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## Evaluation of foliar application of zinc nanoparticles on growth and yield parameters of maize (*Zea mays* L.) grown under greenhouse conditions

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### Abstract

Maize is one of the important crop for human consumption both directly and indirectly. It is also used as major feed source for cattle. After the major nutrients (Nitrogen, Phosphorus and Potassium) micronutrient Zinc, plays critical role in growth and yield of Maize. By owing to zinc importance, the present investigation was conducted to find out the impact of foliar application of zinc nanoparticles. Microbial synthesized zinc nanoparticles (MNP's) in the laboratory and chemically synthesized zinc nanoparticles (CNP's) were foliar applied at 30 and 60 days after sowing in pot culture experiment. Experiment composed of twelve treatments, first treatment was control without any zinc application, second treatment was farmers practice of soil application of zinc sulfate, five treatments were with foliar spray of MNP's (250, 500, 750, 1000 and 1250 ppm), and the other five with CNP's (250, 500, 750, 1000 and 1250 ppm). Among the treatments, the treatments imposed with 750ppm of zinc nanoparticles foliar spray had shown significantly higher growth parameters, chlorophyll content and yield parameters. Application rate of 750 ppm put forward the maximum yield under application of microbial synthesized nanoparticles. Microbial synthesized nanoparticles possess a protein layer from the mother source which helps in improved dispersivity of nanoparticles and increase the active ingredient concentration available to the plant.

**Keywords:** Zinc nanoparticles, MNP's, CNP's, dispersivity and active ingredient

### Introduction

Maize is grown as the top three crop in the world in terms of area and production. Maize is also the basis for the world's poorest countries, where it is, the majority of peoples staple food. In addition, one-third of all malnourished children are found from these countries. Indian average yield is below the world average by ton per hectare. There is a huge scope in the country for the improvement of maize yield and also bio-fortification of the crop (Bodh *et al.*, 2015) [3].

Maize is the indicator plant for the zinc nutrient. Zinc aids in the synthesis of growth hormones and proteins. It is needed in the production of chlorophyll and carbohydrate metabolism. It is essential for the transportation of calcium throughout the corn plant.

It is necessary for cell elongation, the increase in leaf and node size along with grain formation. Also it is the most deficient nutrient in Indian soils. The majorly followed corrective measure is application of 25 Kg of Zinc Sulphate per acre per every two crop seasons. By following this measure the problem would not be solved completely at times because of soil fixation of nutrient, making the applied zinc unavailable to the crop. Foliar application of Zinc even though not a well-known practice in Maize, rarely followed during the symptoms of Zinc deficiency. However it may be given as nutrition also. It is used as a quick method of correcting zinc deficiency foliar application of 2% spray of zinc solution (Singh, 1999; Hotz and Brown, 2004) [13, 7]. A nanoparticle is any matter, which is less than 100 nm at least in one of its dimensions. Nanoparticles were synthesized using top-down approach, with the help of incubation method. The present research was conducted to find out the impact of foliar application of biologically synthesized as well as chemically synthesized nanoparticles on growth and yield parameters of maize.

## Materials and Methods

The fungal isolates that are able to synthesis zinc nanoparticles were isolated from the rhizosphere soil samples based on the available zinc content. The nanoparticles were synthesized biologically using fungi isolates by incubation and enzymatic reduction method (Harish Kumar and Savalagi, 2017) [6]. Chemically synthesized Zinc oxide nanoparticles used were procured from Sigma Aldrich.

A pot culture experiment was conducted to evaluate the influence of zinc nanoparticles on growth and yield parameters of Maize (*Zea mays* L.) in greenhouse conditions at Department of Agricultural Microbiology, University of Agricultural Sciences, Dharwad. Completely randomized design was adapted for pot culture experiment with twelve treatments replicated thrice

Both Microbial synthesized and chemically synthesized zinc oxide nanoparticles were made into formulations in water for dispersible spraying on the foliage. The spray formulations for foliar application was prepared by using ultra-sonification. Foliar application of both the types of nanoparticles was given at 30 and 60 days after sowing (DAS) in the different treatment groups mentioned in the Table 1.

**Table 1:** Details of the treatments used for pot culture experiment

Treatments	Treatment details
T <sub>1</sub>	RPP -ZnSO <sub>4</sub> . 7 H <sub>2</sub> O (Control-Without ZnSO <sub>4</sub> . 7 H <sub>2</sub> O)
T <sub>2</sub>	T <sub>1</sub> + ZnSO <sub>4</sub> . 7 H <sub>2</sub> O (25 kg ha <sup>-1</sup> - Reference)
T <sub>3</sub>	T <sub>1</sub> + ZnO M-NP's 250 ppm
T <sub>4</sub>	T <sub>1</sub> + ZnO M-NP's 500 ppm
T <sub>5</sub>	T <sub>1</sub> + ZnO M-NP's 750 ppm
T <sub>6</sub>	T <sub>1</sub> + ZnO M-NP's 1000 ppm
T <sub>7</sub>	T <sub>1</sub> + ZnO M-NP's 1250 ppm
T <sub>8</sub>	T <sub>1</sub> + ZnO C-NP's 250 ppm
T <sub>9</sub>	T <sub>1</sub> + ZnO C-NP's 500 ppm
T <sub>10</sub>	T <sub>1</sub> + ZnO C-NP's 750 ppm
T <sub>11</sub>	T <sub>1</sub> + ZnO C-NP's 1000 ppm
T <sub>12</sub>	T <sub>1</sub> + ZnO C-NP's 1250 ppm

**Table 2:** Impact of ZnO nanoparticles foliar spray on plant height of maize

Treatment	Plant height (cm)			
	30 Days	60 Days	90 Days	At Harvest
T <sub>1</sub> : RPP – (Without ZnSO <sub>4</sub> )	2 5.54	82.95	108.83	118.53
T <sub>2</sub> : T <sub>1</sub> + ZnSO <sub>4</sub> (25 kg ha <sup>-1</sup> )	27.90	84.28	117.03	123.50
T <sub>3</sub> : T <sub>1</sub> + ZnO M-NP's 250 ppm	25.44	83.48	117.40	122.77
T <sub>4</sub> : T <sub>1</sub> + ZnO M-NP's 500 ppm	25.70	84.25	118.40	125.63
T <sub>5</sub> : T <sub>1</sub> + ZnO M-NP's 750 ppm	25.52	87.93	124.43	129.20
T <sub>6</sub> : T <sub>1</sub> + ZnO M-NP's 1000 ppm	25.34	85.58	120.33	126.37
T <sub>7</sub> : T <sub>1</sub> + ZnO M-NP's 1250 ppm	24.94	84.80	117.77	125.40
T <sub>8</sub> : T <sub>1</sub> + ZnO C- NP's 250 ppm	24.76	83.30	119.67	123.50
T <sub>9</sub> : T <sub>1</sub> + ZnO C- NP's 500 ppm	24.02	84.45	119.20	124.40
T <sub>10</sub> : T <sub>1</sub> + ZnO C- NP's 750 ppm	24.70	86.05	121.30	127.60
T <sub>11</sub> : T <sub>1</sub> + ZnO C- NP's 1000 ppm	25.84	83.68	119.33	127.07
T <sub>12</sub> : T <sub>1</sub> + ZnO C- NP's 1250 ppm	25.62	86.58	120.27	125.27
S.Em ±	0.69	0.59	1.04	1.06
CD (1%)	2.61	2.28	4.14	2.99

## Effect of ZnO nanoparticles foliar spray on chlorophyll content of Maize

At 30 DAS, treatment T<sub>2</sub> recorded higher chlorophyll content with a value of 36.16 compared to all other treatments. Treatment T<sub>5</sub> receiving 750 ppm of M-NP's had shown significantly highest chlorophyll content at 60 DAS with 45.64 followed by T<sub>10</sub> with 45.13. Treatments receiving M-NP's nanoparticle spray had shown higher chlorophyll

**Note:** RPP- Recommended package of practices (Zinc sulphate application excluded)

M-NP's- Microbial synthesized nanoparticles

C-NP's- Chemical synthesized nanoparticles

## Statistical analysis

All treatments in the experiment were replicated thrice and the experimental data generated in pot studies were subjected to one-way analysis of variance (ANOVA). The data interpretation was done as per methods given by Gomez and Gomez (1984) [5].

## Results

The aim of the present study is to test the effect of foliar application of biologically synthesised and chemically synthesised zinc nanoparticles on growth and yield of maize. Observations of plant growth parameters were taken at 30, 60 and 90 days after sowing and yield parameters at harvest.

### Effect of ZnO nanoparticles foliar spray on plant height of Maize

At 30 days after sowing higher plant height was recorded by T<sub>2</sub> (reference treatment). All the treatments were on par with the reference treatment (T<sub>2</sub>) except T<sub>9</sub> (Table 2). At 60 days after sowing (DAS) T<sub>5</sub>, treatment receiving 750 ppm of MNP's recorded significantly higher plant height of 87.93 cm. Treatment T<sub>10</sub> was on par with the treatment T<sub>5</sub>. Least plant height was recorded with treatment T<sub>1</sub> (control). At 90 days after sowing (DAS) T<sub>5</sub>, treatment receiving 750 ppm of M-NP's recorded significantly higher plant height over all other treatments with 124.43 cm. Treatments T<sub>3</sub>, T<sub>4</sub>, T<sub>7</sub>, T<sub>8</sub>, T<sub>9</sub> and T<sub>11</sub> were on par with the reference T<sub>2</sub>, which recorded a value of 117.03 cm. At harvest, treatment T<sub>5</sub> receiving 750 ppm of M-NP's have recorded highest plant height of 129.20 cm followed by treatments T<sub>6</sub>, T<sub>10</sub> and T<sub>11</sub> which are on par with each other. Treatments T<sub>4</sub>, T<sub>6</sub>, T<sub>7</sub>, T<sub>8</sub>, T<sub>9</sub> and T<sub>12</sub> were on par with the reference treatment T<sub>2</sub>.

content than their corresponding counterparts of C-NP's foliar spray, at all the five concentrations of ZnO nanoparticles foliar spray. At 90 DAS, higher chlorophyll content was recorded by T<sub>5</sub> with a value of 47.40. Treatments T<sub>10</sub> was on par with T<sub>5</sub>. At harvest higher value of chlorophyll content was observed with T<sub>5</sub>. Whereas, T<sub>11</sub> was on par with T<sub>5</sub>, which was recorded 15.47 (Table 3).

**Table 3:** Impact of ZnO nanoparticles foliar spray on chlorophyll content of maize

Treatment	Chlorophyll content			
	30 Days	60 Days	90 Days	At harvest
T <sub>1</sub> : RPP – ZnSO <sub>4</sub> (25 kg ha <sup>-1</sup> )	30.66	30.13	24.27	7.70
T <sub>2</sub> : T <sub>1</sub> + ZnSO <sub>4</sub> (25 kg ha <sup>-1</sup> )	36.16	38.32	30.93	9.13
T <sub>3</sub> : T <sub>1</sub> + ZnO M-NP's 250 ppm	31.61	38.50	41.30	14.07
T <sub>4</sub> : T <sub>1</sub> + ZnO M-NP's 500 ppm	31.09	40.45	41.90	14.53
T <sub>5</sub> : T <sub>1</sub> + ZnO M-NP's 750 ppm	32.27	45.64	47.40	17.30
T <sub>6</sub> : T <sub>1</sub> + ZnO M-NP's 1000 ppm	30.91	42.69	43.47	13.60
T <sub>7</sub> : T <sub>1</sub> + ZnO M-NP's 1250 ppm	35.19	39.23	40.75	16.70
T <sub>8</sub> : T <sub>1</sub> + ZnO C- NP's 250 ppm	29.67	35.34	36.57	13.40
T <sub>9</sub> : T <sub>1</sub> + ZnO C- NP's 500 ppm	33.97	37.28	38.27	13.67
T <sub>10</sub> : T <sub>1</sub> + ZnO C- NP's 750 ppm	28.37	41.78	45.13	14.13
T <sub>11</sub> : T <sub>1</sub> + ZnO C- NP's 1000 ppm	23.14	42.16	41.91	15.47
T <sub>12</sub> : T <sub>1</sub> + ZnO C- NP's 1250 ppm	27.02	40.79	42.67	13.33
S. Em ±	0.76	0.16	1.05	0.66
CD (1%)	2.92	0.63	4.19	2.63

### Effect of ZnO nanoparticle foliar spray on yield parameters of Maize

The results pertaining to the impact of the ZnO nanoparticle foliar spray on maize crop was presented in the Table 4.

#### Cob weight

Highest cob weight was recorded in the treatment T<sub>5</sub> receiving 750 ppm of M-NP's with 109.81g followed by T<sub>4</sub> receiving 500 ppm of M-NP's with 108.49g and are on par

with each other. An increasing trend in cob weight for M-NP's foliar spray was observed from T<sub>3</sub> to T<sub>5</sub> then a slight decrease for the next two treatments T<sub>6</sub> and T<sub>7</sub>. Whereas, in case of chemical nanoparticles increasing trend was observed in all the treatments. But comparatively M-NP's foliar spray treatments T<sub>5</sub> was higher compared to all other treatments. Lowest cob weight is observed with the treatment T<sub>1</sub> which is not supplied with any form of zinc with 80.71g.

**Table 4:** Impact of ZnO nanoparticles foliar spray on yield parameters of maize

Treatment	Yield parameters	
	Cob weight (g plant <sup>-1</sup> )	Test weight (g plant <sup>-1</sup> )
T <sub>1</sub> : RPP – ZnSO <sub>4</sub> (25 kg ha <sup>-1</sup> )	80.71	20.52
T <sub>2</sub> : T <sub>1</sub> + ZnSO <sub>4</sub> (25 kg ha <sup>-1</sup> )	90.98	26.35
T <sub>3</sub> : T <sub>1</sub> + ZnO M-NP's 250 ppm	92.36	21.46
T <sub>4</sub> : T <sub>1</sub> + ZnO M-NP's 500 ppm	108.49	26.08
T <sub>5</sub> : T <sub>1</sub> + ZnO M-NP's 750 ppm	109.81	22.31
T <sub>6</sub> : T <sub>1</sub> + ZnO M-NP's 1000 ppm	106.64	28.63
T <sub>7</sub> : T <sub>1</sub> + ZnO M-NP's 1250 ppm	104.38	27.03
T <sub>8</sub> : T <sub>1</sub> + ZnO C- NP's 250 ppm	95.86	23.50
T <sub>9</sub> : T <sub>1</sub> + ZnO C- NP's 500 ppm	99.94	22.45
T <sub>10</sub> : T <sub>1</sub> + ZnO C- NP's 750 ppm	98.79	26.15
T <sub>11</sub> : T <sub>1</sub> + ZnO C- NP's 1000 ppm	105.16	26.83
T <sub>12</sub> : T <sub>1</sub> + ZnO C- NP's 1250 ppm	107.47	25.91
S. Em ±	0.393	0.28
CD (1%)	1.56	1.12

#### Test weight

Test weight was observed significantly highest in the treatment T<sub>5</sub> with 28.63g followed by T<sub>11</sub> with 26.83g. Reference treatment (T<sub>2</sub>) had recorded test weight of 26.35g. Lowest test weight was recorded in the treatment T<sub>1</sub> with 20.52g. There is a significant difference among the treatment groups for the test weight treated with the ZnO nanoparticles foliar spray.

#### Discussion

Microorganisms play a key role in agriculture by various mechanisms. In the rhizosphere they are involved in various relationships out of these, plant microbe symbiosis being the significant. In general the beneficial microorganisms are the major input source in the rhizosphere zone of crop whereas in the present study these beneficial microbes were used for the synthesis of zinc nanoparticles and their impact on growth when applied in the aerial way by foliar application. After the synthesis and characterization of the zinc oxide nanoparticles, the solution form of zinc oxide nanoparticles were converted

to the powder form with the help of lyophilisation. These standardized nanoparticles were applied at five concentrations on maize crop under pot culture experiment, to observe the effects of foliar spray zinc oxide at 30 and 60 days after sowing.

The results of this trial indicated that the treatments receiving 750 ppm and 1000 ppm of mycological synthesized nanoparticles was showing significantly different and higher response to all growth parameters of plant height, number of leaves, total dry matter content with reference treatment receiving soil application of ZnSO<sub>4</sub> at the rate of 25 kg ha<sup>-1</sup> and the treatment control check without any ZnSO<sub>4</sub> application. These results were in supporting with the experiment of foliar application of nano chelate zinc increased the 94% of grain yield in the maize crop performed by (Mosanna and Khalilvand, 2015) [10].

Plant height was recorded highest with the treatment receiving 750 ppm of M-NP's with a value of 129.2 cm at harvest, followed by the 750 ppm of C-NP's 127.6 cm. Almost in all the treatments chemical nanoparticle foliar sprays were

recorded with nearer values to the microbial synthesized zinc nanoparticle spray, but in all the cases the treatments received M-NP's were showing higher values compared to the C-NP's. These results were in accordance with the experimental results of Prasad *et al.* (2012) [11], where 1000 ppm of nano zinc oxide foliar spray at 30 days after sowing recorded the highest plant height. Increase in plant height might be attributed to inter nodal distance as reported by Kaya and Higgs (2002) [8] with Zn application.

The chlorophyll content of the maize recorded significantly highest with treatment T<sub>5</sub> receiving 750 ppm of MNP's at 90 DAS and at harvest, it was also recorded second highest at 60 DAS. The results in the trial indicate the increasing trend of chlorophyll content was observed in all the nanoparticle sprayed treatments till 90 DAS. The chlorophyll content at harvest was also higher in the nanoparticle sprayed treatments. In general, a chlorophyll content of very low value below 10 will be observed at the harvest stage of the crop. The results reveal that with higher zinc content in leaves yield higher chlorophyll content. This conclusion is in accordance with the Akay (2011) [1]. And also these findings are in conformity with the findings of Morteza *et al.* (2013) [9] who reported that effect of nano TiO<sub>2</sub> was significant on total chlorophyll, carotenoids and anthocyanins. The maximum amount of pigment was recorded in the treatment of nano TiO<sub>2</sub> spray at the reproductive stage in comparison with control treatment with water spray. Increase in the biological yield is also attributed to increase in plant height and leaves of corn (Frageria *et al.*, 2006) [4].

Treatment receiving M-NP's spray 750 ppm had recorded higher cob weights. An increasing trend observed in the cob weight in the MNP's receiving treatments till T<sub>5</sub>. The highest grain yield per plant was recorded with the T<sub>5</sub>, 66.15 per cent higher yield when compared to control (T<sub>1</sub>). When compared to the 25 kg ha<sup>-1</sup> of ZnSO<sub>4</sub> soil application 40.46 per cent higher yield. Thus, 750 ppm of M-NP's zinc oxide nano spray showing significantly high grain yield over all other treatments.

Test weight was recorded high value with the treatment T<sub>5</sub>. When compared to control it is 39.52 per cent more. And when compared to T<sub>2</sub> it is 8.65 per cent when compared all other yield parameters test weight was recorded very low significant difference with other parameters. The proper and adequate supply of Zn increased the uptake of N during the grain formation stage and ultimately improved the yield component of maize (Siddiqui *et al.*, 2009) [12]. Higher yield due to Zn fertilization is also attributed to the enhanced synthesis of carbohydrates and their transport to the site of grain production (Babu *et al.*, 2007) [2].

From the comparison of foliar spray of mycologically synthesized and chemically synthesized nanoparticles it has yielded that, mycologically synthesized nanoparticles were found more beneficial to the maize crop than the chemically synthesized nanoparticles. Both the formulations do not put forward any detrimental effects on plant growth and yield till the dosage range of 1250 ppm. In a similar type of research done by using zinc oxide nanoparticles shown that 1000 ppm were proven beneficial for maize seed germination and seedling growth. Stay green character was observed till the harvest of the crops which received the nanoparticle treatment in general but zinc oxide nanoparticles of 2000 ppm were detrimental for maize crop (Tiwari, 2017) [14].

## Conclusion

Nanoparticles can affect plant growth and nutrient content in an agriculturally important crop species, and the charge of these particles influences the colonization bacteria in the rhizosphere. The green synthesized zinc oxide nanoparticles were potential crop nutrients and also non-toxic, as they have enhanced the plant growth and yield parameters in maize under pot culture studies. The effect of these zinc nanoparticles has to be further tested under field conditions.

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