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SYNTHESIS OF NITROGEN NANO FERTILIZER AND ITS EFFICACY

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ABSTRACT

Nitrogen nano fertilizer was synthesized using zeolite as a carrier material at a laboratory scale. X-ray diffraction (XRD) analysis confirmed the incorporation of the fertilizer material into zeolite. Chemical analyses also indicated the sorption of fertilizer material into zeolite. An *in vitro* incubation study was conducted for 30 days at field moisture condition to see the release of the fertilizer materials and was compared with a conventional fertilizer. The release pattern of nutrients from either source showed a substantial decreasing trend with time although the release of N was higher for nano fertilizer than the conventional one. A pot culture experiment with *Ipomoea aquatica* (Kalmi) was also conducted to see the efficacy of the nano fertilizer in the growth promotion of the plant. Analysis showed higher accumulation of N in plants grown with nano fertilizer. Post effect of nano fertilizer application in soil showed better pH, moisture, CEC and available nitrogen under nano fertilizer treatment than the conventional fertilizer.

Keywords: Nano fertilizer, synthesis, efficacy, N-efficiency.

INTRODUCTION

Modern agriculture involves the use of, among others, a substantial amount of inorganic fertilizers - a greater portion of which is removed from the realm of soil once the crop is harvested. Making the plant growth to approach its genetic limit is what the growers are striving for now-a-days (Tisdale *et al*., 1990). Resorting to replace these nutrients is the ultimate choice.

Globally, crop yields have increased by at least 30 to 50% as a result of fertilization (Stewart *et al*., 2005). Agricultural development has provided much evidence that fertilizer application is the most efficient measure for substantially increasing crop production and ensuring food security (Bockman *et al*., 1990) and that sustained yield growth is difficult without fertilizer supply (Larson and Frisvold, 1996). Statistics suggests that, about 40-70% of the nitrogen of the applied fertilizers is lost into the environment and is not utilizable by crops, which not only causes large economic and resource losses but also is instrumental to very serious environmental pollution (Guo *et al*., 2005).

Efforts have been made in the past and are being tried at present to overcome this problem of fertilizer use. Many approaches have been made to increase the fertilizer use economy. Among them the notables are: application of adequate amount of fertilizer (s); deep placement of fertilizer (s); use of granular urea; improving crop response knowledge (Brady and Weil, 2005) and use of slow release nano fertilizer (Ahmed *et al*., 2012).

Nano fertilizer, the most important field of agriculture has been to the attention of the soil scientists as well as the environmentalists due to its capability to increase yield, improve soil fertility, reduce pollution and make a favorable environment for microorganisms (Ahmed *et al*., 2012). In the present study the rate of release of nutrients from laboratory synthesized nano fertilizer and its effects on crop production have been compared with ordinary chemical fertilizer.

MATERIALS AND METHODS

The whole experiment was divided into i. synthesis of nano fertilizer in the laboratory, ii. physical and chemical characterization of the product, iii. release characteristics of the synthesized nano fertilizer in soil and iv. pot experiment with plants. Zeolite was used as the fertilizer carrier. Commercially available zeolite (AnalaR, BDH) was procured from the local market. Synthesis of nano fertilizers was accomplished in two steps:

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(a) Synthesis of Surfactant Modified Zeolite

According to Banishwal *et al.* (2006) surfactant modification of the zeolite was carried out using hexadecyltrimethyl ammonium bromide (HDTMABr). A pre-weighed quantity of zeolite was mixed with HDTMABr solution (200 mg/L) in a 1:100 (solid: liquid) ratio. The mixture was agitated for 7-8 h at 150 rpm on an orbital shaker and then filtered. The solid residue was washed with double-distilled de-ionized water and oven dried for 4-6h. The synthesized Surfactant Modified Zeolite (SMZ) was then mechanically ground with a mortar and pestle into fine particles. As the surfactant is the only source of carbon in the system, the surfactant loading was monitored by total organic carbon (TOC) analysis of the initial and final solutions obtained during the synthesis of SMZ.

(b) Synthesis of Nano Fertilizer

To prepare nano fertilizer, required quantities (~170 g) of SMZ were stirred with 1.0 M solution of $(NH_4)_2SO_4$, for 8 h and filtered, washed three times with de-ionized water, and oven dried. The solid: liquid ratio was 1:10 for the synthesis of nitrogen loaded zeolites. The amount of nitrogen incorporated, was calculated from the difference of the quantities of these elements in the unmodified zeolite and that in the synthesized zeolite (Banishwal *et al.*, 2006). X-ray Diffraction (XRD) analysis of the two zeolites was done to confirm the incorporation of the fertilizer elements.

For the *in vitro* incubation and macrocosm study, soil samples were collected from an agricultural field close the working laboratory following the procedures of USDA (1951). The geo-location of the sampling site is 23°53.147 N and 90°24.809 E. The processing and preservation of the collected soil samples were done as described in Imamul Huq and Didar (2005).

For the pot culture experiment, a leafy vegetables commonly known as Kalmi shak or Kankong (*Ipomoea aquatica*) was used. The treatments were control, conventional fertilizers and the synthesized nano-fertilizer. Urea was used as the conventional fertilizer. The amounts of each nutrient from either source were kept at the same level.

Pots of 2kg sizes were used. The fertilizer requirement was assessed following the Fertilizer Recommendation Guide of BARC (BARC, 2012). The soils in each pot were mixed with the required amounts of fertilizer except for control. The pots were arranged in a completely randomized design and were set in a net house.

Seeds of Kangkong (6-7) were sown in each of the pots and allowed to germinate. After germination, 4 seedlings were kept in each pot. Plants received watering every day. Tap water was used for watering; intercultural operations were carried out whenever necessary.

The plants were harvested carefully from the pots by uprooting them after 30 days of emergence. Processing and preparation of the plant samples are as described elsewhere (Roy *et al*., 2012).

Various physical, chemical and physico-chemical properties of the soil samples were analyzed in the laboratory (Imamul Huq and Didar, 2005). After harvesting of plants the soils were again analyzed to monitor the effect of nano fertilizer on soil after a period of time.

In-vitro incubation study was conducted to see the release characteristics of the elements from the synthesized nano fertilizer using the same categories of soil. 250gm of 5mm sieved soil was used for the study. The procedure followed is similar to what has been described in Chowdhury *et al.* (2010). The period of incubation was 0, 15 and 30 days. Analytical procedures followed were as described earlier.

All data were statistically analyzed by using Microsoft Excel and MINITAB (version 15) packages.

RESULTS AND DISCUSSION

Preparation and characterization of nano fertilizer

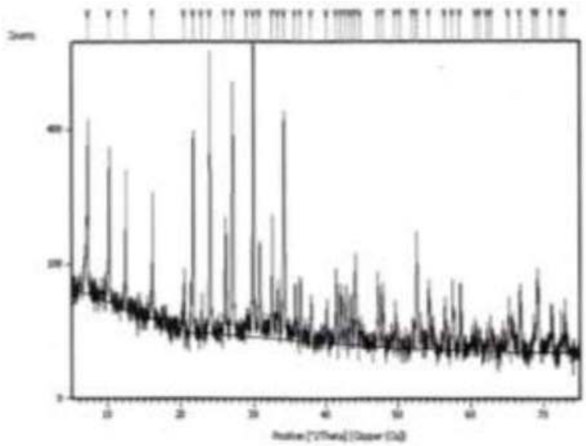
The collected zeolite was analyzed in the laboratory before the synthesis of nano fertilizer. Some properties of the zeolite were measured after which surfactant modification was done. The changes in organic carbon percentage (from 0.084 to 0.21%) and CEC (from 35.71 meq to 48.57% meq) confirmed the modification. The initial N content of the zeolite was very low which was raised to a higher level after the synthesis of the nano fertilizers. This confirmed the successful incorporation of the fertilizer elements onto the modified zeolite (Table 1).

Table 1. Some chemical properties of Zeolite and the Synthesized Fertilizer.

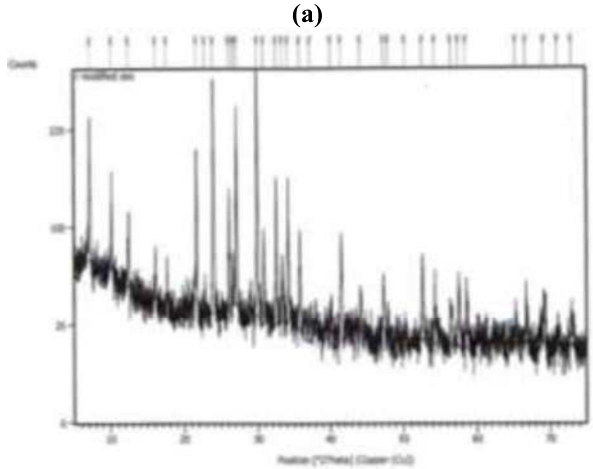
Properties	Zeolite	Synthesized Fertilizer
Total Nitrogen (%)	ND*	1.78
Available Nitrogen (%)	-	0.38

* ND = not detected

The X-Ray diffraction (XRD; Cu Kα as the source for X-rays) analysis of zeolite, surfactant modified zeolite and nano fertilizer was done for final confirmation. The d-spacing values of different samples gave the confirmation. The results of XRD are given in Figure 1(a-c).



(a)



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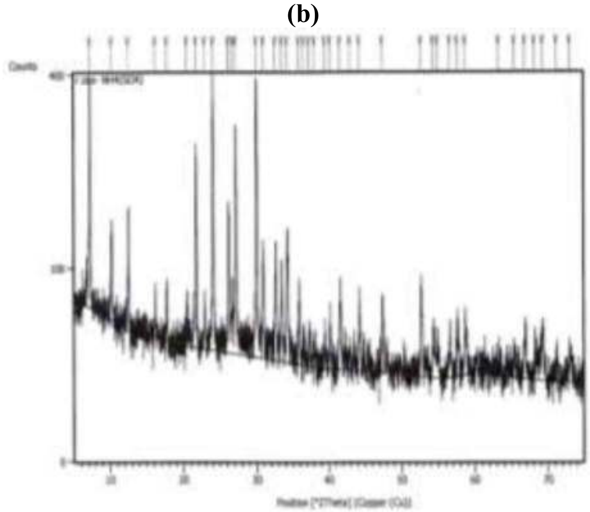

(c)

Fig. 1. XRD of the subsequent materials (a) natural Zeolite and (b) surfactant modified Zeolite and (c) Zeolite Incorporated with $(NH_4)SO_4$

The XRD analysis of surfactant modified Zeolite showed changed position and height of the peak compared to the unmodified Zeolite (Fig. 1a and b). The *d*-spacing values of the prominent peaks are: 12.27±0.01, 8.68, 4.10, 3.70±0.01, 3.28, 2.98, 2.75, 2.68 and 2.62 Å.

Comparing the surfactant modified zeolite with the N incorporated zeolite it has been observed that the position and height of peak has changed in the latter. The peak height showed an increase (Fig. 1b and c). The *d*-spacing values of the prominent peaks are 12.28±0.15, 8.68±0.06, 4.10±0.02, 3.70±0.01, 3.28±0.01, 2.75±0.01, 2.68±0.01 and 2.62±0.01 Å. Closely matched *d*-spacing values of all of these zeolites are suggestive of a containment of the zeolite structure whereas the varied peak height is indicatives of positive modification (Banishwal *et al*., 2006).

Initial characteristics of soil

Some common physical, chemical and physicochemical properties of the soil were analyzed before the setup of the experiment in order to know the initial nutrient status of the soil. The experimental soil was silty clay in texture, acidic in reaction (pH 5.92). The soil contained 1.58% organic matter, total organic carbon 0.92%, total N 0.1%, total P 0.07%, total K 125.85% meq, total S 3.18%, available N 0.002%, available P 0.001%, available K 0.19% meq, available S 0.0007% and CEC 5.79% meq. The moisture content of the soil was 22.54%.

Efficacy of the synthesized nano fertilizer

Efficacy of the nano fertilizer was assessed through (a) *In-vitro* release under laboratory condition and (b) macrocosm study with plant growth.

(a) *In- vitro* incubation study

Soil pH

The pH decreased for any day of sampling (0, 15 and 30). The initial pH of the conventional fertilizer and nano fertilizer treated soil was higher than the control soil. On the following days, pH of all soils regardless of their treatments decreased. However, the decrease was slow in the final phase of the experiment. In every case, the pH of nano fertilizer treated soil was lower than the control soil except for S - nf (0 days) (Table 2).

A higher initial pH due to the application of nano fertilizer could be related to the alkaline nature of zeolite. The reason for decreasing pH may be because of maintaining moist condition. Regression analysis was done for the treatments and the slope is low indicating the fact that the nano fertilizer has very slight positive effect on soil pH.

Soil Moisture

Zeolite can act as water moderator and can absorb it up to 55% of their weight (Pisey *et al*., 2011) so it is likely that zeolite based nano fertilizer application could improve water-holding capacity of a soil. With this view in mind, moisture percentages in the different treated soils were determined after each incubation period. It is interesting to note that, although similar amount of water was added

Table 2. pH of soil at different incubation days after application of nano fertilizer.

Incubation Days	pH		
	Control	Conventional fertilizer	Nano fertilizer S – nf
0	4.96	5.23	5.83
15	4.93	4.47	4.75
30	4.56	4.39	4.50

Table 3. Moisture content of soil after application of nano fertilizer at different incubation days.

Incubation Days	**Moisture Content (%)**		
	Control	Conventional fertilizer	Nano Fertilizer S – nf
0	20.82	20.7	20.52
15	21.56	27.58	30.15
30	13.22	23.53	25.87

Table 4. Available nitrogen of soil after application of nano fertilizer at different incubation days.

Incubation Days	Available nitrogen (mg/kg)		
	Control	Conventional fertilizer	Nano Fertilizer SN –nf
0	20	30	250
15	20	40	200
30	14	20	260

to each soil for moistening purpose, the nano fertilized soils, however, retained more water compared to control and conventional fertilizer applied soils (Table 3). This is an indication that zeolite based nano fertilizer could also improve the water use efficiency (WUE).

Regression analysis shows that the slope is relatively steeper indicating that the nano fertilizer has a positive effect on soil moisture.

Available Nitrogen

The effects of application of nano fertilizer on available nitrogen are presented in Table 4. The release of inorganic nitrogen was prominent in case of nano fertilizer throughout the entire experiment and all the experimental units exhibited the same trend, though at different degrees. The control soil contained less N than the rest. On Day 15 of incubation, the conventional fertilizer treated soil showed an increase followed by an eventual decrease. The nano fertilizer incorporated soils showed slight increase on Day 30 of incubation. The N content in soil remained high in the nitrogen incorporated zeolite and released higher percentage of available nitrogen as compared to the others. This observation is commensurate with that of Junxi *et al*. (2013). Regression analysis shows that the slope is very steep indicating the fact that the nano fertilizer has a positive significant effect on soil mineral nitrogen.

Percent release of nitrogen of conventional fertilizer and nano fertilizer showed that conventional fertilizer has an initial lower value followed by an increase and decrease at 30 days whereas, nano fertilizer showed an initial higher rate followed by a decreasing trend and again an increase at 30 days of incubation and the rate was higher than conventional fertilizer (Fig. 2). This slowdown of the release in nano fertilizer could be due to a tight bondage of the ammonium ions in the nano pores of zeolite. The release is again increased after 30 days indicating the fact that the bonding might have been better and the nano fertilizer thus synthesized have the potential to release nutrients further.

(b) Macrocosm Study

Visual Symptoms

The germination, growth and visual appearance of the Kalmi plants were observed. It appeared visually that the growth of Kalmi was equally better in fertilized (conventional or nano) soils than the control. However, between the conventional fertilizer and nano fertilizer treatments, plant performance was better with the nano fertilizers.

No pest and insect infestations were observed on the leaves of Kalmi plants and soil showed firm consistency, better absorption of water and no subsidence or water logging condition. However, control and conventional fertilizer treated soils showed considerable subsidence.

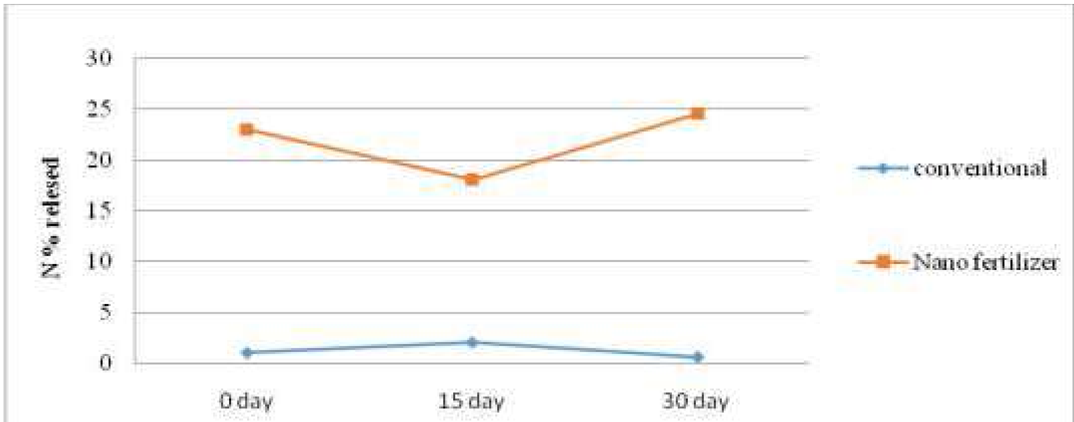

Fig. 2. Percent release of nitrogen by conventional and nano fertilizer at different incubation days.

Fresh and Dry Matter Production of Kalmi

The growths of Kalmi as affected by the various treatments (on fresh and dry weight basis) are shown in table5. It was observed that the fresh weight production of Kalmi was higher in nano fertilizer treated soil compared to the soil without any treatment. The fresh weight production on nano fertilizer treated soil was more or less same to that of conventional fertilizer. In case of dry weight (Table 5) the production was higher in the nano fertilizer treated soil than the untreated soil.

Table 5. Fresh and dry weight (g/100 plants) production of Kalmi plant (*Ipomoea aquatica*).

Treatment	Fresh Weight (g/100 plants)	Dry Weight(g/100 plants)
Control	67.67	4.3
Conventional	75.67	5.1
S –nf	76.00	5.33

Significant difference in fresh matter production could be related to a better water balance in the plants which could further be related to better water retention potentiality of nano fertilizer (Pisey *et al*., 2011).

An analysis of variance test showed that there is a significant effect of the treatments on the fresh weights and dry weight of Kalmi, P value is 0.000 in both cases. To test the efficiency LSD (Lest significance difference) was done and it appeared that the LSD of fresh weight and dry weights are 0.26 at 5% level.

Phytoavailability of Nitrogen

To assess the phytoavailability of nitrogen in the Kalmi plant at different treatments, the concentration and uptake of nitrogen were measured.

The concentration and uptake of nitrogen in Kalmi is presented in Table 6 and from the table it is observed that the nano fertilizer treatments caused an increased nitrogen concentration in the Kalmi plant. Nitrogen concentration was in the minimum for control plant (1.61%). The concentration of nitrogen was the same in case of conventional fertilizer and S - nf treated soil. Uptake of nitrogen (N) by the Kalmi plants was calculated by multiplying the concentration of nitrogen (N) in the plant with their corresponding dry matter production. It is observed from the analysis that uptake of nitrogen by the Kalmi plants was higher in both conventional fertilizer and nano fertilizer over control, however, it was better for the nano fertilizer treatments.

Table 6. The concentration and uptake of N in Kalmi plant.

	Nitrogen (N)	
Treatment	Concentration (%)	Uptake (mg/100 plants)
Control	1.61	69.77
Conventional	1.74	88.74
S –nf	1.74	92.80

ANOVA test indicated that there is a significant effect of the treatments on nitrogen concentration (P value 0.26) and on the uptake by the plant (P value 0.00). The LSD of uptake of N is0.26 at 5% level.

A balance sheet has been prepared to assess the fate of nitrogen in the system and it is presented in Table 7.

From Table 7 it is observed that, all the experimental pot initially contained 60 mg/pot of nitrogen except for control (20 mg/pot). Some of this nitrogen has been taken up by the Kalmi plants. So, the excess amount of nitrogen is supposed to remain in the soil after the crops have been harvested. The calculated values however, indicate that the entire N is not recovered; some amount is missing in the calculations. The percentage of missing nitrogen is 2.03 for nitrogen containing nano-fertilizer treated soil. The better balance sheet for N-containing nano-fertilizer

Table 7. Balance sheet of nitrogen (mg/pot) in different experimental pot (only inorganic fraction is considered).

N (mg/pot)	Experimental Plot		
	Control	Conventional	S –nf
Initial content in the soil	20	20	20
From different fertilizer source	0.00	40	40
Total N content in the pot (a)	20	60	60
Removed through plant uptake (b)	2.09	2.66	2.78
Present in soil after harvest (c)	10	40	56
b+c = d	12.09	42.66	58.78
Amount missing (a-d)	7.91	17.34	1.22
Percent (%) N not accounted for	39.55	28.9	2.03

Table 8. Changes in properties of soil after harvesting of Kalmi plant.

Treatment	pH	Moisture (%)	Organic Carbon (%)	CEC (meq%)	Available N (mg/kg)
Control	5.6 (5.9)	2.8 (4.6)	1.5 (0.92)	6.14 (5.79)	10 (20)
Conventional	5.6 (5.9)	2.7 (4.6)	1.7 (0.92)	5.93 (5.79)	40 (20)
S – nf	4.7 (5.9)	3.0 (4.6)	0.7 (0.92)	6.79 (5.79)	56 (20)

(The figures in the parentheses indicate the initial values)

indicates that the nano fertilizer synthesized was efficient. Moreover, one must understand that in this treatment, the source of N incorporated into nanoparticle was ammonium while in other pots the source of N was urea. Hence, the mineralization process of the added urea needs to be considered too.

After Effects of Nano Fertilizer

After harvesting of the plants, the properties of soils were measured and the changes are monitored in Table 8.

By comparing the properties of after harvest soil with initial soil properties (Table 8) it is observed that pH of the soils decreased slightly. The reason for decreasing pH may be due to root exudates of plant though it is almost in a good range for agricultural production. Regression analysis (R^2 = 100%) shows that the angle of the slope is low for pH indicating that the nano fertilizer has a significant negative effect on pH.

The moisture content of the soils has also decreased comparing with initial. It may be due to uptake of moisture by plants or by evapotranspiration loss. The Regression analysis (R^2 = 88.4%) shows that the angle of the slope is steep indicating that the nano fertilizer has a non-significant positive effect on soil moisture.

In case of organic carbon, treatment shows poor percentage than control comparing with initial. It could be due to release of exudates in soil but the decrease may be because of higher amount of available nitrogen in nitrogen incorporated nano fertilizer. Availability of nitrogen strongly influences the growth and abundance of organisms so that microorganism can release CO_2 as a product of decomposition of organic matter (Vitousek and Howarth, 1991; Shoun *et al*., 1992). Regression analysis (R^2 = 95.9 %) shows that the angle of the slope is low indicating that the nano fertilizer has a significant negative impact on OC.

Zeolites have a high cation exchange capacity and often used as inexpensive cation exchanger Millan *et al.* (2008) and Breck (1974). It may be the reason of increasing CEC of soils which is treated with nano fertilizer than the other. Regression analysis (R^2 = 93.8 %) shows that the angle of the slope is steep indicating that the nano fertilizer has positive significant effect on CEC.

The available nitrogen content of the after harvest soil are much higher than their respective initial values except for control soil. Available nitrogen is much higher in SN - nf treatment than the others. This may be because of the left-over fertilizer in soil and nano fertilizer holds higher amount of inorganic nitrogen than the conventional one. Regression analysis (R^2 = 60.2%) shows that the angle of the slope is steep indicating that the nano fertilizer has significant positive effect on available nitrogen.

CONCLUSION

The growth of Kalmi, its uptake and concentration of nitrogen (N) was better in nano fertilizer treatments than in the conventional fertilizer treatments indicating the fact that there is a scope of nano-fertilizer in crop agriculture. Using this in the farmers' level however, will need pilot scale synthesis of the fertilizer. The present study indicates a bright possibility of using nano technology in the fertilizer sector given the cost effectiveness is assessed.

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