



## Evaluation of urea loaded nanoclay biopolymer composites with Zn and P solubilizing microbes for nitrogen uptake and use efficiency in maize (*Zea mays*)-wheat (*Triticum aestivum*) cropping system

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### ABSTRACT

A field experiment was conducted during rainy (*khari*) 2022 (July 2022–October 2022) and winter (*rabi*) 2022–23 (November 2022–March 2023) seasons at ICAR-Indian Agricultural Research Institute, New Delhi to evaluate a series of Zn and P solubilizing microbial culture enriched nanoclay biopolymer composite (NCBPC) loaded with nitrogenous fertilizer (urea) and the efficiency of the products for maize (*Zea mays* L.) and wheat (*Triticum aestivum* L.). Experiment consisted of 10 treatments, viz. T<sub>1</sub>, Control; T<sub>2</sub>, 100% N through urea; T<sub>3</sub>; T<sub>5</sub>; T<sub>7</sub>; and T<sub>9</sub>, 75% N as urea loaded NCBPC-A (prepared using acrylic acid + acrylamide + mango kernel flour) alone or along with P or Zn or P + Zn solubilizers; T<sub>4</sub>; T<sub>6</sub>; T<sub>8</sub> and T<sub>10</sub>, 75% N as urea loaded NCBPC-B (prepared using acrylic acid + acrylamide + maize flour) alone or along with P or Zn or P + Zn solubilizers in a randomized block design (RBD) and replicated thrice. In both maize and wheat crop, highest grain (5.09 and 5.32 t/ha) and straw yield (6.56 and 7.45 t/ha), apparent N recovery (51.26 and 47.26%) and agronomic efficiency (12 and 13.3 kg grain yield obtained/kg N application) were obtained in treatment T<sub>10</sub> followed by T<sub>9</sub>. In addition, total N uptake significantly enhanced by 20.1–28.4% in maize and 22.1–30.8% in wheat (T<sub>9</sub> and T<sub>10</sub>); apparent nitrogen recovery (ANR) improved by 12.9–18.2 and 15.2–21.1% and agronomic efficiency (AE) triggered by 19.5–21.2 and 15.4–20.8% in maize and wheat crops respectively, under T<sub>9</sub> and T<sub>10</sub> treatments over standard urea fertilization (T<sub>2</sub>). Thus, the study concludes that, 25% N requirement could be cut down through application of 75% N (urea) loaded NCBPCs in conjunction with Zn or P or Zn + P solubilizing microbial culture as compared to sole urea application under maize-wheat cropping system.

**Keywords:** Maize-wheat cropping system, Nano clay bio-polymer composites, Nitrogen uptake, Nitrogen use efficiency, Zn and P solubilizing microbial culture

Prilled urea is commonly used as the primary nitrogen (N) source by farmers, but its rapid hydrolysis in the field leads to substantial nitrogen loss (60–70%) through volatilization, denitrification, nitrate leaching, and nitrous oxide emission. Globally, the synthetic nitrogen fertilizer supply chain is accountable for 10.6% of agricultural greenhouse gas (GHG) emissions, of which with 38.8% emissions stemming from N fertilizer production (Menegat *et al.* 2022). Several slow or controlled release fertilizers are developed to deal with these issues. Generally, slow-release fertilizers are produced by applying various coating material over urea granules to regulate N release at slower rate in soil systems so that a greater proportions of hydrolyzed

products become available to plants at critical growth stages. Recent research has concentrated on adding fertilizers into superabsorbent polymeric networks in order to deliver both nutrients and water with a single formulation (Zhan *et al.* 2004 and Guo *et al.* 2005). However, preparing those superabsorbent polymer compounds from only pure organic chemicals is non-economical (Mohan *et al.* 2005).

Nanoclay polymer/biopolymer composites (NCPCs/NCBPCs) were prepared by combining inorganic clays (kaolinite, illite and smectites) alone or along with naturally available starch (such as maize flour and mango kernel flour) into polymeric networks of acrylic acid and acrylamide (substituting a portion of acrylamide from polymer networks) could lower the cost of production, and retain sufficient amount of water and nutrients after loading. The NCPC/NCBPC fertilizers shown its effectiveness as slow-release fertilizer (Liang and Liu 2007) which can release absorbed nutrients at a slower rate as compared to conventional fertilizers in soil environment. The ability of NCPCs/

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NCBPCs for nutrient delivery has been well established by many researchers (Saurabh *et al.* 2019); but their suitability as carrier material for nutrient solubilizing microbes not studied thoroughly. Phosphorus solubilizing bacteria (PSB) and zinc solubilizing bacteria (ZSB) belongs to the genera such as *Bacillus* and *Pseudomonas*, can solubilize insoluble occluded phosphorus (P) and zinc (Zn) compounds and enhance its availability to plants in soil (Supanjani *et al.* 2006). It was reported that, addition of P and Zn solubilizing microbes in soil enhances microbial activity in root rhizosphere; produces plant growth hormone (indole-3-acetic acid) and various soil enzymes (phosphatase, protease, urease) had role in N transformation which helps in better plant growth and N uptake (Bargaz *et al.* 2021 and Lv *et al.* 2022). Thus, it is expected that application of 75% N loaded NCBPs along with P + Zn solubilizing microbes may produce similar or better crop yield, N uptake and N-use as compared to standard urea application under maize (*Zea mays* L.)-wheat (*Triticum aestivum* L.) crops. Therefore, an attempt has been made to study the feasibility and efficiency of urea loaded with NCBPCs along with Zn + P microbial cultures, on the yield, nitrogen uptake and use efficiency under maize-wheat crops.

MATERIALS AND METHODS

*Field experimental design and crop management practices:* A field experiment was conducted during rainy (*khariif*) 2022 (July 2022–October 2022) and winter (*rabi*) 2022–23 (November 2022–March 2023) seasons at ICAR-Indian Agricultural Research Institute, New Delhi to evaluate urea loaded NCBPCs in conjunction with Zn and P solubilizing microbial cultures as nitrogen and microbial culture delivery material involving maize-wheat crops (grown in sequence on the same piece of land). The experimental location has a subtropical climate, with hot and dry spell in April to June to wet summer spell in July to September and a cool and dry winter spell from October to March. Average rainfall ranged from 800–1200 mm, 75–80% of which occurred during monsoon season. The mean annual summer temperature ranges between 35–45°C (April–July) and whereas in winter temperature ranged from 8–20°C (December–February). The soil of the experimental field was sandy loam, low in organic carbon (0.39%) with slightly alkaline pH (7.82) (Supplementary Table 1). The experiment was laid out in randomized block design (RBD) with 10 treatments in three replications (5 × 5 m<sup>2</sup>) (Table 1).

Maize var. Pusa HQPM5 Improved (Maize) and wheat var. HD-2967 were sown as *khariif* (11<sup>th</sup> July, 2022) and *rabi* crops (13<sup>th</sup> November, 2022) in all the treatments, respectively. The recommended dose of fertilizer for both maize and wheat crops were 150:60:60 NPK kg/ha; nitrogen applied in 3 splits, 50% as basal and remaining in two equal splits at 30 days after sowing (DAS) and 60 DAS respectively (T<sub>2</sub>). In T<sub>3</sub>–T<sub>10</sub> treatments, 50% N applied as basal and remaining N applied at 35 DAS through NCBPCs. The P and K supplied by single super phosphate (SSP) and muriate of potash (MOP); full P and K applied as basal dose.

Table 1 Treatment details

T <sub>1</sub>	Control
T <sub>2</sub>	100% N through urea
T <sub>3</sub>	75% N (urea) loaded nanoclay biopolymer composite prepared using mango kernel flour (NCBPC-A)
T <sub>4</sub>	75% N (urea) loaded nanoclay biopolymer composite prepared using maize flour (NCBPC-B)
T <sub>5</sub>	75% N (urea) + P solubilizing culture loaded NCBPC-A
T <sub>6</sub>	75% N (urea) + P solubilizing culture loaded NCBPC-B
T <sub>7</sub>	75% N (urea) + Zn solubilizing culture loaded NCBPC-A
T <sub>8</sub>	75% N (urea) + Zn solubilizing culture loaded NCBPC-B
T <sub>9</sub>	75% N (urea) + P and Zn solubilizing culture loaded NCBPC-A
T <sub>10</sub>	75% N (urea)+ P and Zn solubilizing culture loaded NCBPC-B

Treatment details are given under Materials and Methods.

Both maize and wheat crops were harvested (October 2022 and April 2023, respectively) manually. After harvesting and threshing, grains were sun dried and the grain yield was reported at 12% moisture content.

*Synthesis of modified fertilizers and urea and microbes loading:* The NCBPCs were synthesized by graft polymerization reaction of acrylic acid and acrylamide along with bentonite clay in the presence of nitrogen gas (Liang and Liu 2007). Acrylic acid (AA) (5.76 g) and acrylamide (AM) (1.15 g) dissolved in 15 ml distilled water, then neutralized with liquid ammonia (60% neutralization reaction) in a four-neck flask equipped with a thermo-magnetic stirrer and nitrogen gas supply line. Required amount of nanoclay powder, starch, N, N-methelenebisacryamide (NN-MBA) and ammonium persulphate (APS) added in partially neutralized monomer solution of AA and AM; the final product is heated at 70°C until polymer product is formed. Maize flour and mango kernel flour at 20% by weight of acrylic acid were used as the sources of starch materials. The finished product is oven dried and grinded in to powder before loading. Nitrogen (urea) loading was done by adding required amount of urea solution (1:10 ratio) in fine grained NCBPC at 1:1 N to NCBPC (weight) ratio in a tray and kept for 24–48 h at room temperature.

Microbial bio-inoculum and urea loading was done through adding of pre-weighed dry gels into the solution of urea. After urea loading, required quantities of microbial inoculum (P/Zn/P + Zn both) added in the swollen polymer materials (containing sufficient moisture) and mixed properly so that microbial formulation got retained in the surface and pores of the swollen polymer materials. Thereafter, the N + microbial inoculum loaded swollen gels were air dried for 3–7 days and used in the field.

*Plant sampling and analysis:* For chlorophyll estimation, fresh plant leaf samples were collected and determined by

non-maceration method (Hiscox and Israelstam 1979). For estimation of N in grain and straw samples crops were harvested at maturity. The collected plant biomass dried under diffused sunlight followed by oven drying at 70°C. Total nitrogen content of grain and shoot samples were analyzed by Kjeldahl digestion–distillation method (Buresh *et al.* 1982). Root samples were collected by uprooting fresh plants by digging surrounding soil carefully and after soil particles adhered to roots removed by washing with clean water. Later root surface area and volume was measured by root scanner.

**Statistical analysis:** The data obtained from the field experiment was subjected to analysis of variance appropriate to the experimental design. The means of all the treatments were separated by using LSD test at 5% level of significance using software Statistical Analysis System (SAS). The apparent N recovery and agronomic N use efficiency were calculated as:

$$\text{Apparent N recovery Efficiency (ANR) (\%)} = \frac{N \text{ uptake in fertilized pot} - N \text{ uptake in control pot}}{\text{Amount of fertilizer application}} \times 100 \quad (\text{Eq. 1})$$

$$\text{Agronomic nitrogen use efficiency (ANUE) (kg grain produced/kg N}_2 \text{ fertilizer application)} = \frac{\text{Yield under fertilized plot} - \text{Yield under control plot}}{\text{Amount of fertilizer application}} \quad (\text{Eq. 2})$$

## RESULTS AND DISCUSSION

**Plant growth and root parameters:** Application of urea + Zn + P solubilizing microbial culture loaded NCBPCs

significantly ( $P < 0.05$ ) influenced plant chlorophyll content, plant height, root volume and root surface area in wheat and maize crops (Supplementary Table 2 and 3). The total chlorophyll content of the maize and wheat crops varied from 1.38 ( $T_1$ )–2.37 ( $T_{10}$ ) mg/g and 1.23 ( $T_1$ )–2.15 ( $T_{10}$ ) mg/g, respectively (Supplementary Table 2). In both wheat and maize crops, highest total chlorophyll content, Chl a and b were in both 40 and 60 DAS respectively recorded under  $T_{10}$  treatment followed by  $T_9$ , where urea + Zn + P solubilizer loaded NCBPCs were applied together (Supplementary Table 2). Highest plant height and total root surface area in both wheat and maize crops were recorded under  $T_{10}$  followed by  $T_9$  treatment (Supplementary Table 3). This may be explained by the biopolymer composites' ability to supply nitrogen in maize and wheat crops with a slower rate and consistent basis. Our results are in agreement with Zhao *et al.* (2013) that that application of controlled release nitrogenous fertilizer enhanced photosynthetic rate in maize due to higher chlorophyll content as compared to conventional nitrogenous fertilizer. Likewise, Ghafoor *et al.* (2021) reported that application of coated nitrogenous fertilizer improved chlorophyll content in wheat over standard urea. Application of P and Zn solubilizing microbes along with NCBPCs enhanced solubilization and mobilization of insoluble P and Zn compounds formed in soil (like initial fertilizer reaction products, like  $\text{CaHPO}_4$ ,  $2\text{H}_2\text{O}$  and  $\text{ZnCO}_3$ ) thus exhibits higher root volume and surface area under  $T_9$  and  $T_{10}$  (Khourchi *et al.* 2022).

**Crop yield and yield components, nitrogen uptake and nitrogen use efficiency:** The highest maize seed index and wheat grain test weight followed by grain and straw yield, grain N content, N uptake, ANR and ANUE for both the

Table 2 Maize grain and straw yield, N content, N uptake, apparent N recovery and agronomic efficiency under different treatments

Treatment	Number of grains per cobs	Test weight (Weight of 100 seeds) (g)	Grain yield (t/ha)	Straw yield (t/ha)	Grain N content (%)	Straw N content (%)	Grain N uptake (kg/ha)	Straw N uptake (kg/ha)	Total N uptake (by grain and straw) (kg/ha)	Apparent N recovery (%)	Agronomic efficiency (kg grain yield obtained/kg N applied)
$T_1$	276.00	22.54	3.09	5.26	1.21	0.31	43.56	14.35	52.46		
$T_2$	308.00	22.96	4.73	5.67	1.5	0.41	76.35	26.57	96.08	30.00	9.93
$T_3$	311.00	23.03	4.77	5.74	1.54	0.45	78.54	31.64	100.17	34.84	10.00
$T_4$	313.00	23.25	4.82	5.91	1.59	0.45	81.57	32.00	103.42	37.10	10.20
$T_5$	317.00	23.23	4.89	6.00	1.59	0.49	82.20	35.04	107.62	39.55	10.47
$T_6$	319.00	23.47	4.93	6.11	1.62	0.52	84.40	37.34	112.07	42.55	10.73
$T_7$	314.00	23.18	4.87	5.97	1.57	0.49	80.86	34.99	106.06	38.62	10.33
$T_8$	318.00	23.40	4.91	6.08	1.6	0.51	83.04	36.47	110.48	41.06	10.60
$T_9$	321.00	23.53	5.05	6.20	1.65	0.52	87.78	37.91	115.43	45.18	11.47
$T_{10}$	324.00	23.65	5.09	6.56	1.72	0.56	92.88	41.72	123.36	51.12	12.00
SEM±	4.66	0.32	0.05	0.08	0.02	0.01	0.74	0.63	1.54	0.49	0.04
LSD ( $P=0.05$ )	13.83	0.96	0.14	0.24	0.06	0.02	2.21	1.88	4.58	1.45	0.13

Treatment details are given under Materials and Methods. LSD test ( $P=0.05$ ) was performed for isolation of mean.

crops were registered under T<sub>10</sub> followed by T<sub>9</sub> in both the experiments followed by grain yield (Table 2 and 3). On an average T<sub>10</sub> and T<sub>9</sub> produced 7.61 and 6.77% higher maize yield, and 13.16 and 11.59% more wheat grain yield compared to T<sub>2</sub> receiving 100% N through urea (5.09 t/ha and 4.73/ha wheat and maize grain yield) respectively. Further, grain and straw yield obtained (in both maize and wheat crops) under treatments receiving 75% N through urea loaded NCBPCs along with single microbial culture (T<sub>5</sub>–T<sub>8</sub>) found statistically superior than those obtained at T<sub>2</sub>. As compared T<sub>2</sub> total N uptake (grain and straw combined) by maize and wheat registered to be 20–28% and 22–31% higher under T<sub>9</sub> and T<sub>10</sub> respectively. The apparent N recovery (ANR) obtained at T<sub>9</sub> and T<sub>10</sub> found greater by 10–16.28% in wheat and 10–15.4% more in maize over T<sub>2</sub>. Likewise, T<sub>10</sub> and T<sub>9</sub> showed 1.47 to 2 kg/kg greater agronomic N use efficiency (ANUE) in wheat and 1.87 to 2.13 kg higher ANUE in maize over T<sub>2</sub>. Similarly, total N uptake, ANR and ANUE recorded under T<sub>5</sub>–T<sub>8</sub> treatments found slightly higher than T<sub>2</sub> or statistically similar in T<sub>3</sub> under both the crops.

Better yield, nitrogen uptake and nitrogen recovery *vis-a-vis* agronomic efficiency under urea loaded NCBPCs + microbial culture loaded treatments T<sub>9</sub> and T<sub>10</sub> might be due to the following reasons i.e. (a) slower release of nitrogen from polymer composites and (b) greater availability of P and Zn to plants along with their indirect contribution to N uptake by crops. It was reported that addition of nanoclays (kaolinite/illite/bentonite) in the polymeric structure of acrylic acid and acrylamide reduces diffusion co-efficient of urea in soil and water from urea loaded nanoclay composite

formulation and regulate its release rate in soil environment (Sarkar *et al.* 2014). Substitution of a portion of acrylamide with starch materials (maize flour/mango kernel flour) further reduces N release rate from NCBPCs due to reduction in overall porosity of the polymer material. In our study, urea loaded NCBPCs render plant available NH<sub>4</sub><sup>+</sup> ion in soil for longer duration as compared to 100% urea treated T<sub>2</sub> which benefitted the crop plants to draw more N in important crop growth phases (Supplementary Fig. 1 and 2) as evidenced by Sarkar *et al.* (2014) in pearl millet crop. From a multi locational trial conducted in China, Ma *et al.* (2022) reported that use of slow-release fertilizers over commercially available urea improved wheat grain yield by 7.76% while reducing over overall N requirement by 15% over treatment received 100% recommended dose of fertilizer nitrogen. Similarly, Guan *et al.* (2014) used attapulgite based slow-release fertilizer, observed that 15.1–18.4% yield increase and 10–26.7% greater partial factor productivity of applied N fertilizers associated with control release fertilizers over control treatments where conventional uncoated urea fertilizers were used as nitrogen source. On the similar lines, in our study we obtained 6.5–7.67 and 11.5–13.1% higher grain yield in maize and wheat while reducing 25% N fertilizer requirement using urea loaded NCBPCs application. Other hand, further, NCBPCs absorbed almost 15 to 20 times more water (data not shown) and acts as good carried material for Zn and P solubilizing microbial culture by providing suitable micro environment and protection of microbe’s *vis-a-vis* supply water along with nutrients to crops under moisture stress conditions (acts as micro reservoir); therefore, enhance nutrient use efficiency at

Table 3 Wheat grain and straw yield, N content, N uptake, apparent N recovery and agronomic efficiency under different treatments

Treatment	Number of effective tillers	Test weight (weight of 1000 seeds) (g)	Grain yield (t/ha)	Straw yield (t/ha)	Grain N content (%)	Straw N content (%)	Grain N uptake (kg/ha)	Straw N uptake (kg/ha)	Total N uptake (by grain and straw) (kg/ha)	Apparent N recovery (%)	Agronomic efficiency (kg grain yield obtained/kg N applied)
T <sub>1</sub>	6.72	41.10	3.6	4.63	1.17	0.31	36.15	16.31	57.91		
T <sub>2</sub>	7.72	43.75	5.09	6.48	1.36	0.56	64.33	31.75	102.92	29.08	10.93
T <sub>3</sub>	8.15	44.08	5.1	7.03	1.39	0.59	66.30	33.87	110.18	31.81	11.20
T <sub>4</sub>	8.52	44.12	5.13	7.11	1.41	0.6	67.96	35.46	113.56	33.98	11.53
T <sub>5</sub>	8.60	44.30	5.17	7.15	1.44	0.62	70.42	37.20	117.24	36.77	12.00
T <sub>6</sub>	8.72	44.65	5.21	7.18	1.48	0.64	72.96	39.10	121.74	39.74	12.27
T <sub>7</sub>	8.58	44.18	5.15	7.14	1.43	0.61	69.64	36.42	115.84	35.73	11.87
T <sub>8</sub>	8.60	44.45	5.19	7.15	1.47	0.63	72.18	38.30	119.51	38.68	12.13
T <sub>9</sub>	9.62	44.89	5.32	7.29	1.5	0.64	75.75	39.68	125.69	41.98	13.07
T <sub>10</sub>	9.73	45.22	5.4	7.45	1.56	0.67	79.40	43.95	134.60	47.26	13.33
SEm±	0.12	0.67	0.07	0.10	0.02	0.01	0.74	0.63	1.54	0.77	0.05
LSD (P=0.05)	0.35	1.99	0.21	0.30	0.06	0.02	2.21	1.88	4.58	2.29	0.46

Treatment details are given under Materials and Methods. LSD test (P=0.05) was performed for isolation of mean.

the same time (Liang *et al.* 2007). After establishment, the nutrient solubilizing microbes solubilize P from insoluble P compounds (like  $\text{CaHPO}_4$ ,  $\text{CaH}_2\text{PO}_4$ ) and produced bacterially derived plant growth hormone (indole-3-acetic acid), made available to plants which results in better plant growth and yield under  $T_5$ ,  $T_6$ ,  $T_9$  and  $T_{10}$  treatments (Bargaz *et al.* 2021). Higher P availability helps crops to extract more P from soil ultimately more nitrogen uptake by triggering N transformation in soil through enhancing microbial activities in soil (Xia *et al.* 2023). Like PSB, ZSB solubilize  $\text{ZnCO}_3$  and increases Zn availability to plants; higher availability of Zn in soil triggers production of various soil enzymes such as protease and ureases play an important role in N transformation and increases the abundance of some microbes contributed in mineralization of soil organic matter like *Sphingomonas*, *Gemmatirosa* and *Flavisolibacter* etc. thereby increases availability of organic N to crop plants (Lv *et al.* 2022). Further greater availability of Zn to crop plants improved anthesis which showed positive influence on the number of seed per tiller (wheat) or cobs (maize); indirectly improved grain yield in both the crops (Mutambu *et al.* 2023).

From the result it can be concluded that use of 75% N (as urea) + Zn + P solubilizing microbial culture loaded NCBPCs prepared using mango kernel flour or maize flour can reduce N requirement by 25% over standard urea fertilization; could be recommended for enhanced crop production and for N use efficiency under maize and wheat crops. However further research is needed to reduce the overall production cost of NCBPC material and multi-locational field trials in different agro-ecosystems are required for further validation and broad recommendation.

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