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Effect of zinc oxide nanoparticles (ZnONPs) on yield attributes and yield of hybrid maize (*Zea mays* L.)

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Abstract
The field study was carried out at the Department of Agronomy, Eastern Block Farm in Tamil Nadu Agriculture University Coimbatore, to study the effect of Zinc oxide nanoparticles (ZnONPs) on yield attributes and yield of hybrid maize. Total fourteen treatment were followed out of which twelve treatments were of ZnONPs at 50,100,200 and 300 ppm treatment were administered in two stages *viz.*, knee height stage (30-35 DAS) and tasseling stage (46-56 DAS) and one treatment of foliar spray of 0.5% $ZnSO_4$ and one treatment of control water spray with three replications. Among the different treatment foliar application of ZnONPs @ 300 ppm at knee height and tasselling stage give the significantly recorded higher yield attributes *viz.*, cob length (20.21cm), cob weight (217.17 g), cob girth (16.8 cm) as well as the grain yield (5,213 kg ha^{-1}) and stover yield (13,417 kg ha^{-1}).

Keywords: ZnONPs, maize, foliar application

Introduction
Maize (*Zea mays* L.) is the third most significant cereal, next to rice and wheat, in the world as well as in India. One of the most versatile crops, it has a variety of uses in human nutrition, animal feed and as a source of raw materials for numerous industrial goods. It can be grown in a variety of environmental circumstances (Ayyar *et al.*, 2019) [2]. It is grown in more than 130 countries and is referred to as a "wonder crop" and the "queen of cereals" due to its huge potential (Suganya, 2015) [20]. Maize is now gaining relevance due to its prospective use in the production of starch, resins, syrups, ethanol and other products in addition to its usage as food and fodder (Ayyar and Appavoo, 2016) [1]. As a C_4 plant, maize is effective at turning ingested nutrients into food. There is widespread agreement that maize grains are essential to the diet of three billion people worldwide. In India, maize is grown on 9.09 million ha, producing 23.29 million tonnes with a productivity of 2,563 kg ha^{-1} (Suganya *et al.*, 2020) [21]. Because of the state's growing non-vegetarian population, changing eating patterns and rapidly expanding poultry sector, maize grain is increasingly in demand as poultry feed. Currently, 35% of the maize grown in India is utilised for human consumption, 25% is used for animal feed (cattle and poultry) and 15% is used for food processing (corn flakes, popcorn *etc*.,) and other sectors (starch, dextrose, corn syrup, corn oil *etc*.,) (Balakrishnan and Subramanian, 2012) [3].
Some of the essential elements needed for maximum plant growth, development and productivity are nitrogen (N), phosphorous (P), sulphur (S), zinc (Zn) and iron (Fe). Any of these nutritional deficiencies may result in defective plant development and reduced productivity (Kumar *et al.*, 2021) [10]. The maize crops have relatively modest micronutrient needs and the intervals between their deficiency and toxicity in soil and plants are quite small. The hidden hunger of micronutrient deficiencies affects far more individuals badly and has long-lasting effects on humankind and their cultures, in contrast to malnutrition that is caused by a lack of food and has received global attention (Patel *et al.*, 2011; Šimić *et al.*, 2012) [13, 18]. After the green revolution, high nutrient intensive crop rotations such as rice-wheat were adopted, along with unbalanced fertilization and high doses of nitrogen, nutrient uptake by both grain and straw from the field and very little or no application of organic manures. This is the main cause of the widespread emergence of zinc deficiency in the country (Ranjith *et al.*, 2011) [16].
Zinc is among the 17 essential elements necessary for the regular growth and development of plants. It is one of the eight micronutrients that plants require. Zinc plays a key role in plants with enzymatic reactions involved in carbohydrate metabolism, protein synthesis, gene

expression, auxin (growth regulator) metabolism, pollen formation, maintenance of biological membranes, protection against photo-oxidative damage and excessive heat, resistance to infection by certain pathogens (Marschner, 2011 and Cakmak, 2002) [12, 5]. Zinc deficiency in plants slows down photosynthesis and nitrogen metabolism, inhibits blooming and fruit development, lengthens growth periods (leading to delayed maturity), diminishes yield and quality and causes less efficient nutrient usage. Some of the typical signs of zinc deficiency in plants include light green, yellow or bleached spots in the interveinal areas of older leaves; emerging leaves are smaller and frequently called "little leaves," and they display rosetting, which is when the internodal distance gets so small that all the leaves appear to emerge from the same point (Brennan *et al.*, 1993) [4]. In India, agricultural losses due to zinc shortage were estimated to be significant (Kumar *et al*., 2019) [9].

Nanotechnology is a potentially useful instrument that might useful in a new era of precise farming methods, making it a potential remedy to these issues. Even in difficult circumstances, nanotechnology may boost agricultural potential to provide larger yields in an environmentally sustainable manner (Sugunan and Dutta, 2008) [22]. Even though research into the use of NPs in crop development is still ongoing, we may anticipate seeing their frequent application in farmer fields in the near future. In response to various signals including heat, wetness and other abiotic stress, the nanofertilizers release the nutrients in a controlled way. Nanofertilizers may control the release of nutrients, give the right amount of nutrients to crops in the right proportions and increase yield while protecting the environment (De Rosa *et al*. 2010) [6].

Zn nanoparticle application has been more popular in recent years as a way to improve plant absorption. The agronomic efficiency of Zn fertilizers may be impacted by particle size (Kumar *et al*., 2021) [11]. Reduced particle size increases the number of particles per unit weight of applied Zn and a fertilizer's specific surface area, both of which speed up the dissolving of fertilizers with low water solubility, such Zn oxide (ZnO). Compared to conventional agriculture systems, nanotechnology offers a much greater ability to solve many of the issues associated to agriculture. It is a relatively new practise to utilise nanoparticles to stimulate plant growth. The impacts of nanoparticles on the growth of maize have not been extensively studied. Due to their diverse characteristics, Zn-oxide nanoparticles stand out among metal oxides. The United States Food and Drug Administration (USFDA) recognises zinc oxide as one of the safest substances. The current study examines the impact of ZnO nanoparticles (ZnONPs) on maize production and yield-related characteristics.

Material and Methods-

The field study was carried out at the Department of Agronomy, Eastern Block Farm, Tamil Nadu Agricultural University Coimbatore. The field is located at a height of 427 metres above mean sea level in Tamil Nadu's Western Agro-climatic Zone with latitudes of 12°16'N and longitudes of 76°58'21E. Before the field experiment, a soil sample from the experimental site was collected and examined for a number of physico-chemical parameters. The soil type at the experimental site is sandy clay loam. The experimental plot had a low concentration of soluble salts (EC - 0.299 dSm^{-1}) and an extremely alkaline pH of 9.25. The soil has low nitrogen availability (209 kg ha^{-1}), medium phosphorus availability (19 kg ha^{-1}), moderate potassium availability (753 kg ha^{-1}) and low zinc availability (0.81 mg kg^{-1}).

The experiment had fourteen treatments and three replications, and it was set up using a Randomised Block Design, Zinc oxide nanoparticles with a specific surface area of 10–25 m^2g^{-1} and a particle size of less than 100 nm were imported from Sigma Chemical in the United States. Treatment was administered in two stages *viz*., knee height stage (30-35 DAS) and tasseling stage (46-56 DAS) These are the details of the treatment;- T_1-Foliar spray of ZnONPs @ 50 ppm at Knee height stage, T_2-Foliar spray of ZnONPs @ 50 ppm at Tasseling stage, T_3-Foliar spray of ZnONPs @ 50 ppm at Knee height and Tasseling stage, T_4-Foliar spray of ZnONPs @ 100 ppm at Knee height stage, T_5-Foliar spray of ZnONPs @ 100 ppm at Tasseling stage. T_6-Foliar spray of ZnONPs @ 100 ppm at Knee height and Tasseling stage, T_7-Foliar spray of ZnONPs @ 200 ppm at Knee height stage, T_8-Foliar spray of ZnONPs @ 200 ppm at Tasseling stage, T_9-Foliar spray of ZnONPs @ 200 ppm at Knee height and Tasseling stages,T_{10}-Foliar spray of ZnONPs @ 300 ppm at Knee height stage, T_{11}-Foliar spray of ZnONPs @ 300 ppm at Tasselling stage,T_{12}-Foliar spray of ZnONPs @ 300 ppm at Knee height and Tasseling stage,T_{13}-Foliar spray of ZnSO4 @ 0.5% during zinc deficiency and T_{14}-Control (Water Spray).

At harvest, samples from each plot were taken in order to record the yield related variables, such as the number of cobs $plant^{-1}$, cob weight, length, girth, number of rows cob^{-1}, and test weight. Recorded yields included the biological yield, grain yield and stover yield. The ratio of the economic yield (grain yield) to the biological yield (grain + stover) was used to calculate the harvest index.

Data from various observations were gathered and statistically analysed using a randomised block design given by Gomez and Gomez (1984) [7]. A critical difference (CD) of 5% was determined wherever the treatment differences were found to be significant. Non-significant treatment differences were denoted by the probability level NS.

Results and Discussion

The yield attributes and yield have ultimately been affected by the plant's overall increase in growth. The results (Table 1 and 2) showed that foliar treatments of ZnONPs and $ZnSO_4$ as well as control (water spray) varied significantly from one another. There is significant difference in cob length, foliar application of ZnONPs @ 300 ppm at knee height and tasseling stage (T_{12}) recorded the highest cob length (20.21 cm) which was significantly higher (26%) compared to control (16.01 cm). Similarly there was significant difference in the girth of the cob, the highest cob girth (16.28 cm) was recorded at application of ZnONPs @ 300 ppm at knee height and tasseling stage (T_{12}) which was on par with T_9 (14.97 cm) application of ZnONPs @ 200 ppm at knee height and tasseling stage similar result was obtained by Satdev *et al*. (2020) [17]. Whereas single cob weight (217.17 g) was recorded the highest with the application of ZnONPs @ 300 ppm at knee height and tasseling stage (T_{12}) which was on par with T_9 (202.46 g) . Application of ZnONPs of 300 ppm at both knee height and tasseeling stage resulted in producing more number of rows (15.62) cob^{-1} which was significantly higher than T_{14}-Control (30%) and T_{13}-0.5% $ZnSO_4$ (15%) (Table1). More number of grains row^{-1} (29.45) was produced with the application of ZnONPs @ 300 ppm at knee height and tasseling stage (T_{12}) and was significantly differ from control (32%) and 0.5% $ZnSO_4$ (21%). However the application of ZnONPs of 200 ppm at knee height and tasseling stage recorded (26.44) grains per row. The highest total grain cob^{-1} (460.68) was recorded in application of ZnONPs @ 300 ppm at knee height and tasseling stage (T_{12}) which was on par with T_9 (389.52), Similar result was obtained by Subbaiah *et al.* (2016) [19]. There was no significant difference found in the test weight of grain. When Zn-foliar application was increased, P content in maize shoots and grains also increased simultaneously and significantly. This is due to Zn's stimulation of P transporter (Khan *et al.*, 2014) [8] and an increase in the active loading of Zn2+ (metal cations) to apoplastic xylem. Watts-Williams *et al.* (2014) [23] observed that such a synergistic effect did not conflict with P's antagonistic effect on Zn absorption.

The number of cobs per plant recorded (one) were same in all treatments (Table 2) .The other cobs that were produced by the plants were not considered as they were very small and without matured grains. The highest grain yield (5,213 kg ha^{-1}) was obtained in application of ZnONPs @ 300 ppm at knee height and tasseling stage (T_{12}) which was significantly higher to control (55%) and 0.5% $ZnSO_4$ (26%). Similarly the highest stover yield was also obtained in application of ZnONPs @ 300 ppm at knee height and tasseling stage (T_{12}), which was significantly higher to T_{14} - Control (33%) and T_{13} - 0.5% $ZnSO_4$ (25%). However the harvest index was the highest in ZnONPs @ 200 ppm at knee height and tasseling stage (T_9) which was on par with T_{10}-ZnONPs @ 300 ppm at knee height stage (29.26) and T_6 -ZnONPs @ 100 ppm at knee height and tasseling stage (29.20). Positive results may be attributed due to the rapid transport and assimilation of Zn nanoparticles, which leads to the expression of growth-promoting enzymatic activity and auxin metabolism in plants. The inefficiency of bulk $ZnSO_4$ is most likely due to its high solubility and short retention duration in the plant system. As a result, the bioavailability/sustained availability of bulk particles within the plant system and at the site of uptake is not proven. However, due of its nano size, high surface-to-volume ratio, and high reactivity, nano ZnO absorbs rapidly by the leaf surface and is metabolised faster than bulk $ZnSO_4$. Similar result was obtained by several researcher the effect of different Nano scale material in increasing yield of different crops. Prasad *et al.* (2012) [15] found that foliar application of zinc oxide nanoparticle is more effective than the soil application and there was an improvement of pod yield and zinc content in peanut by zinc oxide foliar application. Satdev *et al.* (2020) [17] reported that foliar application of ZnONPs @ 250 ppm enhances the yield and yield attributes of sweet corn. Poornima and Koti (2019) [14] reported that application of nano zinc oxide recorded more yield and growth of sorghum.

Table 1: Effect of Zinc Oxide NPs on yield attributes of hybrid maize (*Zea mays*)

Treatments	Cob length (cm)	Cob girth (cm)	Weight cob^{-1} (g)	No. of rows cob^{-1}	No. of grains row^{-1}	Total No. of grain cob^{-1}	Test Weigh (g)
T_1-Foliar spray of ZnONPs @ 50ppm at Knee height stage	16.13	12.24	166.87	12.18	22.71	272.60	30.68
T_2-Foliar spray of ZnONPs @ 50ppm at Tasseling stage	16.28	13.77	169.29	13.46	23.75	320.66	30.49
T_3-Foliar spray of ZnONPs @ 50ppm at Knee height and Tasseling stage	16.44	14.20	182.95	13.74	24.45	337.87	30.94
T_4-Foliar spray of ZnONPs @ 100ppm at Knee height stage	17.64	14.13	179.47	12.40	25.86	322.39	31.42
T_5-Foliar spray of ZnONPs @ 100ppm at Tasseling stage	16.76	14.03	175.00	13.35	25.21	335.89	31.61
T_6-Foliar spray of ZnONPs @ 100ppm at Knee height and Tasseling stage	18.04	14.26	200.90	14.02	26.49	372.29	32.16
T_7-Foliar spray of ZnONPs @ 200ppm at Knee height stage	17.52	14.17	187.81	13.23	25.86	342.37	31.50
T_8-Foliar spray of ZnONPs @ 200ppm at Tasseling stage	17.31	14.03	184.00	13.78	25.53	352.40	31.92
T_9-Foliar spray of ZnONPs @ 200ppm at Knee height and Tasseling stages	19.14	14.97	202.46	14.73	26.44	389.52	32.78
T_{10}-Foliar spray of ZnONPs @ 300ppm at Knee height stage	17.80	14.27	193.83	13.57	25.21	341.93	31.41
T_{11}-Foliar spray of ZnONPs @ 300ppm at Tasselling stage	17.52	14.18	189.63	12.98	25.65	332.37	32.12
T_{12}-Foliar spray of ZnONPs @ 300ppm at Knee height and Tasseling stage	20.21	16.28	217.27	15.62	29.45	460.68	33.29
T_{13}-Foliar spray of $ZnSO_4$ @ 0.5% during zinc deficiency	16.48	13.97	171.47	13.54	24.24	328.74	30.31
T_{14}-Control (Water Spray)	16.01	12.11	153.55	12.05	22.25	267.26	29.89
S.Ed	1.16	0.94	13.55	0.87	1.70	35.23	2.01
CD (P=0.05)	2.38	1.93	27.86	1.80	3.50	72.41	4.13

Table 2: Effect of Zinc Oxide NPs on yield of hybrid maize (*Zea mays*)

Treatments	No. of Cobs Plant^{-1}	Grain yield (kg ha^{-1})	Stover yield (kg ha^{-1})	Harvest Index
T_1-Foliar spray of ZnONPs @ 50ppm at Knee height stage	1.00	3400	10435	24.59
T_2-Foliar spray of ZnONPs @ 50ppm at Tasseling stage	1.00	3448	10664	24.45
T_3-Foliar spray of ZnONPs @ 50ppm at Knee height and Tasseling stage	1.00	3676	11309	24.53
T_4-Foliar spray of ZnONPs @ 100ppm at Knee height stage	1.00	4573	11498	28.46
T_5-Foliar spray of ZnONPs @ 100ppm at Tasseling stage	1.00	4222	11367	27.04
T_6-Foliar spray of ZnONPs @ 100ppm at Knee height and Tasseling stage	1.00	4871	11787	29.20
T_7-Foliar spray of ZnONPs @ 200ppm at Knee height stage	1.00	4567	11439	28.56
T_8-Foliar spray of ZnONPs @ 200ppm at Tasseling stage	1.00	4456	11454	28.01
T_9-Foliar spray of ZnONPs @ 200ppm at Knee height and Tasseling stages	1.00	5011	12096	29.35
T_{10}-Foliar spray of ZnONPs @ 300ppm at Knee height stage	1.00	4703	11403	29.26
T_{11}-Foliar spray of ZnONPs @ 300ppm at Tasselling stage	1.00	4640	11555	28.66
T_{12}-Foliar spray of ZnONPs @ 300ppm at Knee height and Tasseling stage	1.00	5213	13417	28.00
T_{13}-Foliar spray of $ZnSO_4$ @ 0.5% during zinc deficiency	1.00	4138	10710	27.93
T_{14}-Control (Water Spray)	1.00	3361	10109	25.06
S.Ed	-	282.78	774.09	1.19
C.D (P=0.05)	-	581.25	1591.16	2.42

Conclusion

According to the study, applying nano ZnO particles to maize resulted in more yield attributes and yield than using bulk $ZnSO_4$. As a result, applying very little fertilizer can reduce fertilizer application doses, fertilizer waste and environmental risks and boost nutrient usage efficiency. The impacts of nano zinc oxide particles on soil-based beneficial microorganisms and various beneficial processes, such as nitrification, nitrogen fixation, organic matter breakdown, mineralization and immobilisation, need to be studied. To improve crop production, it is also necessary to standardise the doses of Nano fertilizers for different crops and the ideal growth stage. It is also necessary to understand the intra- and extracellular mechanisms involved in the uptake and translocation of nanoparticles. In conclusion application of ZnONPs @ 300 ppm at knee height and tasseling stage increases the yield attributes and yield of hybrid maize.

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