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Synthesis of nano NP and nano P fertilizers to find out its effect on growth and nutrient content of wheat (*Triticum aestivum* L.) under controlled condition

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Abstract
Nano fertilizers are synthesized or modified form of traditional fertilizers by different chemical, physical, mechanical or biological methods with the help of nanotechnology used to improve soil fertility and productivity of agricultural produces. Nanostructure fertilizer exhibits novel physico-chemical properties, which determines their interaction with biological substances and processes. The study entitled was carried out in three parts, (i) Synthesis and characterization of nano NP and nano P fertilizers, (ii) optimization of nano NP and nano P fertilizers dose for wheat under *in vitro* and (iii) in green house study with using pots of 13 kg capacity of soil at Anand Agricultural University, Anand, Gujarat during 2021. In laboratory, the nano NP and nano P fertilizers were synthesized of different concentrations *viz*., 0, 500, 1000, 2000, 3000 and 4000 ppm by using direct precipitation method. The *in vitro* experiment conducted in laboratory was comprised of six levels of nano NP (0, 500, 1000, 2000, 3000 and 4000 ppm) and nano P (0, 500, 1000, 2000, 3000 and 4000 ppm) with 36 treatment combinations laid out in factorial completely randomized design (FCRD) with three repetitions. The greenhouse experiment was carried out based on the *in vitro* study. From the *in vitro* study, seed treatment of $NP_3 + P_2$ (nano NP @ 2000 ppm + nano P @ 1000 ppm) was found optimum dose for the wheat seedlings in terms of growth parameters. The results of greenhouse experiment reveled that foliar fertilization of $NP_3 + P_2$ (nano NP @ 2000 ppm + nano P @ 1000 ppm) and $NP_4 + P_2$ (nano NP @ 3000 ppm + nano P @ 1000 ppm) was found better at 21, 35 and 60 DAS than rest of the treatments in terms of growth, dry matter yield, nutrient content and uptake by plant.

Keywords: Nano fertilizer, growth, wheat and nutrient content

Introduction
Nano-fertilizers are the important tools in agriculture to improve crop growth, yield and quality parameters with increase nutrient use efficiency, reduce wastage of fertilizers and cost of cultivation. The main reason for high interest in nano-fertilizers is mainly their penetration capacity, size and very high surface area which is usually differ from the same material found in bulk form. Nano-fertilizers are not only eco-friendly but also reduce environment pollution. Nano-fertilizers are highly effective for precise nutrient management in precision agriculture with matching the crop growth stage for nutrient and may provide nutrient throughout the crop growth period. Nano-fertilizers increase crop growth up to optimum concentrations further increase in concentration may inhibit the crop growth due to the toxicity of nutrient. Nitrogen absorbed in soil comes in two inorganic forms: ammonium (NH^{+4}) and nitrate (NO^{-3}). N intensifies the green colour of the leaves, constituent of essential cellular components, controller of P, K and other nutrients, improves the succulence of many crops and promotes photosynthesis. Phosphorus is also an essential nutrient, vital component of ATP and require in processes from the beginning of seedling growth through to the formation of grain and maturity. In soils, inorganic P shows low diffusion and high fixation rates through ligand exchange by 1:1 clay minerals, Fe and Al oxides and hydroxides and thus precipitated as Fe, Al, and Ca phosphates. In light of these problems related to the conventional P application, the nano-sized apatite particles could be as effective in providing the nutrient P as the commonly-used soluble P fertilizers while minimizing the secondary contamination risks (e.g., Eutrophication) and the delivery problem associated with it.
An improved understanding of the interactions between nanoparticles (NPs) and plant responses, including their uptake, localization and activity could revolutionize crop production through increased disease resistance, nutrient utilization and crop yield (Wang *et al*., 2016) [20].

The nano-fertilizer mainly controls and/or delays the release of the nutrients and prolongs effectiveness of the applied fertilizers (Tarafdar and Adhikari, 2015) [19]. It is observed that seed treatment by nanoparticles can promote the crop growth, increase the yield and improve the quality of many crop products, including cereal crops and cash crops. Although nanotechnology application in food and agriculture is in its budding stage (Hong *et al.*, 2014) [6].

Wheat (*Triticum aestivum* L.) is the second most important cereal staple crops, after rice and it contributes significantly to total cereal production contributes nearly (*i.e.*, 35% to the national food basket) and country's food security. Wheat cultivation in India occupies 34.50-million-hectare area with the production of 108.75 million tonnes and productivity of 33.70 q/ha. In Gujarat, wheat is grown in 13.66-million-hectare area with production of 43.78 million tonnes and productivity of 32.03 q/ha (Anonymous, 2021) [2]. Productivity of wheat can only be enhanced by application of scientific tools and techniques in agriculture. Modern science basically deals with three areas *i.e.* information technology, biotechnology and nanotechnology. These three sciences proved their worth in every sector of society, but agriculture is still lagging behind. Since, the soil application of nitrogen and phosphorus nano-fertilizers is not much accepted by scientists, but seed treatment and foliar spray is effective in increasing growth of crops throughout their life period.

Materials and Methods

The experiment comprised of synthesis and characterization of nano NP and nano P fertilizers that were conducted in laboratory of the Department of Nanotechnology and Centre for Advanced Research in Plant Tissue Culture, Anand Agricultural University, Anand. The Commercial Urea source of Nitrogen is used for synthesis of nano NP fertilizer which is prepared by using direct precipitation method and Hydroxyapatite (HA) source of Phosphorus is used for synthesis of nano P fertilizer which is prepared by using Wet chemical precipitation method as developed by Kottegoda *et al.*, 2017 [8]. After the preparation of nano NP and nano P fertilizers different characterization techniques were used to investigate their particle size (nm), poly dispersive index (PDI), count rate (Kcps), zeta potential and FTIR at same place where nano NP and nano P were synthesized.

The synthesized 3.0% nano NP and 0.5% nano P stock solution were tested under *in vitro* condition at same place. The *in vitro* experiment was comprised of six levels of 3.0% nano NP (0, 500, 1000, 2000, 3000 and 4000 ppm) and 0.5% nano P (0, 500, 1000, 2000, 3000 and 4000 ppm) fertilizers with 36 treatment combinations laid out in factorial completely randomized design (FCRD) with three repetitions in plastic cups at laboratory. The wheat variety GW-451 (Gujarat wheat 451) was used in the present investigation as a test crop. Seed treatment with synthesized nano particles of NP and nano P was given by seeds soaking as per treatment for 2 h and shade-dried for an hour. These seed were used for sown in cocopit filled plastic cup. Total 540 Number of plastic cup will be taken for *in vitro* study and filled with 100 g prepared cocopit collected from Department of Nanotechnology and Centre for advanced Research in Plant Tissue Culture, Anand Agricultural University, Anand. In experiment five cup in each repetition were used and each cup there is two seed was sown. The plastic cups were place in germination chamber that was maintained temperature at 20 ± 2 °C with humidity 40 to 50%. Plastic Cup was regularly watered and maintains field capacity of soil till 30 days of study.

The green house experiment was carried out based on the *in vitro* study. The results of *in vitro* experiment were optimized and best treatments were taken out for the green house experiment. Based on *in vitro* experiment, the greenhouse experiment was comprised of five levels of 3.0% nano NP (0, 500, 1000, 2000 and 3000 ppm) and 0.5% nano P (0, 500, 1000, 2000 and 3000 ppm) fertilizers with 25 treatment combinations laid out in factorial completely randomized design (FCRD) with two repetitions in pots. The wheat variety GW-451 (Gujarat wheat 451) was used in the present investigation as a test crop at Department of Nanotechnology and Centre for advanced Research in Plant Tissue Culture, Anand Agricultural University, Anand. The foliar application of nano NP and nano P was carried out at 21, 35 and 60 DAS. The earthen pots were filled with 13 kg composite soil, collected from Regional Research Station, AAU, Anand. Collected soil was mixed before transferring the soil to the pots (table 1). The pots were brought to field capacity by proper watering; five seeds of wheat were sown in each pot. After germination, wheat plants were thinned to two plants per pot. The pots were place in greenhouse that was maintained temperature at 25 ± 2 °C with humidity 50 to 65% and also having fan-pad system for cools down temperature. Pots were regularly watered and weed free condition was maintained till achieved harvesting stage of wheat crop. When the wheat was at harvest stage, the plants were uprooted carefully. The fresh and oven dry weight of plant were recorded from each pot. The wheat plants were harvested at 101 DAS.

Table 1: Initial physico – chemical properties of the soil used for pot study

Sr. No.	Characteristics		Method
1.	**Mechanical analysis**		
	Coarse sand (%)	3.88	International pipette method
	Fine sand (%)	76.12	
	Silt (%)	11.95	
	Clay (%)	6.49	
	Textural class	Loamy sand	
2.	Bulk density (g/cc)	1.29	Core sampler method
3.	MWHC (%)	33.29	Brass cup method
4.	pH (1:2.5)	8.0	Potentiometric
5.	EC (1:2.5) dS/m	0.23	Conductimetric
6.	Organic carbon (%)	0.33	Chromic acid wet oxidation method
7.	Available N (kg/ha)	198	Alkaline permanganate method
8.	Available P_2O_5 (kg/ha)	39.04	Olsen's Method
9.	Available K_2O (kg/ha)	271	Neutral Normal Ammonium acetate method

Growth parameters

In *in vitro* experiment, days to 50% germination was counted based on number of days for each treatment up to the 50% germination was attained, germination and mortality percentage were recorded at 4th and 8th day of sowing in each plastic cup. The vigour index, shoot and root length were measured at 12 DAS in each plastic cup and average was calculated.

Vigour index = seedling length* germination percentage

In greenhouse study, days to emergence was counted based on number of days for each treatment to emerge out plant, germination percentage were recorded at 4th and 8th day of sowing in each pot, chlorophyll content and leaf area index at 45 DAS, plant height, number of tillers and dry matter yield at 100 days were measured in each pot and average was calculated. Nutrient content and uptake of whole plant was found after 105 DAS.

Statistical analysis

The data were subjected to statistical analysis as per the methods suggested by Steel and Torrie (1982) [17]. The value of "F" was worked out and compared with value of "F" at 5% level of significance. The values of standard error (mean) (S.Em.±), critical difference (C.D.) and coefficient of variation (C.V. %) were also calculated and appropriately used for interpretation of data, which are presented in respective tables.

Results and Discussion

Characterization of nano NP and nano P fertilizers in laboratory

Characteristic of nano NP and nano P: The synthesized nano NP and nano P fertilizer of different concentration have a particle size (0 to 709 nm), count rate (0 to 272), poly dispersity index (0 to 0.40) and zeta potential (0 to -57.88 mV) are within the standard range of nanoparticles. This indicated that the particles remain in dispersed form in all the samples. From the analysis, the zeta potential value was revealed that better stability of synthesized nano NP and nano P in aqueous suspension. Present findings are in close agreement with Mehta *et al*. (2023) [10] and Patil *et al*. (2020) [12]. The FTIR spectrum of synthesized nano NP (pH 8) shows absorption bands at wave number of 3431.27, 3335.11, 1677.53, 1593.13, 1460.14, 1150.57, 1027.86, 787.07 and 555.81 per cm. The peak was found at 1027.86 and 787.07 per cm is the characteristic absorption of the H-O bond. For the nano P absorption bands at 3479.65, 1647.37, 1212.63, 1123.28, 1054.07, 983.77, 871.38, 781.50, 656.27, 604.06, 574.73 and 521.29 per cm. FTIR characterization suggests that H-bonding modes are present as well. This results are close conformity with Kottegoda *et al*. (2011) [9] that noted the N–H stretching frequency of pure urea appeared as a doublet at 3430 and 3340 per cm, which in urea bonded to HA nanoparticles was shifted to 3200 per cm, where noticeable peak broadening had occurred. This suggests significant hydrogen bonding between N–H groups of urea with O–H groups of HA nanoparticles. Although the band for the N–H bending motion of urea had shifted from 1590 to 1627 per cm after the surface modification process, it was a clear indication of the presence of free N–H bonds even after adsorption of urea onto HA nanoparticles. They gave the statement that most prominent sharp and intense absorption band located around 1050 per cm represents the PO_3^{-4} ions in hydroxyapatite. The broad absorption band centered on 3500 per cm confirmed the presence of hydroxyl groups.

Table 2: Characterization of nano NP and nano P fertilizers for particle size, count rate, poly Dispersity index and zeta potential

Treatments	Particle size (nm)	Count rate (Kcps)	Poly dispersity index	Zeta potential (mV)
Standard range	**1 to 1000**	**100 to 500**	**0-1**	**+30 to -30**
Concentrations of Nano NP				
NP_0 (0)	0	0	0	0
NP_1 (500)	303	233	0.30	-35.50
NP_2 (1000)	408	240	0.33	-41.17
NP_3 (2000)	455	272	0.34	-41.17
NP_4 (3000)	512	258	0.32	-40.70
NP_5 (4000)	547	229	0.31	-40.63
Concentrations of Nano P				
P_0 (0)	0	0	0	0
P_1 (500)	449	179	0.24	-57.10
P_2 (1000)	634	227	0.38	-57.67
P_3 (2000)	654	263	0.40	-57.88
P_4 (3000)	696	246	0.22	-56.23
P_5 (4000)	709	169	0.22	-55.87

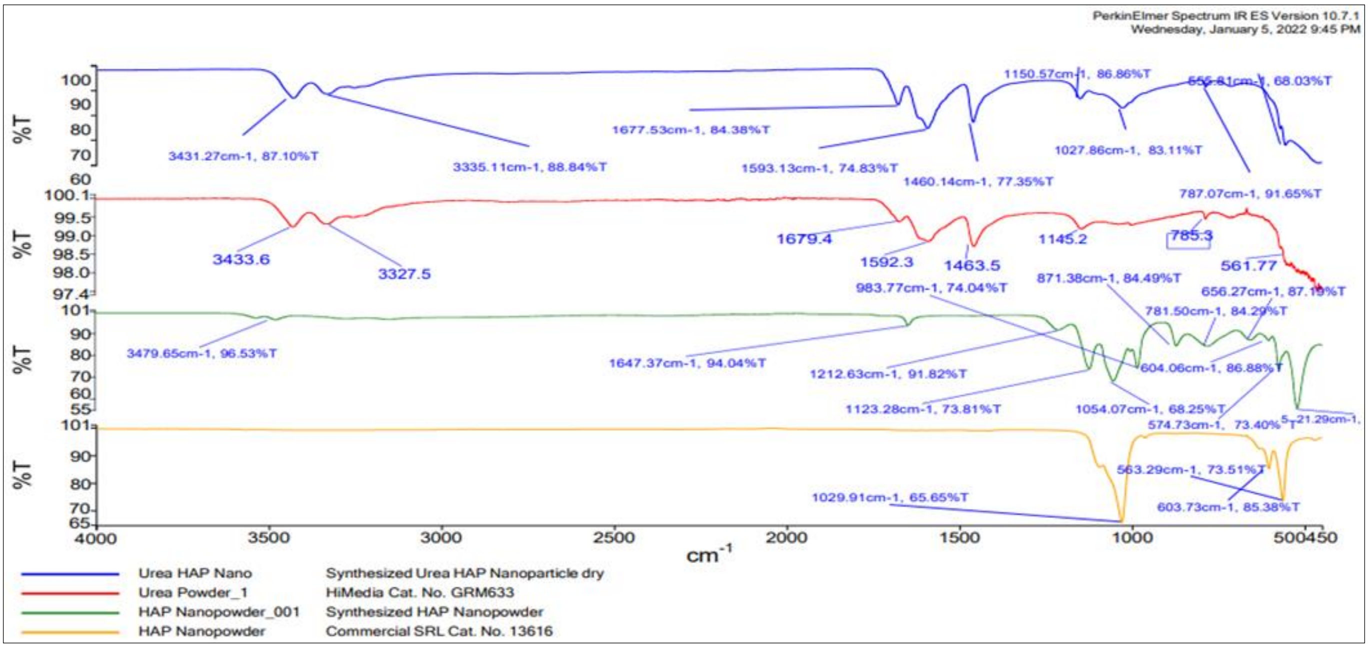

Fig 1: FTIR spectrum of nano NP (pH-8) and nano P (pH-10) fertilizers with Urea and HAP powder

Optimization of nano NP and nano P fertilizer doses for wheat under *in vitro* condition

The data regarding to mortality (%) of wheat as influenced by seed treatment of nano NP and nano P was recorded at 8 days after sowing (DAS). The mortality percentage of seeds was found zero percent at 8th day after sowing in all treatments.

Effect of nano NP

The influence of seed treatment of nano NP was found significant on days to 50% germination, germination percentage, vigour index, shoot and root length of wheat.

The significantly lower days to 50% germination (2.00 days) and highest germination (85.56 and 95.00% at 4th and 8th day of application, respectively) were observed with seed treatment of nano NP @ 2000 ppm, but days to 50% germination was at par with seed treatment of nano NP @ 1000 ppm (2.28 days) over the other treatments. It might be due to that synthesized nano urea was encapsulated into the HA were safe for germination of seeds and enhance the property of slow and sustained release of nitrogen to the plants. The results were conformity with finding of Badran and Savin (2018) [3] in almond.

Significantly higher shoot length (17.50 cm) was observed with seed treatment of nano NP @ 2000 ppm, but it was at par with treatment application of nano NP @ 3000 ppm (17.02 cm) as compared to other treatments and the highest root length (14.34 cm) and vigour index (3032) were observed with seed treatment of nano NP @ 2000 ppm over the other treatments. It might be due to the germinated seeds under doses of nano-fertilizers gave strong seedlings which had a tallest length of shoot and also gave the highest root. It can be attributed to nano-fertilizers are available for absorption and can provide nutrients that required for plant growth in a wide range of period. It might be due to that well grown seedling is the result of better germination, efficient nutrient release and demand of plant which increasing the vigour of plant.

Effect of nano P

Seed treatment of nano P was found significant on days to 50% germination, germination percentage, vigour index, shoot and root length of wheat.

Significantly a lower day to 50% germination (3.06 days) and higher germination (70.56% at 4th day of application) were observed with seed treatment of nano P @ 1000 ppm, but it was at par with seed treatment of nano P @ 3000 ppm (3.17 days to 50% germination), 2000 ppm (3.17 days to 50% germination and 68.89% germination) and 500 ppm (3.11 days to 50% germination and 68.89% germination) as compared to other treatments. Whereas, at 8th day of application, significantly higher germination (84.44%) was observed with seed treatment of nano P @ 2000 ppm, but it was at par with nano P @ 1000 ppm (83.33%) These results were in close conformity with finding of Subbaiyal *et al*. (2012) [18] in green gram. It might be due to significantly affect their biological activity most probably by enhancing the amount of water with nutrient that penetrates inside the seeds during the germination period. Whereas nanoparticles can create new pores for water permeation by penetration of seed coat. This process can increase germination and growth rate of wheat seedlings.

Significantly higher shoot length (18.06 cm) was observed with seed treatment of nano P @ 2000 ppm, but it was at par with application of nano P @ 1000 ppm (17.44 cm). Whereas significantly the highest root length (14.68 cm) was observed with seed treatment of nano P @ 1000 ppm over the rest of treatments. Significantly a higher vigour index (2685) was observed with seed treatment of nano P @ 1000 ppm, but it was at par with application of nano P @ 2000 ppm (2609) as compared to other treatments. This result was conformity with Priya *et al*. (2015) [13] in Mung, Urd and Cowpea.

Interaction Effect

The interaction effects of nano NP and nano P were found to be significant on days to 50% germination, germination percentage and vigour index of wheat.

Significantly lower days to 50% germination (2.00) and higher vigour index (3402) was found in treatment combination of $NP_3 + P_2$ (nano NP @ 2000 ppm + nano P @ 1000 ppm) over the rest of treatment combinations.

Table 3: Effect of seed treatment of nano NP and nano P fertilizers on days to 50% germination, germination percentage, vigour index, shoot and root length of wheat

Treatments	Days to 50% germination	Germination %		Vigour Index	Shoot length (cm)	Root length (cm)
		4th day	8th day			
Level of Nano NP (ppm)						
NP_0 (0)	3.72	62.78	72.78	2084	16.52	12.05
NP_1 (500)	3.06	69.44	84.44	2490	16.59	12.79
NP_2 (1000)	2.28	77.22	86.67	2604	16.69	13.31
NP_3 (2000)	2.00	85.56	95.00	3032	17.50	14.34
NP_4 (3000)	4.00	58.89	73.33	2219	17.02	13.17
NP_5 (4000)	4.83	47.22	60.00	1722	16.36	12.31
S. Em. ±	0.10	0.88	0.72	32	0.22	0.18
CD (P=0.05)	0.29	2.48	2.02	89	0.63	0.51
Level of Nano P (ppm)						
P_0 (0)	3.83	61.67	74.44	2231	16.58	13.25
P_1 (500)	3.11	68.89	78.89	2436	16.94	13.81
P_2 (1000)	3.06	70.56	83.33	2685	17.44	14.68
P_3 (2000)	3.17	68.89	84.44	2609	18.06	12.71
P_4 (3000)	3.17	66.67	76.67	2168	16.08	12.09
P_5 (4000)	3.56	64.44	74.44	2022	15.58	11.42
S. Em. ±	0.10	0.88	0.72	32	0.22	0.18
CD (P=0.05)	0.29	2.48	2.02	89	0.63	0.51
Interaction						
NP X P	Sig.	Sig.	Sig.	Sig.	NS	NS
S. Em ±	0.25	2.15	1.76	77	0.55	0.44
CD (P=0.05)	0.70	6.07	4.95	218	NS	NS
CV %	12.98	5.57	3.87	5.68	5.65	5.88

Table 4: Interaction effect of seed treatment of nano NP and nano P fertilizers on days to 50% germination and vigour index of wheat

Nano P (ppm)	Nano NP (ppm)											
	NP_0 (0)	NP_1 (500)	NP_2 (1000)	NP_3 (2000)	NP_4 (3000)	NP_5 (4000)	NP_0 (0)	NP_1 (500)	NP_2 (1000)	NP_3 (2000)	NP_4 (3000)	NP_5 (4000)
P_0 (0)	4.00	3.00	2.67	2.00	4.00	7.33	1977	2354	2531	2871	2126	1527
P_1 (500)	3.67	3.00	2.00	2.00	4.00	4.00	2176	2368	2780	3284	2187	1819
P_2 (1000)	3.33	3.00	2.00	2.00	4.00	4.00	2390	2847	2881	3402	2596	1994
P_3 (2000)	3.67	3.00	2.33	2.00	4.00	4.00	2212	3027	2728	3333	2497	1860
P_4 (3000)	3.67	3.00	2.33	2.00	4.00	4.00	1947	2259	2490	2683	1995	1633
P_5 (4000)	4.00	3.33	2.33	2.00	4.00	5.33	1799	2084	2213	2619	1916	1500
S. Em. ±	0.25						77					
CD (P=0.05)	0.70						218					

Significantly increasing effect on germination (100.0%) was found in treatment combination of $NP_1 + P_3$ (nano NP @ 500 ppm + nano P @ 2000 ppm), $NP_3 + P_1$ (nano NP @ 2000 ppm + nano P @ 500 ppm), $NP_3 + P_2$ (nano NP @ 2000 ppm + nano P @ 1000 ppm) and $NP_3 + P_3$ (nano NP @ 2000 ppm + nano P @ 2000 ppm) at 8th day of application over the rest of treatment combinations.

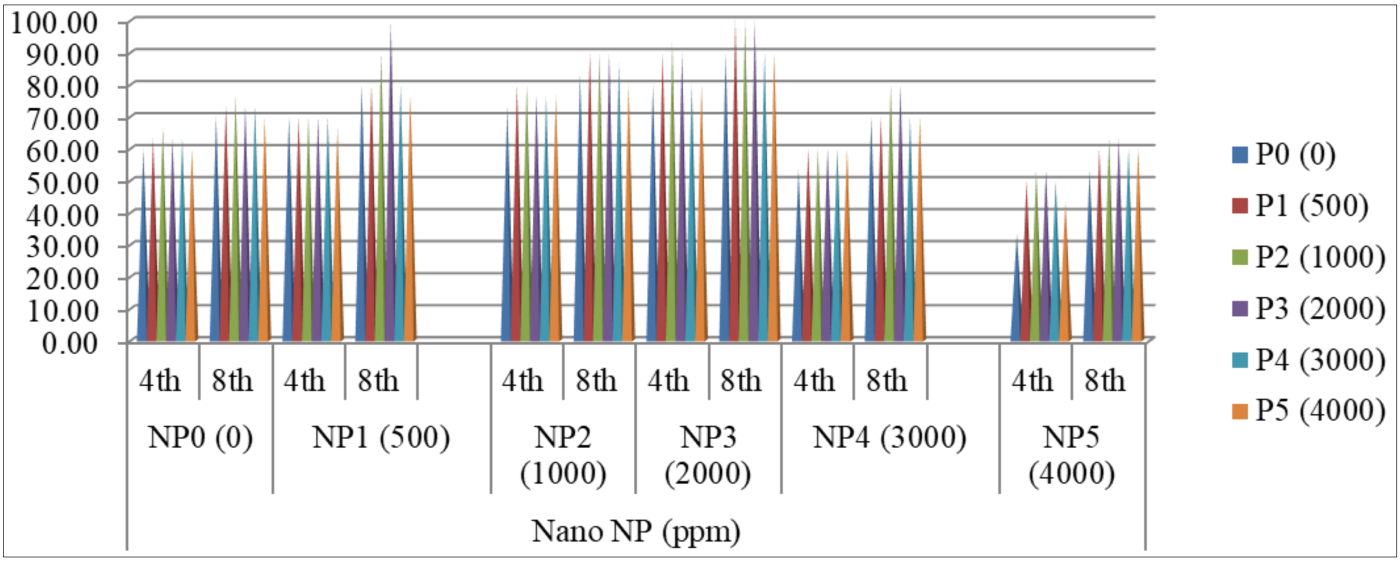

Fig 2: Interaction effect of seed treatment of nano NP and nano P fertilizers on germination percentage of wheat at 4th and 8th day

Effect of nano NP and nano P fertilizer doses for wheat under greenhouse condition

The data regarding two days to emergence and germination (%) of wheat were recorded at 8 days after sowing (DAS). The emergence of seeds was found 100 percent at 8th day after sowing in all treatments. Similarly, germination percentage of seeds at 8th day after sowing was also found 100 percent in all treatments. These may be due to the all the treatment were homogenous in nature up to 21 DAS and at 21 DAS treatments were imposed. The plant height (cm), K content (%) at harvest and available N, P_2O_5 and K_2O after harvest of wheat showed non-significant results with the application of different levels of nano NP and nano P.

Effect of nano NP

The significantly higher chlorophyll content (18.40 mg/g) at 45 DAS and number of tillers (7.90/plant) at harvest was found in wheat with foliar application of nano NP @ 2000 ppm, but it was at par with foliar application of nano NP @ 3000 ppm (18.33 mg/g and 7.60/plant, respectively). Whereas the highest leaf area index (1.08) and dry matter yield (3.43 g/pot) of wheat was noted with foliar application of nano NP @ 2000 ppm over the rest of treatments. It might be due to that chlorophyll is the light harvesting pigment responsible for photosynthesis. Sufficient amount of chlorophyll means greater production of photosynthates responsible for the growth and development of the plant. Higher chlorophyll content means higher available N preventing premature senescence. Also urea plays a crucial role in meristematic growth through its effect on the synthesis of photo-hormones. Among various plant hormones, cytokinin plays important role in growth of tillers. These findings are in accordance with the finding of Benzon *et al.* (2015) [4] in rice, Rathnayaka *et al.* (2018) [15] in rice and Abdel *et al*. (2018) [1] in wheat.

The significantly highest N (2.76%) and P (0.78%) content in plant was registered with the foliar application of nano NP @ 3000 ppm over rest of other treatments. The reason behind this is foliar application of nanoparticles with high concentration increased the amount of nutrient content and decreased losses by effectively translocation of foliar spray from source to sink. That gave the better performance in harvested plant of wheat. These findings are in accordance with the finding of Abdel *et al*. (2018) [1] and Manikandan and Subramanian (2016) [11] in maize. The data of nitrogen (88.61 mg/pot) and phosphorus (25.05 mg/pot) uptake by wheat, was found higher with the foliar fertilization of nano NP @ 3000 ppm over rest of the treatments, but it was at par with treatment application of nano NP @ 2000 ppm (86.14 mg/pot and 24.06 mg/pot, respectively). While, significantly higher potassium (19.40 mg/pot) uptake was found with foliar application of nano NP @ 2000 ppm, but it was at par with treatment application of nano NP @ 3000 ppm (19.22 mg/pot) over rest of the treatments. This finding was supported by Rajonee *et al*. (2016) [14] in Kalmi.

Table 5: Effect of foliar application of nano NP and nano P fertilizers on chlorophyll content, leaf area index, plant height, number of tillers and dry matter yield of wheat

Treatments	Chlorophyll content (mg/g)	Leaf area index	Plant height (cm)	Number of tillers/plant	Dry matter yield (g/pot)
	At 45 DAS		At 100 DAS		At 105 DAS
Level of Nano NP (ppm)					
NP_0 (0)	13.45	0.73	26.73	6.55	2.10
NP_1 (500)	15.54	0.83	26.91	6.90	2.51
NP_2 (1000)	16.63	0.95	27.34	7.05	3.05
NP_3 (2000)	18.40	1.08	27.52	7.90	3.43
NP_4 (3000)	18.33	0.96	27.27	7.60	3.24
S. Em. ±	0.11	0.02	0.67	0.20	0.06
CD (P=0.05)	0.31	0.07	NS	0.57	0.17
Level of Nano P (ppm)					
P_0 (0)	16.55	0.90	27.04	7.05	2.94
P_1 (500)	17.02	0.94	27.21	7.55	3.05
P_2 (1000)	17.21	0.98	27.33	7.85	3.12
P_3 (2000)	15.91	0.87	27.25	6.95	2.70
P_4 (3000)	15.66	0.86	26.96	6.60	2.51
S. Em. ±	0.11	0.02	0.67	0.20	0.06
CD (P=0.05)	0.31	0.07	NS	0.57	0.17
Interaction					
NP X P	Sig.	Sig.	NS	NS	Sig.
S. Em ±	0.24	0.06	1.50	0.44	0.13
CD (P=0.05)	0.70	0.16	NS	NS	0.38
CV %	2.07	8.71	7.83	8.67	6.36

Effect of nano P

Data clearly indicated that significantly higher chlorophyll content (17.21 mg/g), leaf area index (0.98), number of tillers (7.85/plant) and dry matter yield (3.12 g/pot) of wheat was found with foliar application of nano P @ 1000 ppm, but it was at par with application of nano P @ 500 ppm (17.02 mg/g, 0.94, 7.55/plant and 3.05 g/pot, respectively). It might be due to that high reactivity because of more specific surface area, more density of reactive areas or increased reactivity of these areas on the particles surface. It may be due to HAP adequately and timely supply of P might have favored net assimilation and partitioning of photosynthates to various metabolic sinks, which ultimately increased growth and yield attributes. These findings are in accordance with the finding of Hasaneen *et al.* (2016) [5] in French bean and Junxi *et al.* (2013) [7] in kale plant. Dry matter increase due to that phosphorus is a vital component of ATP, which forms during photosynthesis has crucial role in biomass production. Thus lead to dry matter production in plant. These findings are in accordance with the finding of Manikandan and Subramanian

(2016) [11] in maize.

The N (2.37%) and P (0.66%) content of wheat plant was found significantly higher with foliar application of nano P @ 3000 ppm, while it was at par with nano P @ 1000 ppm (2.23% and 0.63% respectively), 2000 ppm (2.31% and 0.65% respectively) and 500 ppm (0.63% P only) than rest of treatments. It might be due to that the increasing in performances of nano P formulations is due to higher supply of nano P_4 than the P_0, P_1, P_2 and P_3. It might be due to that higher P content in plant from coated nano NP formulations is either due to synchronization of nutrient release and plant demand or fewer fixations and leaching of nutrients from soil solution. These findings are in accordance with the finding of Sarkar *et al.* (2020) [16] in wheat. The nitogen (71.71 mg/pot), phosphorus (20.41 mg/pot) and potassium (17.27 mg/pot) uptake by wheat was noted significantly higher with nano P @ 1000 ppm, moreover it was at par with level of nano P @ 500 ppm (69.11 mg/pot, 19.74 mg/pot and 16.81 mg/pot, respectively) and 0 ppm (16.13 mg/pot potassium only) over rest of the treatments.

Table 6: Effect of foliar application of nano NP and nano P fertilizers on N, P and K content and uptake of wheat plant

Treatments	After harvest of plant content			After harvest of plant uptake		
	N (%)	P (%)	K (%)	N (mg/pot)	P (mg/pot)	K (mg/pot)
Level of Nano NP (ppm)						
NP_0 (0)	1.74	0.48	0.51	36.57	10.06	10.67
NP_1 (500)	2.03	0.58	0.53	50.91	14.50	13.23
NP_2 (1000)	2.20	0.65	0.55	67.11	19.80	16.74
NP_3 (2000)	2.51	0.69	0.57	86.14	24.06	19.40
NP_4 (3000)	2.76	0.78	0.59	88.61	25.05	19.22
S. Em. ±	0.05	0.01	0.02	2.17	0.51	0.67
CD (P=0.05)	0.16	0.04	NS	6.32	1.47	1.96
Level of Nano P (ppm)						
P_0 (0)	2.12	0.60	0.54	64.90	18.42	16.13
P_1 (500)	2.19	0.63	0.54	69.11	19.74	16.81
P_2 (1000)	2.23	0.63	0.55	71.71	20.41	17.27
P_3 (2000)	2.31	0.65	0.55	63.61	17.99	15.00
P_4 (3000)	2.37	0.66	0.56	60.00	16.91	14.04
S. Em. ±	0.05	0.01	0.02	2.17	0.51	0.67
CD (P=0.05)	0.16	0.04	NS	6.32	1.47	1.96
Interaction						
NP X P	NS	NS	NS	Sig.	Sig.	NS
S. Em ±	0.12	0.03	0.05	4.85	1.13	1.51
CD (P=0.05)	NS	NS	NS	14.14	3.30	NS
CV %	7.56	7.55	11.86	10.42	8.56	13.45

Table 7: Effect of foliar application of nano NP and nano P fertilizers on available N, P_2O_5 and K_2O after harvest of wheat

Treatments	N	P_2O_5	K_2O
	(kg/ha)		
Initial	198.49	39.04	271.68
Level of Nano NP (ppm)			
NP_0 (0)	201.10	41.45	284.80
NP_1 (500)	196.20	40.82	279.50
NP_2 (1000)	192.85	39.77	272.80
NP_3 (2000)	186.85	39.00	266.80
NP_4 (3000)	185.50	38.98	265.70
S. Em. ±	4.31	0.90	6.39
CD (P=0.05)	NS	NS	NS
Level of Nano P (ppm)			
P_0 (0)	193.40	40.03	273.50
P_1 (500)	192.45	39.82	272.60
P_2 (1000)	190.22	39.64	271.20
P_3 (2000)	192.83	40.22	275.40
P_4 (3000)	193.6	40.32	276.70
S. Em. ±	4.31	0.90	6.39
CD (P=0.05)	NS	NS	NS
Interaction			
NP X P	NS	NS	NS
S. Em ±	9.63	2.00	14.30
CD (P=0.05)	NS	NS	NS
CV %	10.22	7.06	5.64

Interaction Effect

The fertilization of NP_4 + P_2 (nano NP @ 3000 ppm + nano P @ 1000 ppm) was found significantly higher chlorophyll content (20.73 mg/g) and leaf area index (1.19) over the rest of treatments, but in case of leaf area index, it was at par with combination treatments of NP_3 + P_0 (nano NP @ 2000 ppm +

nano P @ 0 ppm), $NP_3 + P_1$ (nano NP @ 2000 ppm + nano P @ 500 ppm), $NP_3 + P_2$ (nano NP @ 2000 ppm + nano P @ 1000 ppm), $NP_3 + P_3$ (nano NP @ 2000 ppm + nano P @ 2000 ppm), $NP_3 + P_4$ (nano NP @ 2000 ppm + nano P @ 3000 ppm), $NP_4 + P_0$ (nano NP @ 3000 ppm + nano P @ 0 ppm) and $NP_4 + P_1$ (nano NP @ 3000 ppm + nano P @ 500 ppm), while in case of chlorophyll content, it was at par with $NP_4 + P_1$ (nano NP @ 3000 ppm + nano P @ 500 ppm).

Significantly higher dry matter yield (3.92 g/pot) was found with combined application of $NP_4 + P_2$ (nano NP @ 3000 ppm + nano P @ 1000 ppm), but it was at par with treatments combination of $NP_4 + P_0$ (nano NP @ 3000 ppm + nano P @ 0 ppm) and $NP_4 + P_1$ (nano NP @ 3000 ppm + nano P @ 500 ppm) over rest of treatments. The reason behind higher dry matter yield obtained from nano urea and nano HAP treated plant may be attributed to the increased N availability due to reduced ammonia loss. The activity of nutrients after foliar application of nano-fertilizer was increased and N and P were absorbed into the plants with the absorbing of water, thus the dry matter production was also increased.

Table 8: Interaction effect of foliar application of nano NP and nano P fertilizers on chlorophyll content and leaf area index at 45 DAS of wheat

Nano P (ppm)	Nano NP (ppm)									
	Chlorophyll content					Leaf area index				
	NP_0 (0)	NP_1 (500)	NP_2 (1000)	NP_3 (2000)	NP_4 (3000)	NP_0 (0)	NP_1 (500)	NP_2 (1000)	NP_3 (2000)	NP_4 (3000)
P_0 (0)	13.44	15.05	16.40	18.26	19.60	0.70	0.79	0.92	1.03	1.06
P_1 (500)	13.70	15.58	16.75	18.49	20.59	0.74	0.80	0.95	1.10	1.11
P_2 (1000)	13.79	15.78	16.93	18.80	20.73	0.76	0.87	0.97	1.13	1.19
P_3 (2000)	13.31	15.71	16.74	18.38	15.45	0.74	0.86	0.95	1.09	0.74
P_4 (3000)	13.05	15.58	16.33	18.07	15.27	0.74	0.83	0.95	1.07	0.73
S. Em. ±	0.24					0.06				
CD (P=0.05)	0.70					0.16				

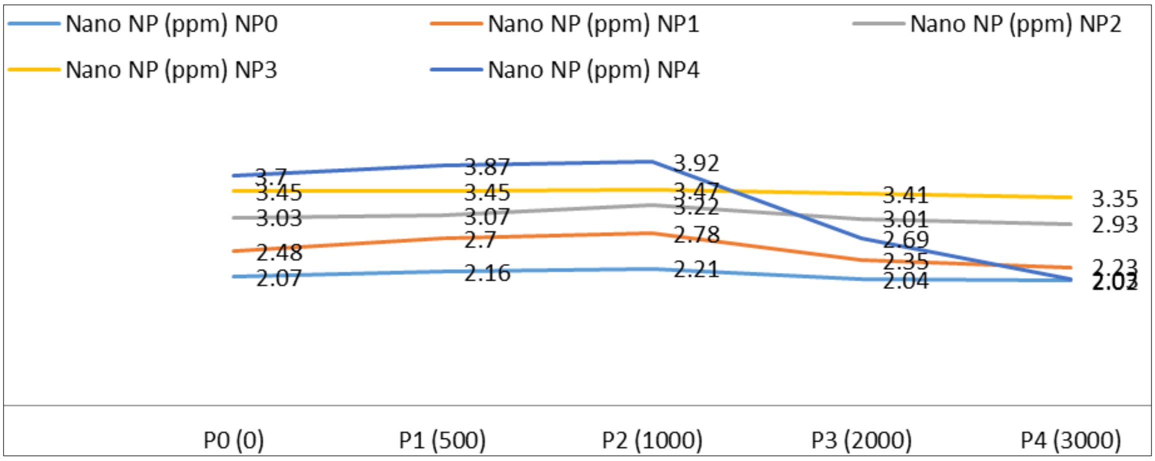

Fig 3: Interaction effect of foliar application of nano NP and nano P fertilizers on dry matter yield at 105 DAS of wheat

The significantly higher nitrogen (106.47 mg/pot) and P (30.20 mg/pot) uptake was noted with the combined application of $NP_4 + P_2$ (nano NP @ 3000 ppm + nano P @ 1000 ppm) and both was at par with treatments combination of $NP_4 + P_0$ (nano NP @ 3000 ppm + nano P @ 0 ppm) and $NP_4 + P_1$ (nano NP @ 3000 ppm + nano P @ 500 ppm).

Table 9: Interaction effect of foliar application of nano NP and nano P fertilizers on N and P uptake by wheat plant

Nano P (ppm)	Nano NP (ppm)									
	N uptake by plant					P uptake by plant				
	NP_0 (0)	NP_1 (500)	NP_2 (1000)	NP_3 (2000)	NP_4 (3000)	NP_0 (0)	NP_1 (500)	NP_2 (1000)	NP_3 (2000)	NP_4 (3000)
P_0 (0)	31.37	49.41	63.67	82.56	97.51	8.34	13.68	19.07	23.43	27.56
P_1 (500)	36.37	54.16	65.77	85.10	104.16	10.05	15.38	19.64	24.06	29.57
P_2 (1000)	39.03	56.13	70.47	86.46	106.47	10.34	16.17	21.01	24.35	30.20
P_3 (2000)	37.67	48.35	67.86	88.44	75.74	10.73	13.68	19.87	24.26	21.42
P_4 (3000)	38.42	46.49	67.79	88.17	59.17	10.85	13.58	19.43	24.21	16.49
S. Em. ±	4.85					1.13				
CD (P=0.05)	14.14					3.30				

Conclusion

The nano NP and nano P having particle size (0 to 709 nm), count rate (kcps) (0 to 287), poly dispersity index (0 to 0.46) and zeta potential (0 to -57.88 mV) were found in standard range of nano particles, when it were synthesized using direct precipitation method and characterized through DLS and FTIR.

From the *in vitro* study, seed treatment of $NP_3 + P_2$ (nano NP @ 2000 ppm + nano P @ 1000 ppm) was found optimum dose for the wheat seedlings in terms of growth parameters.

The results of greenhouse experiment reveled that foliar fertilization of $NP_3 + P_2$ (nano NP @ 2000 ppm + nano P @ 1000 ppm) and $NP_4 + P_2$ (nano NP @ 3000 ppm + nano P @ 1000 ppm) was found better at 21, 35 and 60 DAS than rest of the treatments in terms of growth, dry matter yield, nutrient content and uptake by plant.

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