the preparation of nano-fertilizer tracer study. In order to assess the toxicity of nano-zeourea with different biosafety studies were conducted against maize seed (NK 6240) germination (Phytotoxicity test) for 5 days with 6 levels of fertilizers, soil beneficial microorganism culture for 3 days with pre-weighed quantity of fertilizers by 6 levels, bacterial and fungal assays with agar diffusion method for 3 days and earthworms exposed for 14 days (Acute toxicity- growth test). All experiments were studied using standard OECD methods. Significant results were recorded with maize NUE, recovery and retention in soil. The tracer study revealed that the increased NUE of nano-zeolite based nano N fertilizer. With the use of ^{15}N tracer the exact pathway of movement of N from source to sink has been identified in plant in the present study. Among the treatments, the seeds treated with @ 500 mg of nano-zeourea were recorded maximum germination (100 %) while it was minimum in seeds treated with @1000mg (90 %) observed after 5 days and germination percentage was maintained 96.7 % on higher concentration of nano-zeourea @ 1250mg and 1500 mg.The healthy roots were developed regardless of treatments.The results of germination test revealed that use of nano-zeourea safe on maize. The bacteria and fungi were not inhibited by nano-zeourea treatments. We had not observed any inhibition zone regardless of different treatments. The result showed that the nano-zeourea treatments were not toxic to the bacteria and fungi. The average weight of 10 earthworms in each treatment was increased @ 4-6 g than the initial weight on nano-zeourea treatments. Weight was improved after 14 days (4.13 g) @ 250 kg N of nano-zeourea than (2.73 g) @ 250 kg N of urea fertilizer application. As a result the increased earthworm length was recorded (0.3 cm) @ 250 kg N of nano-zeourea than 250 kg N of urea (0.01 cm) and which indirectly explained that there is nil effect earthworm survival rate and growth.Our preliminary studies have shown that nitrogen nano-formulations are safer to beneficial soil microorganism's bacteria and fungi (*Enterobactercloacae*, *Trichoderma harianum*), earthworms (*Eiseniafoetidae*) and maize (*Zea mays*) seed germination.The use of nanoporous zeolite could be beneficial with respect to nutrient slow release, retention in soil and their use efficiency. Despite the fact that the N use had improved tremendously, more research need to be undertaken under protected conditions and open field situations to validate the results obtained.

Key words: Nano-fertilizers, Nano-zeolite, Nano-zeourea,^{15}N isotope, Toxicity tests

Introduction

Nitrogen (N) is considered as "*element of sun"* which is important constituent of chlorophyll and it captures the energy of the sun for aiding plant growth. On plant nutrition, among the nitrogenous fertilizers urea (46% N) is highest N containing solid conventional

fertilizer. White coloredprill urea is water soluble (52% at 20ºC). The nitrogen use efficiency (NUE) by crops is very low (30-35%) due to the loss of N to the tune of 50 - 70% by leaching, volatilization and microbial mineralization and other transformation. Indiscriminate use of N fertilizers caused major detrimental impacts on the soil biodiversity and functioning of the non-agricultural ecosystems due to eutrophication of aquatic systems. Particularly it causes detrimental effect on subsoil and aquatic system of proximal fields. In addition, there can be gaseous emission of N reacting with the stratospheric ozone and the liberation of toxic ammonia into the atmosphere. In order to combat this, newer fertilizer compounds that are needed to improve their use efficiency. Conventional N fertilizers are solid and low efficiency with improper release as their own is a serious problem to every field that wants to be taken seriously. Nitrogen losses can be reduced through various inputs and methods (Manikandan*et al.*, 2013).

Cyrus and Reddy (2011) reported that zeolite could be a good substrate for slow N release in soil. We had taken practical feasible steps in improving the efficiency and also to mitigate the practice's impact on crop production. We utilized the available natural materials like micro and nano formulations of zeolite to increase the nutrient use efficiency using nano and mesopores (Manikandan and Subramanian, 2014). In order to improve, slow and controlled release fertilizer compounds like nanoporous zeolite based N fertilizers were developed for crop production.Utilizing novel nanotechnology in fertilizer research to their best advantage and ensuring the improved nutrient use efficiency in agricultural sciences. Nanocomposites and Nano-fertilizers are symbol of protected and sustainable agriculture prosperity (Subramanian and Tarafdar, 2009 and 2011).

Nano-fertilizers and nano composites can be used to control the release of nutrients (De Rosa *et al.*, 2010) from the fertilizer granules so as to improve the nutrient use efficiency while preventing the fixation or loss of nutrients to the environment (Subramanian and Tarafdar, 2009) and supply with range of nutrients in desirable proportions (Datta, 2011). Zeolite and nanoporous zeolite used as a slow release fertilizer in farming (Gholizadeh, 2008: Ramesh *et al.*, 2010). Zeolite incorporated urea, potassium sulphate and calcium hydroxyapatite as a slow release nano-fertilizer increased availability for 60 days (Kottegoda*et al.*, 2011). Sarkar (2011) synthesised clay polymer nutrient nanocomposite using crystalline and non-crystalline components of soil clays increased biomass yield. Nanocomposite fabricated using nanoclays and zeolite for maize as a slow release fertilizer which regulated N availability up to 45-49 days (Sharmila, 2010).

Komarneni (2009) demonstrated that modified zeolites with occluded ammonium and nitrate showed good promise to be a slow-release N fertilizer. Nanotechnology could be applied in environmental soil science with respect to slow-release fertilizers in crop production and pollutant remediation at industrial areas.Kardaya*et al.* (2012) reported that zeolite or urea-impregnated zeolite as slow-release ammonia or SRU agent was potential in decreasing ruminal ammonia, pH, acetate to propionate ratio, methane, and maintaining low plasma urea within its physiological range. They reduced loss of N on limited extent. But the fate of crop uptake, retention pattern, recovery and NUE need to be known. Nano-materials exhibits large surface area and its toxicity on environmental issues and concerns must be assessed prior to commercialization (Lakshmanan et al 2012). Chakhalyan*et al.* (2008) reported that complex of zeolite and nitrogen fixing microorganisms exceeding the efficiency of the known bacterial nitrogen fertilizers and ecologically safe biofertilizer. The stimulating action was observed of zeolites upon the growth and propogation of *Azotobacterchroococcum*.In this study our objective is to gain information on effect of fabricated nitrogen nano-fertilizers i.e. nan-zeoureaon their fate of ^{15}N labelled nanozeourea, recovery and NUE of maize seed germination, soil microorganisms and earthworms.

Materials and Methods

All the biosafety studies were performed with maize [*Zea mays*] Hybrid NK6240 and light textured soil collected from BhavaniSagar. Experiments were performed under net house.

Enumeration of total bacterial population

All glassware were sterilized in a hot air oven at180°C for three hours or autoclaved at 1

atm for 15 min according to the requirement. All growth media, broth and water blanks were steam sterilized in an autoclave at 1 atm pressure for 15 min and all microbiological procedures were carried out in the laminar flow champer. Rhizosphere soil sample was serially diluted up to 10^{-6} and the aliquots of 10^{-6}, 10^{-4} and 10^{-3} were transferred in 1 ml quantities to sterile petridishes to assess the bacterial, fungal and actinomycetes populations. Five replications were maintained per dilution and poured with soil extract agar medium (Allen, 1953) and incubated at 28±1°C. After 24-48h of incubation total bacteria appearing in plates were counted and the population was estimated on soil dry weight basis, expressed in $cfug^{-1}$ of dry soil.

Nitrogen use efficiency- ^{15}N isotope study

A pot study was conducted with maize hybrid NK 6240 on loamy sand soil collected from Agricultural Research Station, Bhavanisagar. This soil is classified as Irugur Series in alfisols. The ^{15}N urea fertilizer obtained from M/s Rashtriya Chemicals & Fertilizers Limited., Mumbai, were used for ^{15}N labelling and ^{15}N dilution experiment. To know the fate of ^{15}N-urea loaded nano-zeolite fertilizer and nitrogen use efficiency. Applications of labeled fertilizer N were made as per the treatment and ^{15}N applied plants were removed at harvest stage. Treatments consisted of control, 100% N as urea, 50% N as nano-ferilizer, 100% N as nano-fertilizer replicated 5 times in a CRD. Leaf, stalk, cob sheath and grains were separated dried, weighed and ground for analysis. The post-harvest soil samples were also collected. Plant parts and soil were subjected to ^{15}N assay. ^{15}N labelled urea (10 % atom ^{15}N excess) was used instead of ordinary urea for the preparation of nano-fertilizer as per the treatment.

Nitrogen was applied through ^{15}N urea (10% atom excess), equivalent to 50 and 100 % of nano-fertilizer applied at the recommended dose of 250 kg ha^{-1}. Split dose of 250 kg N ha^{-1} was applied on soil weight basis to the treatments at the time 10, 25, 45 and 60^{th} DAS in near the root zone of plants. The ^{15}N isotope composition of plant was determined by mass spectrometry.Plant samples collected at the harvest stage were segregated as leaf, stover, cob sheath and grains and analysed for their total N. Thereafter, the ^{15}N assay was made. The non-applied plants were taken as the reference crop and compared with applied plants.

The plant samples were digested by adopting micro-kjeldahl digestion procedure (Piper, 1966). The digested samples were distilled into 2% boric acid solution containing double indicator (Bromocresol green and Methyl red) and titrated with 0.1N H_2SO_4 (Bures*het al*., 1982). The acidified boric acid solution containing ^{15}N as ammonium sulphate was evaporated at 90°C on a sand bath to reduce the volume to about 3 ml. The contents were then evaporated at room temperature until it dried to flakes. The boric acid flakes were transferred to screw cap vials, stored and used for ^{15}N assay.The urea samples were fed in micromass 622 VG ISOGAS mass spectrophotometer to quantify ratio analysis was performed as per the procedure outlined by Buresh et al. (1982) and Prude*net al.* (1985). The urea reacted with sodium hypobromite to evolve nitrogen gas which was fed in the inlet of the mass spectrometer under vaccum condition. The current of major ion beam (M) and minor ion beam (m) were recorded and ^{15}N abundance was calculated.

Isotopic abundance was expressed in term of ^{15}N atom per cent and calculated using the following relationship.

$$^{15}N \text{ atom } \% = \frac{R}{R+2} \quad X \quad 100$$

Where,R = Measured ratio between the ion currents corresponding to the mass 28 ($^{14}N^{14}N$ and mass 29 ($^{14}N^{15}N$)

$$R = \frac{m}{(M+1)\ 100}$$

Where, m = current of minor ion beam ($^{14}N\ ^{15}N$)

M = current of major ion beam ($^{14}N\ ^{14}N$)

The labeled^{15}N recovered by crop as well as retained in soil were calculated as detailed in the technical document IAEA (1983).

Phytotoxicity test

Biosafety of nano zeolite fortified N fertilizer against maize seeds conducted at biosafety laboratory of the Department of Nano Science and Technology. Seed germination considered as a rapid phytotoxicity test method.

The 10 seeds were put into petri dishes on three layers of blotter paper together with 5 mL treatment solution (a suspension that contains 0.5g of substrates and 20mL deionised water). Seeds were kept in dark at ambient temperature for 5 days. Every day we poured deionised water for control and treatment solution for the other variants to determine seed germination. The experiment contacted with six concentrations of nanozeourea 250, 500, 750, 1000, 1250, 1500 mg per kg seeds along with control. The experiment replicated for three times with completely randomised block design (CRD) design. Seed germination rate, emergence of seedlings, root elongation and growth in seedling stage were studied on synthesised fertilizers.

Against soil beneficial microorganisms

Biosafety of nano-zeolite fortified N fertilizer against soil beneficial microorganisms was studied for 3 days at Central Institute for Cotton Research, Nagpur, as per the OECD guidelines. Microbial culture was prepared poured small quantity in petridish. Nanozeourea did not get dissolved and therefore nano-fertilizers were added in pre-weighed quantity of agar medium. Before pouring into plates at different concentrations of nanozeourea are incorporated viz 0 (T_1), 5 (T_2), 10 (T_3), 15 (T_4), 20 (T_5), and 25mg (T6) respectively, per petridish with 3 replications of completely randomised block (CRD) design. Hence the microorganisms are exposed to the nanozeourea preferably for the period of 3 days.

Antibacterial and Antifungal assay

The antibacterial activities were determined using agar diffusion method. Bacterial culture was grown in nutrient broth at room temperature (28°C) for 60 to 72 h. *Enterobacter cloacae* bacterial culture of having cell density 10^7 cells per ml was used. 100 microlitre of culture is spread over the plate with the help of 'L' shaped spreader. The plates were incubated at room temperature for 60 to 72 h. Finally, the diameter of inhibition was measured (Boakye*et al*., 1977). The antifungal assay was carried out using agar diffusion method. Sterile dimethyl sulfoxide (DMSO) was used to dissolve the test sample. Sabouraud dextrose agar (SDA) was prepared by mixing Sabouraud 3% glucose agar and agar-agar in distilled water. The required amount of fungal strain (*Trichoderma harzianum*) was suspended in 2 ml Sabouraud dextrose broth (SDB). This suspension was uniformly streaked on petri plates containing SDB media by means of sterile cotton swab. Compounds were applied into agar medium using same technique for bacteria. These plates were then checked for the presence of zone of inhibition and result was noted (Hadacek and Greger, 2000).

Against Earthworm

Biosafety of nano-zeolite fortified N fertilizer against earthworm was studied for 14 days as per the OECD guidelines. Three hours prior to being placed in the test vials, worms are placed on moist filter paper so they can void their guts, and are then washed and dried before use. Soil was blended with nano-fertilizer @ 0 (T_1), urea (T_2), 50 (T_3), 100 (T_4), 150 (T_5), 200 (T_6) and 250 (T_7) Kg Nha^{-1} with 3 replications of completely randomised block (CRD) design Red earthworm (*Eisieniafoetida)* was introduced @10 nos. per pot measuring a dimension of (30 cm dia x 10 cm height) introduced after one week. The earthworms were measured for their behaviour with length and weight gain besides survival rates on 7^{th} and 14^{th} day of experiment (OECD, 1984 and Edwards *et al*., 1995). The data collected from phytotoxicity and acute toxicity of earthworm experiments were subjected to analysis by variance (P = 0.05) with mean separation by least significant difference (LSD) as well as by DMRT as per Panse and Sukhatme (1978).

Results and Discussion

Perspective on fabricated fertilizers (nanozeourea) toxicity effects on seed, microorganisms and earthworm were studied using OECD methods. Decreased particle size and increased surface area of nanozeourea play a major role in physiochemical and growth and other behavioral properties of test material.The nanozeourea (1:1 ratio) had 5 % moisture content on oven-dry basis (w/w). The colour of the zeourea was 5YR 7/2. The bulk density and particle density were 0.49 and 0.62Mg m^{-3} respectively. The percent porespace and pore volume were 10 % and 0.5 ml, respectively. It had slightly alkaline (pH 7.6) and low EC 0.27dSm^{-1}. The cation exchange capacity of the nano-zeo-urea was 63 $cmol_{(+)}$ kg^{-1}. The N content of nano-zeo urea was 28.5%.

The microbial population of soils was significantly influenced by different forms of urea formulations with the exception of fungal population in Inceptisol (Table 1). The highest bacterial and actinomycetes populations of 25.9 x 10^{-6} and 4.2 x 10^{-3}, respectively, in Inceptisol that received nano-zeo-urea fertilization. The microbial population was 46.7% and 47.6% higher than urea fertilized soils. Even zeo-urea fertilized soils had higher values by 40.5% and 21.4% for these microbial populations in comparison to urea fertilized soils. Zeolite blending with urea also had an effect on microbial populations. All the three microbial populations were significantly influenced by fertilizer formulations. The response to nano-zeo-urea fertilization was more pronounced than other urea formulations. The best treatment registered higher values for bacteria, fungi and actinomycetes. The microbial populations enumerated in the best treatment were 26.9 x 10^{-6}, 5.3 x 10^{-4} and 4.2 x 10^{-3}, respectively. The microbial populations were 49%, 5.6% and 54.7% higher in nano-zeo-urea fertilized soils in comparison to urea fertilized soils. Microbial response to added urea formulations was less pronounced for zeolite blended urea formulations.

Nitrogen contents in stover and grains were significantly higher in nano-zeo-urea than other treatments (Table 2). In grains, nano-zeo-urea fertilized maize plants had 28% higher N content (0.76%) in comparison to urea fertilized plants (0.48%).Nano-zeo urea fertilized plants @ 50% and 100% were compared. The data on dry matter, total N and N uptake were consistently higher for 100% than 50% fertilization regardless of any portion of the maize plants such as grain, leaf, cobsheath and stover. Major portion of dry matter accumulated in grain, followed by stover, leaves and cobsheath. Similarly, N content and uptake were also followed the similar trend of response. The percentage N derived from the fertilizer was computed using the standard set of formulae. The highest Ndff was registered in stover of both set of treatments.One-fifth of N in got accumulated in the nano-zeo-fertilized stover of maize plants while it was one-fourth when the fertilization was done at 50% dose. On the other hand, grains Ndff was higher in 100% (9.1%) than 50% (19.6%). The recovery of N was the higher in grains of 50% fertilized plants. Overall, the N use efficiencies in the order of 46.1, 17.49, 4.67 and 3.40% for grains, stover, leaves and cobsheath, respectively. The cumulative N use efficiency was 71.7% while it is reduced to 48.7% when the fertilization was done @ 100% nano-zeo-urea. The highest population of bacteria, fungi and actinomycetes were observed in treatment involving nanozeourea. The lowest population was registered in the urea. Soil beneficial microbial activities increased with use of fabricated fertilizers. Nanozeourea slow release fertilizer stimulated the growth of microbes by providing nutrients and directly increased the population. An increased availability of N, which is preferentially assimilated by microorganisms (Paul and Clark, 1996), but normally rather limited in soil, enables an increase in activity of the microbial biomass. Andronikashvili*et al.* (2008) found that zeolite application increased quantity of the soil microflora especially on azotobacter.

With the use of ^{15}N tracer the exact pathway of movement of N from source to sink has been identified in plant in the present study. Grain N, being the ultimate sink gains translocated N from the major source of N contained in stover and cob sheath. Uptake of N in stover was high, and fertilizer N contributed for the same. This has been evidenced from %Ndff of stem which was moderate. One-fifth of N in got accumulated in the nano-zeo-fertilized stover of maize plants while it was one-fourth when the fertilization was done at 50% dose. On the other hand, grains Ndff was higher in 100% (9.1%) than 50% (19.6%). The recovery of N was the higher in grains of 50% fertilized plants. Overall, the N use efficiencies in the order of 46.1, 17.49, 4.67 and 3.40% for grains, stover, leaves and cobsheath, respectively.The cumulative N use efficiency was 71.7% while it is reduced to 48.7% when the fertilization was done @ 100% nano-zeo-urea.

The true efficiency of added N fertilizer measured as total %15N recovery (NUE) ranged from 36.5 to 53.8. The 30 per cent assessed by the contribution of urea alone. Application of 50% nanozeourea registered the highest overall N use efficiency (53.8 %) assessed by the contribution of urea and nanozeolite fortification. Split and Close placement of nutrients in the nanozeourea near the surface where roots proliferate caused very

high mgNdff that has been reflected in ^{15}N recovery under nanozeourea. The tracer study revealed that the increased nitrogen use efficiency of zeolite based nano N fertilizer.The use of nanozeolite could be beneficial with respect to nutrient slow release, retention in soil and their use efficiency.

Phytotoxic effects of nanozeourea were tested with maize seeds. The results of maize germination test explained that the use of nanozeourea not showed any detrimental effect on germination. Germination test was carried out for 5 days. Among the treatments, the seeds treated with @ 500 mg of nanozeourea were recorded maximum germination (100 per cent) while it was minimum in seeds treated with @1000mg (90 per cent) observed after 5days. They germination percentage was maintained 96.7 % on higher concentration of nanozeourea @ 1250mg and 1500 mg. The healthy roots were developed regardless of treatments. The results of germination test revealed that use of nanozeourea safe on maize (Table 3). The results of seed germination showed no effect of seed germination. The critical seed germination fixed at 10% and we recorded 96 to 100% germination. The risk of ammonia evolution from urea reduced by impregnation with nanozeolite and thus reduced the ammonia volatilization with the meager slow release behavior and thus nil effect on maize seed germination. But, ammonia inhibitory effect, ammonium fertilizer band application is highly toxic to seed germination andammonia enters in plant system through passive diffusion along with ammonium ion. Similar results were observed by aerobic rice conditions by Bremner and Krogmeier (1989), Zhang *et al.* (2006), Oancea and Oancea (2010) and Haden *et al.* (2011).Bremner and Krogmeier (1989) reported that the adverse effect of urea fertilizer on seed germination in soil is due to ammonia formed through hydrolysis of urea by soil urease (biuret/ phenylphosphorodiamidate). Zhang *et al.* (2006) recorded the slow /controlled release fertilizers cemented and coated by 4 kinds of cementing and coating agents were safe to wheat seed emergence and Oancea and Oancea (2010) on corn seed germination.

The interactions of nanozeourea on beneficial soil microorganisms were demonstrated with bacteria (*Enterobactercloacae*) and fungi *(Trichoderma harianum)*. Experiment was conducted for 3 days with @ 0, 5, 10, 15, 20, 25 mg of nanozeourea. The bacteria and fungi were not inhibited by nanozeourea treatments (Table 4). We had not observed any inhibition zone regardless of different treatments (Table 5). The result showed that the nanozeourea treatments were not toxic to the bacteria and fungi. The soil beneficial microorganism growth was recorded on culture treated against nanozeourea of 0 to 25mg on 3rd day. All the concentration did not cross the minimum inhibitory concentration. The microorganism growth was observed on all surface of agar medium. Zone of inhibition not occurred within the 0-25mg concentration of nanozeourea against bacteria and fungi. Colony forming units were observed on all concentration. Similar results were found in Wei *et al.* (2011) reported the colonisation of clinoptilolite particles by microorganisms was demonstrated, revealing spontaneous adhesion to preferentially sheltered areas as pits. The microbial abundance ranged from single cells to dense biofilm-like aggregations on the cratered zeolite's surface.Idoko*et al.* (2013) furfural-urea used as the primary ligand in this study is a slow release nitrogen fertilizer which releases nitrogen by hydrolysis and microbial activities. Contradictory results shown that NO-loaded Zn^{2+} exchanged zeolite: PTFE (50:50) materials have potent anti-bacterial properties against examples of Gram-negative and Gram-positive bacteria (Fox *et al*., 2010).

The study of the toxicity of nanozeourea treatments had showed that the treatments had no effect on the earthworms *Eiseniafoetidae* on survival rate (Table 6). The experiment was carried out for 14 days. There was no mortality in nanozeourea treatments @ 50, 100 and 200 kg N on 7th and 14th days of experiment. The results recorded that the earth worm survival rate was high (96.7, 93.3 %) on 7th and 14th days after experiment of 250 kg N of nanozeourea. The reduction on population was observed @ 150 and 250 kg higher dose of nanozeourea. With respect to urea the earth worm survival rate was low (83.3, 80.3 %) on 7th and 14th days after experiment of 250 kg N of urea. The earthworm growth was observed all the treatments. The average weight of 10 earthworms in each treatment was increased @ 4-6 g than the initial weight on nanozeourea treatments. Weight an improved after 14 days (4.13 g) @ 250 kg N of

nanozeourea than (2.73 g) @ 250 kg N of urea fertilizer application. As a result the increased earthworm length was recorded (0.3 cm) @ 250 kg N of nanozeourea than 250 kg N of urea (0.01 cm) and which indirectly explained that there is nil effect earthworm survival rate and growth. The obtained results of the present study indicate that 0-250 kg of nanozeourea in their acute toxicity to earthworm.

Higher concentrations of ammonia evolution caused earth worm dermal abrasion and at extreme case impart death. Although ammonia highly toxic to earthworms the evolution of reduced concentration of ammonia volatilization from nanozeourea did not show any impact on earth worm survival and growth increased with nanozeolite also served as a food substrate to earth worm. Different results observed by Iordache and Borza (2010) the highest number of earthworms was recorded in the treatment with the largest dose of nitrogen fertilizer (by 85.85% higher compared to the control treatment). Abbiramy and Ronald Ross (2013) reported that the lethal toxic concentration of urea to *E.fetida* was evaluated as 28µg/cm^2 and the lethal concentration for 48h was 10mg/5ml concentration. Despite these formulations enhanced the nutrient use efficiencies, more research is requited to validate the results of greenhouse protected environmental studies.

Conclusion

With the use of ^{15}N tracer the exact pathway of movement of N from source to sink has been identified in plant in the present study. The use of nanoporous zeolite could be beneficial with respect to nutrient slow release, retention in soil and their use efficiency.Theresults of this study have shown that nano-formulation is safer to beneficial soil microorganisms (bacteria *Enterobactercloacae* and fungi *Trichoderma harianum*), earthworms *Eiseniafoetidae* and maize seed germination.

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Table 1. Effect of zeolite based N fertilizers in soil microorganism (CFU g^{-1}) on post-harvest soil

Treatments	Inceptisols			Alfisols		
	Bacteria (10^{-6})	Fungi (10^{-4})	Actinomycetes (10^{-3})	Bacteria (10^{-6})	Fungi (10^{-4})	Actinomycetes (10^{-3})
T_1- Urea	13.8	5.8	2.2	13.7	5.0	1.9
T_2-Zeolite +Urea	19.6	4.9	1.4	19.7	4.3	2.0
T_3- Nanozeolite + Urea	22.5	5.2	1.8	23.3	4.4	2.6
T_4- Zeourea	23.2	4.1	2.8	22.8	5.7	2.4
T_5-Nanozeourea	25.9	4.5	4.2	26.9	5.3	4.2
S.Ed	2.13	0.61	0.28	1.26	0.36	0.43
CD (0.05)	4.36	NS	0.58	2.59	0.75	0.88

Table 2. Recovery of added ^{15}N labeled nanozeourea(NZU) fertilizer in maize parts and post-harvest soil

Treat.	Portion	Dry matter (g)	TN (%)	mg N in plant/soil	%Ndff	mg Fertilizer N added	mgNdff	Recovery	NUE
^{15}N Recovery of maize									
50% NZU	Grain	46.4	1.55	717.8	19.6	300	138.4	46.1	71.7
	Leaf	25.2	0.88	219.6	6.7	300	14.0	4.7	
	Cobsheath	10.2	0.74	75.6	13.5	300	10.2	3.4	
	Stover	33.5	0.71	239.1	21.9	300	52.5	17.5	
100 % NZU	Grain	65.6	1.72	1130.9	9.1	600	101.8	17.0	48.7
	Leaf	33.1	1.15	381.8	12.4	600	47.7	7.9	
	Cobsheath	12.6	0.73	92.1	17.1	600	15.9	2.6	

	Stover	44.3	1.08	478.8	26.5	600	126.8	21.1	
Soil Retention									
	Soil	Weight (g)	N %	N (g)	%Ndff	Fert N	mgN dff	Retention	
50% NZU	Soil	5000	0.12	5875	2.5	300	146.6	48.8	-
100 % NZU	Soil	5000	0.13	6500	2.1	600	136.3	22.5	-

Table 3. Phyto toxicity of nano-zeourea formulation on maize germination

Treatments (mg/1000 seeds)	**T_1- Control (water alone)**	**T_2- NF-250**	**T_3-NF-500**	**T_4- NF-750**	**T_5- NF-1000**	**T_6- NF-1250**	**T_7- NF-1500**	**S.Ed**	**CD (0.05)**
Maize Germination (%)	96.7	96.7	100	96.7	90	96.7	96.7	10.66	NS

Table 4. Minimum Inhibitory concentration (mg) of nano-zeourea fertilizer

Microorganism	**Concentration/Agar medium (mg)**				
	5	**10**	**15**	**20**	**25**
Enterobacter cloacae	+	+	+	+	+
Trichoderma harzianum	+	+	+	+	+

+ Presence of growth, -Absence of growth

Table 5. Antimicrobial activity (mm) of nano-zeourea fertilizer

Microorganism	**Concentration/Agar medium (mg)**				
	5	**10**	**15**	**20**	**25**
	Zone of Inhibition (mm)				
Enterobacter cloacae	-	-	-	-	-
Trichoderma harzianum	-	-	-	-	-

Table 6. Acute toxicity of nano-zeourea against earthworm

Treatments (Kg N ha^{-1})	**Survival rate (%)**		**Weight(g)**			**Length (cm)**		
	7^{th}day	**14^{th} day**	**Initial**	**14^{th} day**	**Increased**	**Initial**	**14^{th} day**	**Increased**
T1-Control	100	100	5.44	11.51	6.07	20.92	22.06	1.14
T2-Urea-250	83.3	83.3	5.68	8.41	2.73	21.17	21.18	0.01
T3-NF-50	100	100	5.34	11.08	5.75	19.92	20.45	0.53
T4-NF-100	100	100	5.49	10.96	5.47	19.61	20.19	0.58
T5-NF-150	93.3	93.3	5.62	10.26	4.64	20.57	20.84	0.27
T6-NF- 200	100	100	5.58	10.03	4.45	19.64	20.08	0.44
T7- NF-250	96.7	93.3	5.35	9.48	4.13	19.89	20.19	0.3
S.Ed	10.69	10.60	-	-	0.55	-	-	0.06
CD (0.05)	NS	NS	-	-	1.18	-	-	0.13