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A comparative evaluation of the effects of seed invigoration treatments with precursor zinc salt and nano-sized zinc oxide (ZnO) particles on vegetative growth, grain yield, and quality characteristics of *Zea mays*

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Abstract

Introduction: The zinc micronutrient fertilizers have a critical impact on the grain productivity and quality attributes of maize. However, the low use-efficiency issues of the applied Zn-fertilizers are required to be addressed through the development of novel formulations and alternative application techniques.

Objectives: This field study investigates the comparative impact of seed invigoration (including seed priming and coating) treatments with bulk zinc and ZnO nanoparticles (ZnONPs).

Methods: The two seed treatments with two different zinc sources at three different concentrations of 0, 20, and 40 mg L^{-1} each, for a total of ten treatments, were evaluated for vegetative growth, photosynthetic pigments, grain yield, and quality traits in *Zea mays*.

Results: The total chlorophyll content was improved by ZnONPs seed priming at the V8 stage. However, there were plants that grew tall, bearing longer ears with bulk $ZnSO_4$ and the untreated control. Yield-contributing factors like number of seeds per cob, and 1000-grain weight were marginally improved by ZnONPs treatment. Overall, only cob weight, starch, total soluble protein, and soil nutrient (N, P, K, and Zn) content were significantly enhanced by ZnONPs treatment. Furthermore, no negative effect was recorded on the soil microbiological and enzymatic activities seed treatment with both the zinc sources.

Conclusion: The seed treatment, i.e. coating and priming with ZnONPs, did not significantly alter the grain yield, but the seed starch and total soluble protein content were improved.

Keywords: Seed priming and coating, Soil nutrient, Yield characters, *Zea mays*, ZnO NPs

Introduction

Maize (*Zea mays* L.) is very responsive to water and nutrient application. It exhibits high nutrient requirements at all crop stages. As grain productivity is often dependent on the optimum establishment of vigorous seedlings (Larrosa and Borrás 2022; Shuai et al. 2019), adequate nutrient availability is a prerequisite (Nciizah et al. 2020; Tondey et al. 2021). Micronutrients are

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essential for proper growth and development. Though these are required in small quantities but can enhance grain yield by up to 50% and improve grain nutrient quality (Ullah et al. 2020). Zinc (Zn) is a micronutrient required for better metabolic activity and has a vital role in ascertaining the quality and productivity of food crops. It is involved in many aspects of plant metabolic and structural activity including photosynthesis, respiration, chlorophyll synthesis, and chloroplast functioning (Zulfiqar et al. 2020). It also plays an important role in enzyme activation, carbohydrate metabolism, and protein synthesis (Rehman et al. 2018b; Rehman et al. 2018a; Ullah et al. 2020) as well as in fertilization and pollen function (Pandey et al. 2006).

Maize crop exhibits Zn-deficiency, which results in reproductive impairment, reduced yield potential, and nutrient contents (Itroutwar et al. 2020b). The grain yield losses incurred may exceed to even 80% due to Zn-deficiency besides compromising the grain Zn-contents (Cakmak 2008; Suganya et al. 2020). Hence, this may lead to a substantially high economic loss for the maize growers. This nutrient deficiency can be remediated by the application of micronutrient-containing fertilizer(s). The micronutrient(s) can be applied to crops in various ways, such as soil application, foliar spray, and seed treatment, but each approach has its benefits and drawback (Tondey et al. 2021; Wasaya et al. 2017). The basal/soil application of micronutrient fertilizers is not recommended but can prove useful for soils deficient or exhibiting low micronutrient status (Liu et al. 2020). Though in calcareous soils, soil application technique suffers from issues such as fixation or precipitation of the applied fertilizers rendering these unavailable to the crop plants (Rengel 2015). Therefore, the foliar fertilization technique is preferred to address the micronutrient deficiency and enhance the produce/grain micronutrient content—the biofortification aspect (Prasad et al. 2014). However, the foliar-specific dose or concentration is generally low as higher concentrations can damage the foliage (Nable et al. 1990). Also, the requirement for multiple applications at critical growth stages of the crop makes it time-intensive. Hence, the seed treatment methods are being utilized to improve the percent germination, seedling vigour, and plant stand establishment (Mondal and Bose 2019; Nciizah et al. 2020).

Recently, nanoscale particle formulations have emerged as an advanced seed invigoration method in precision agriculture (Itroutwar et al. 2020a; Pereira et al. 2021; Tondey et al. 2021). The nano-dimensions of these formulations are expected to improve the micronutrient uptake by the plant besides enhancing the use efficiency of the applied micronutrients (Kalia and Kaur 2019a). Seed nano-invigoration is anticipated to exhibit positive effects by altering the seed metabolism and signalling pathways (Pereira et al. 2021). Further, seed treatments with nanoscale particles can improve the rate of emergence and subsequent growth, yield, and quality of the crop (Pereira et al. 2021; Tondey et al. 2021). Seed nano-priming, one of the seed nano-invigoration techniques, involves seed surface treatment with nano-formulation of the active ingredient(s). In contrast, the seed coating requires the adsorption of substances such as nutrients, chemicals, plant growth regulators mixed with an organic or inorganic polymer on the surface of seeds as an outer covering (Farooq et al. 2012; Ur Rehman et al. 2011). Various seed priming and coating agents have been evaluated to improve seed germination and prevent seed ageing such as microbial or algal extracts and nanoparticles of metal and/or metal oxides (Mahakham et al. 2017).

Previously published literature has indicated the enhancement in the germination of the seeds and seedling root and shoot growth parameters by the application of zinc oxide nanoparticles (ZnONPs) as seed priming agents in rice (Itroutwar et al. 2020a), wheat (Munir et al. 2018; Rai-Kalal and Jajoo 2021), maize (Itroutwar et al. 2020b), and broccoli (Awan et al. 2021). Also, seed priming with ZnONPs may enhance the plant Zn-content as depicted in a study on the use of bare and dextran functionalized ZnONPs in wheat seedlings (Elhaj Baddar and Unrine 2018). The seed priming treatment with nano-Zn and nano-Fe fertilizers improved the grain yield and productivity of red bean seeds (Bayat et al. 2020). Further, the ZnONPs seed priming can also help to ameliorate the negative effects of salt stress (Abou-zeid et al. 2020) and heavy metal toxicity in fragrant rice (Li et al. 2021). It was interesting to identify that most of these research papers reported the superior role of ZnONPs over the macro-sized or bulk zinc salt treatment involving an effect on the vegetative, biochemical, physiological, and zinc nutrient status of the treated plants. For example, in an in vitro filter paper germination assay, seed priming treatment with the biogenically synthesized ZnO nanoparticles (at 100 mg L^{-1}) in maize more effectively improved the vegetative traits compared to the ionic salt priming and hydropriming treatments (Itroutwar et al. 2020a). Similarly, an improved wheat seed germination rate was reported by seed priming treatment with nano-zinc oxide particles which was 14% higher over the zinc sulphate salt treatment (Rai-Kalal and Jajoo 2021). Though these studies illustrated results on concentration-dependent response of ZnONPs as seed priming agents, but fall short for providing a differential response for the seed priming and coating treatments with both the bulk Zn-salt and ZnONPs at different working concentrations. Also, the information on the extent of the benefits imparted by seed invigoration treatments with

ZnONPs under field application conditions could not be identified as the majority of the studies have been performed under controlled conditions including the results obtained from in vitro filter paper germination assays or pot culture in soil-less or alternative substrates under glass- or poly-house conditions. Therefore, the role or function of soil, a multi-component dynamic matrix comprised of both biotic and abiotic components; vis-a-vis the effect of Zn-seed invigoration treatments on these components is also required to be determined. Prior published reports on ZnONPs-field studies provide information on the effect of foliar application treatments on cereal crops (Dapkekar et al. 2018; Deshpande et al. 2018), while segmented reports have been published illustrating the effect of soil treatments with ZnONPs on soil chemical and nutritional status, microbial population, and enzyme activities (Shah et al. 2022; Shemawar et al. 2021; Shen et al. 2015). The researchers have provided negative impact of nano-ZnO particle soil treatment (100 or 1000 mg L^{-1}) on these parameters (Shah et al. 2022). Another research group mentioned the effect to be transient and ephemeral (Shemawar et al. 2021). These observed negative effects may be due to the soil application of higher concentration of the ZnONPs rendering direct contact of the ZnONPs with various soil components. The foliar ZnONPs treatment studies rather showed an eliciting impact on the soil microflora and enzymatic activities (Bala et al. 2019).

As outlined above, a comprehensive field study deciphering the effect of seed invigoration treatments with ZnONPs on vegetative, agronomic, plant and soil nutrition status, grain quality, and soil microbiological and enzymatic parameters in maize has not been presented. The previous literature lacks determination of all the key factors or the traits affected by use of the ZnONPs under field cultivation conditions. Therefore, this study identified a specific hypothesis involving evaluation of the nano-ZnO particles to exhibit a differential response compared to bulk Zn-salt (zinc sulphate, $ZnSO_4$) in a concentration-dependent or independent mode. Also, the role of method of application (seed priming or coating) and the alteration in the benefits imparted if any by the two seed invigoration with the $ZnSO_4$/ZnONPs to the maize crop cultivated under field conditions was assessed. The overall aim of the current study was to evaluate the comparative effect of the application of two different zinc sources, i.e. zinc sulphate and nano-zinc oxide (ZnO) particles as seed priming and coating agents on vegetative growth, quality, and yield-attributing traits, soil nutrient, microbiological and soil enzymatic activities of grain maize (PMH-1) under the field conditions. Further, it was performed to identify the interactive effect(s), if any, for the two types of seed invigoration treatments, Zn-sources, and their concentrations in this field experiment.

Results

Characterization of the ZnO NPs

The scanning electron microscopy characterization of the ZnONPs depicted irregular to quasi-spherical aggregate shape (Fig. 1a), while the TEM showed an average size of 65 ± 9.0 nm (Fig. 1b, c). The average hydrodynamic diameter of the zinc oxide nanoparticles was 72.14 ± 5.0 nm, and the zeta potential of -17.1 mV was recorded for the ZnONPs synthesized through the wet chemical technique.

Vegetative growth parameters

A non-significant effect of application of different concentrations of ZnONPs on several vegetative growth parameters was observed. A statistically non-significant effect was recorded for the mean germination percentage on different seed treatments (Table 1, Additional file 1: Figure S1). In addition, the number of plants per

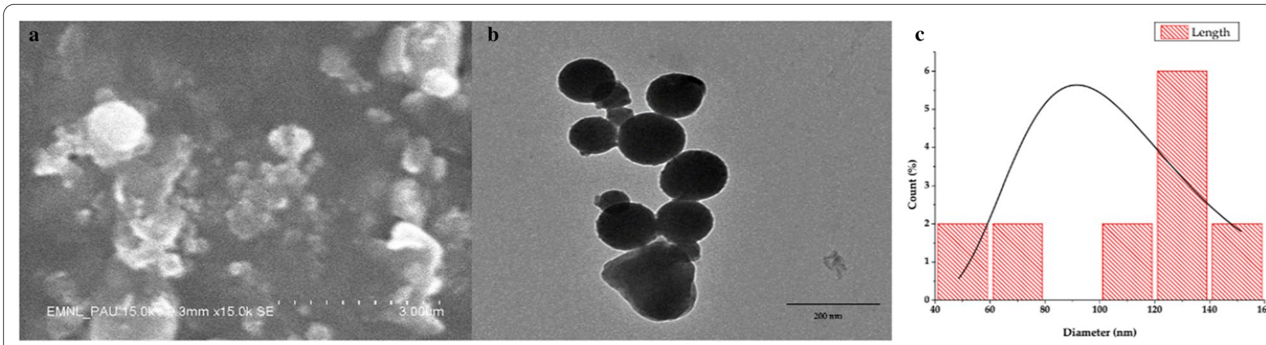

Fig. 1 Electron microscopy characterization **a** scanning electron micrograph depicting small-sized aggregates, **b** transmission electron micrographs of zinc oxide nanoparticles showing spherical shaped nanoparticles, **c** histogram showing size distribution curve

Table 1 Effect of seed treatments and various concentrations of Zn-sources applied on vegetative growth parameters in maize var. PMH-1 under field conditions

Seed treatment	Zinc source concentration	Percent germination	Number of plants per plot	Plant height (cm)	Number of leaves
Priming	Control	54.6 ± 4.0	60.25 ± 4.9	227.00 ± 6.25	12.75 ± 1.53
	$ZnSO_4$_20	50.0 ± 3.6	58.25 ± 6.2	216.25 ± 9.01	13.25 ± 2.08
	$ZnSO_4$_40	57.0 ± 5.6	55.25 ± 4.51	221.75 ± 3.05	13.25 ± 0.58
	ZnONP20	56.0 ± 2.0	53.25 ± 8.02	218.75 ± 2.9	12.50 ± 2.08
	ZnONP40	56.6 ± 12.7	56.75 ± 4.7	220.00 ± 5.0	13.75 ± 1.53
Coating	Control	56.3 ± 4.9	63.25 ± 2.52	225.50 ± 6.8	12.50 ± 2.01
	$ZnSO_4$_20	50.0 ± 7.5	63.75 ± 9.01	224.25 ± 2.9	13.50 ± 2.03
	$ZnSO_4$_40	58.0 ± 3.0	50.50 ± 8.4	233.00 ± 7.5	13.25 ± 2.08
	ZnONP20	53.7 ± 1.5	57.75 ± 4.6	223.75 ± 8.3	14.25 ± 1.53
	ZnONP40	54.2 ± 2.6	58.75 ± 5.3	229.25 ± 5.0	13.50 ± 1.15
LSD = $p \leq 0.05$		6.61	11.936	13.428	2.988

Values represent the average mean ± Standard deviation

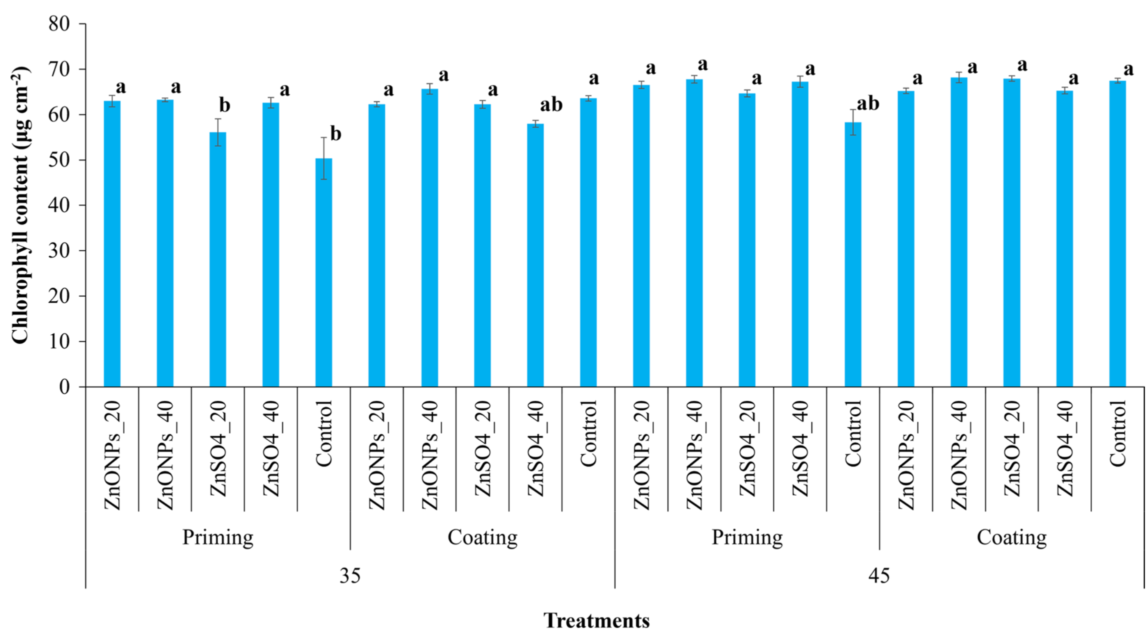

Fig. 2 Effect of application of Zn-sources (Zn-salt and ZnONPs) on chlorophyll content (µg cm^{-2}) of maize cv. PMH-1 cultivated under field conditions

plot remained statistically at par in seed treatments. The seed treatment with either $ZnSO_4$ or ZnONPs did not alter the number of plants per plot. The application of different concentrations of Zn-sources could only marginally affect the chlorophyll content of maize var. PMH-1 with maximum chlorophyll content (µg cm^{-2}) recorded at 45 days after sowing (DAS) (Fig. 2). There was no significant variation observed for this parameter with only an equivalent effect recorded for ZnONPs treatment with respect to the control treatment.

Yield and yield-attributing characters

Zinc micronutrient application via seed coating and priming treatments non-significantly affected the total cob yield, cob weight, and 1000-seed weight (Table 2). The trend obtained for yield-affecting attributes indicated

Table 2 Effect of seed treatments and different concentrations of Zn-sources applied for yield-attributing parameters in maize var. PMH-1 cultivated under field conditions

Seed treatment	Zinc source concentration	Total cob yield (100 kg/ha)	Three cobs wt. (g)	Total grain wt. (100 kg/ha)	1000-grain wt. (g)
Priming	Control	7.63 ± 0.93	697.0 ± 14.3	5.37 ± 1.19	306.0 ± 11.2
	$ZnSO_4$_20	7.81 ± 0.46	745.0 ± 19.1	4.19 ± 0.17	310.5 ± 15.1
	$ZnSO_4$_40	8.24 ± 0.63	748.0 ± 16.7	5.45 ± 0.12	333.5 ± 8.1
	ZnONP20	8.31 ± 0.68	756.0 ± 13.1	5.15 ± 0.92	331.2 ± 16.2
	ZnONP40	8.56 ± 0.93	735.5 ± 15.01	4.66 ± 0.66	315.5 ± 13.3
Coating	Control	7.80 ± 1.18	757.5 ± 4.2	5.46 ± 0.36	321.0 ± 10.6
	$ZnSO_4$_20	8.90 ± 1.65	747.5 ± 5.01	5.36 ± 0.73	342.5 ± 22.0
	$ZnSO_4$_40	8.46 ± 0.48	763.5 ± 7.02	5.01 ± 0.53	322.0 ± 20.9
	ZnONP20	8.93 ± 0.3	785.0 ± 10.5	4.58 ± 0.39	325.5 ± 12.0
	ZnONP40	9.13 ± 0.54	745.0 ± 13.7	5.44 ± 0.54	324.0 ± 26.0
	LSD = $p \leq 0.05$	1.885	60.09	1.438	31.916

Values represent the average mean ± standard deviation

negligible or even no significant increase in various yield-attributing traits particularly the total cob yield and total grain weight by the seed invigoration treatments (Table 2, Additional file 1: Figure S2).

Among the ten treatments, the seed priming and coating treatment with the lower ZnONPs concentration (20 mg L^{-1}) treatment exhibited the highest three cob yields. However, no significant improvement in the total grain weight was recorded by treatment with ZnONPs. Both seed treatments exhibited at par results for the 1000-grain weight with numerical increase observed for the seed coating treatment. Among the Zn-source concentrations, no significant variation was recorded (Table 2).

Quality parameters

The grain total starch, total soluble proteins, and N, P, K, and Zn content exhibited a positive effect of the Zn-sources (Table 3). It can also be identified that the grain quality parameters were recorded to be higher for the seed coating treatment as compared to the priming treatment. Similarly, among the Zn-source concentrations significantly higher NPK, Zn, starch, and protein contents were recorded in ZnONPs (40 mg L^{-1}) treatment (Table 3).

The effect of Zn-source on a variety of functional groups (primary groups: carbohydrates, lipids, proteins) in the ground maize seed flour was identified through FT-IR spectroscopy studies (Fig. 3). The sharp peaks at

Table 3 Effect of seed treatments and concentration of Zn-source on grain nutrient content, starch, and total soluble protein content of maize crop (variety PMH-1) grown under field conditions

Seed treatment	Zn-source Concentration	Starch content (mg g^{-1})	Protein content (mg g^{-1})	N (g kg^{-1})	K (g kg^{-1})	P (g kg^{-1})	Zn (mg kg^{-1})
Priming	Control	626.68 ± 0.12	46.83 ± 0.15	0.34 ± 0.015	4.05 ± 0.015	0.87 ± 0.01	0.42 ± 0.020
	$ZnSO_4$_20	653.45 ± 0.14	49.15 ± 0.35	0.38 ± 0.010	6.65 ± 0.015	0.96 ± 0.01	0.75 ± 0.015
	$ZnSO_4$_40	654.25 ± 0.21	58.12 ± 0.27	0.49 ± 0.010	7.43 ± 0.015	1.14 ± 0.02	0.25 ± 0.010
	ZnONPs 20	654.25 ± 0.19	61.32 ± 2.33	0.64 ± 0.010	8.02 ± 0.010	1.23 ± 0.02	1.30 ± 0.010
	ZnONPs 40	680.29 ± 0.12	66.81 ± 4.72	0.71 ± 0.010	9.35 ± 0.010	1.16 ± 0.01	1.65 ± 0.015
Coating	Control	647.29 ± 0.13	58.12 ± 2.25	0.47 ± 0.010	6.15 ± 0.010	1.01 ± 0.01	0.83 ± 0.012
	$ZnSO_4$_20	680.26 ± 0.39	61.59 ± 3.13	0.62 ± 0.015	7.09 ± 0.153	1.17 ± 0.01	1.16 ± 0.015
	$ZnSO_4$_40	680.75 ± 1.53	69.67 ± 1.81	0.74 ± 0.010	9.46 ± 0.025	1.25 ± 0.02	1.25 ± 0.021
	ZnONPs 20	690.43 ± 1.75	76.37 ± 1.75	0.78 ± 0.010	10.43 ± 0.025	1.27 ± 0.02	1.54 ± 0.010
	ZnONPs 40	704.14 ± 1.82	76.48 ± 2.21	0.87 ± 0.010	11.15 ± 0.010	1.35 ± 0.01	2.04 ± 0.010
LSD = $p \leq 0.05$		23.42	6.65	0.019	0.086	0.021	0.024

Values depict the average mean ± Standard deviation of three replications

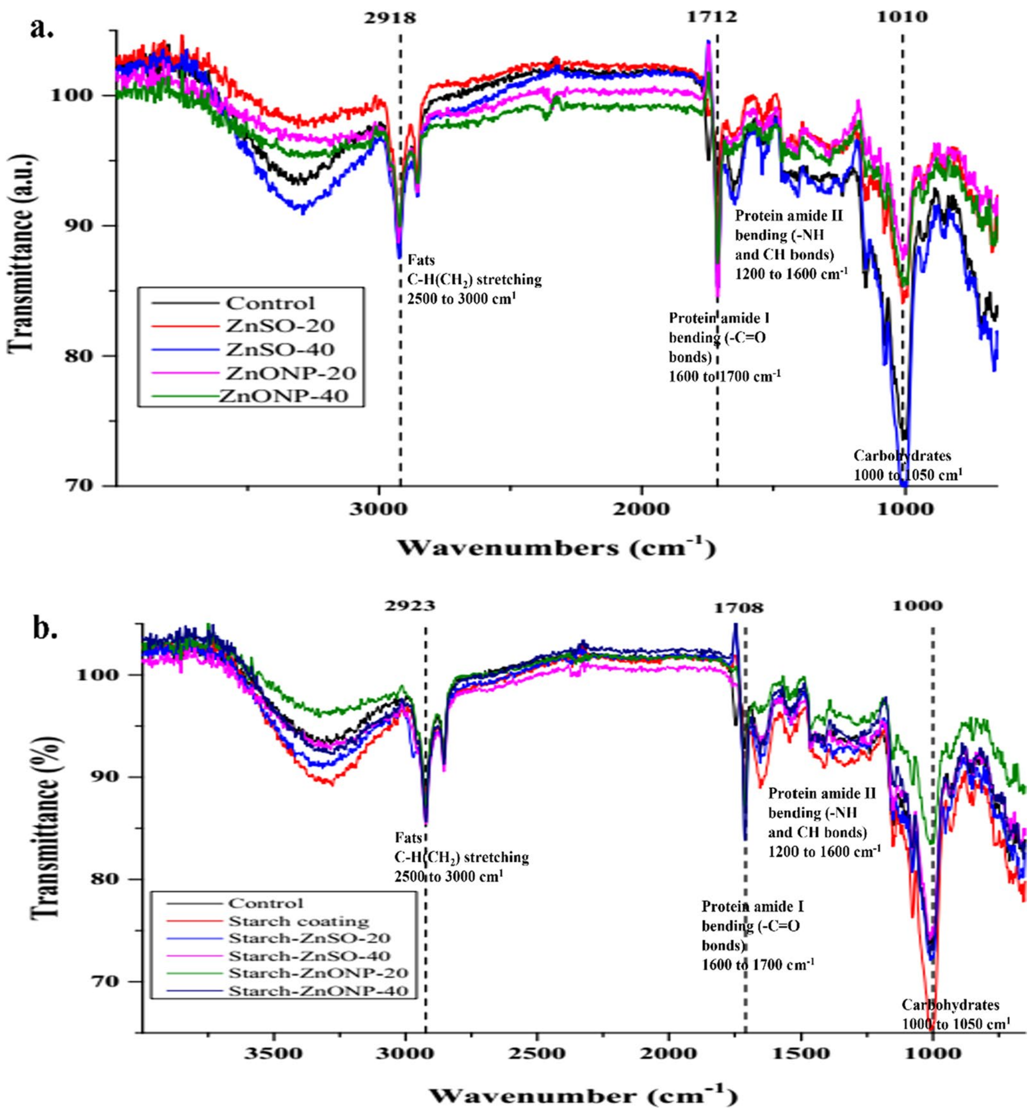

Fig. 3 Fourier-transform infrared spectra of the maize flour depicting the effect of zinc source seed priming (**a**) and coating **b** treatments on chemical functional group changes

2923 and 2918 cm^{-1} depicting the symmetric and/or asymmetric stretching vibrations of the $-(CH_2)_n$ bond of lipids appeared for the seed coating and priming treatments with ZnONPs/Zn-bulk sources, respectively. Seed priming treatment with $ZnSO_4$ (40 mg L^{-1}) and ZnONPs (20 mg L^{-1}) exhibited several intense peaks (2918, 2854, 1637, 1540, and 1010 cm^{-1}) compared to the control treatment. Seed coating treatment exhibited more discrete variations. The $-C-CH_3$ symmetric stretching peak at 2854 cm^{-1} indicating the lipid components in the flour can be observed to be more intense in $ZnSO_4$ and ZnONPs at 40 mg L^{-1}seed coating treatments (Fig. 3b). The $-C{=}O$ stretching (carboxylic acids) vibrations at 1337 cm^{-1}can be identified to be most intense in starch coating only treatment followed by starch + $ZnSO_4$ (20 mg L^{-1}) treatment (Fig. 3b). Furthermore, the fundamental peaks representing the bond vibrations of the peptide–protein, lipid, and carbohydrate groups at 1643, and 1537, 2923, 1710, and 1010 cm^{-1} were also recorded in these treatments. Also, antisymmetric (peak at 1241 to 1243 cm^{-1}) and symmetric (1076 cm^{-1}) stretching vibrations of phosphoryl or phosphoanhydride ($RO\text{-}PO_2\text{-}OR$) functional group which is a constituent of nucleic acids (DNA, RNA), membrane phospholipids, and phosphorylated protein were also recorded to be intense for priming and coating with zinc salt treatments compared to the respective control treatments. In addition, a strong band centred at 1005 to 999 cm^{-1} indicating the short-range structure (crystallinity) of the starch was observed to be more intense for seed coating treatments with ZnONPs and $ZnSO_4$ compared to the control treatment.

Soil nutrient and microbiological properties

The ANOVA results depicted a significant effect of sources of variation, i.e. DAS, seed treatments, and Zn-source concentrations on soil nutrient content (Table 4) and microbiological properties (Additional file 1: Table S2). The mean effect of the sources of variations exhibited specific trends. Among the DAS, significantly highest soil N, P, K, and Zn contents were recorded at 35 DAS (Table 4). Seed coating treatment significantly increased the available N, P, K, and Zn contents compared to the seed priming treatment. Among the Zn-source concentrations, a 1.12-, 1.15-, 1.12-, and 1.19-fold increase in the N, P, K, and Zn contents was recorded in the ZnONPs (40 mg L^{-1}) treatment as compared to the control treatment.

Zinc application via seed coating and priming significantly affected the microbial population (Additional file 1: Table S2). A temporal variation was also observed for the various Zn-source seed treatments with the highest average fungal, bacterial, and actinobacterial and pseudomonads and non-symbiotic nitrogen fixer viable cell counts recorded at 35, 60 and 90 DAS, respectively. Seed coating treatment led to a significant increase in the average viable cell counts of all the microorganisms evaluated compared to the seed priming treatment. Among the ZnONPs concentration, ZnONPs (at 40 mg L^{-1}) showed significantly higher mean viable cell counts for all the microorganisms evaluated compared to bulk $ZnSO_4$ and control. In terms of a per cent increase, the ZnONPs (at 40 mg L^{-1}) treatment exhibited 4.70, 22.70, 21.86, 6.90, and 25.79% increase in the viable cell counts of bacteria, fungi, pseudomonads, actinobacteria, and non-symbiotic N-fixers, respectively, over control (Additional file 1: Fig. S3).

Similar to microbial viable cell count trends, significant positive effects on the soil enzyme activities, i.e. dehydrogenase and phosphatase (acid and alkaline) activities were recorded in the seed coating treatment. The DAS also significantly affected the evaluated soil enzyme activities with significantly highest dehydrogenase and acid phosphatase activities observed at 60 DAS. In comparison, an increased alkaline phosphatase activity was recorded

Table 4 Mean effect of DAS, seed treatments, and concentration of Zn-source on soil nutrient content in maize crop (variety PMH-1) grown under field conditions

Sources		N (kg ha^{-1})	K (kg ha^{-1})	P (kg ha^{-1})	Zn (mg ha^{-1})
Days after sowing (DAS)					
35		170.44a	136.91a	18.32a	1.022a
120		162.03 b	126.09b	17.44b	0.739b
Seed treatment					
Priming		157.50 b	131.36a	15.084b	0.922b
Coating		167.97 a	131.64a	17.68a	1.041a
Zn-source concentration (mg L^{-1})					
0		150.35 e	124.39d	16.51d	0.918d
$ZnSO_4$_20		155.30 d	129.12c	17.33c	0.911e
$ZnSO_4$_40		159.29 c	129.80c	17.26c	0.925c
ZnONPs 20		167.21 b	134.08b	18.73b	1.055b
ZnONPs 40		169.02 a	140.12a	19.09a	1.0991a
ANOVA results					
Source	DF				
DAS	1	70,201.657***	1755.977***	518.263***	3.528***
Treatment	1	49,530.520***	1.229*	318.136***	0.210***
DAS*Treatment	1	317.860***	7.079**	0.142*	0.004***
Concentration	4	7847.062***	420.265***	117.935***	0.093***
DAS*Concentration	4	2445.266***	7.852***	11.176***	0.003***
Treatment*Concentration	4	6356.658***	143.180***	14.550***	0.053***
DAS*Treatment*Concentration	4	1162.322***	10.150***	2.651***	0.043***

Means within the sub-factor followed by the same letter in a column are not significantly different at $p \leq 0.05$ according to pair-wise comparison of least square means

* = $p \leq 0.05$, ** = $p \leq 0.01$, *** = $p \leq 0.001$, *ns* not significant

at 90 DAS (Additional file 1: Table S2). Among the Zn-source concentrations, seed treatment with the ZnONPs (40 mg L^{-1}) led to 8.11 and 22.62 per cent increase in the dehydrogenase and acid phosphatase activities over the control treatment. At the same time, 2.26-fold increase over control in the alkaline phosphatase activity was observed in the ZnONPs (20 mg L^{-1}) treatment.

Discussion

Nanomaterials exhibit unique and size-dependent properties compared to their bulk counterparts (Kalia and Kaur 2019b). The nanoscale dimensions of the nanoparticles endow them the ability to readily be taken up by the living cells. Besides the nano-size, the surface charge or zeta potential of the nanomaterials substantially affects their adsorption and agglomeration properties (Kalia et al. 2020). These aspects have been harnessed for the development of novel formulations of the nutrient fertilizers particularly the micronutrient nanoparticles (Kalia and Kaur 2019a). Zinc nanoparticles have been most extensively evaluated for their nutrient delivery prospects under different growing conditions for a variety of crop plants. The ZnONPs synthesised in the present study exhibited an average size of 70.0 nm with a net negative zeta potential. The DLS analysis of the aqueous nanoparticle suspension exhibited an average hydrodynamic diameter which was less than 100 nm. The size of these nanoparticles appears to be appropriate for adsorption and internalization through the pericarp and seed coat layers of the maize grain. The pericarp of the maize seed is comprised of layers of tubular structures of mostly dead cells which form microcavities with interface pore dimensions ranging from 1 to 2 µm (Segatto et al. 2020, 2018). Adsorption of the ZnONPs on the edges of these microcavities followed by internal movement through microchannels represents the apoplastic pathway of NPs movement (Segatto et al. 2018). As the wet chemical method was used for generation of the ZnONPs, zeta potential has been observed to be comparable (−22.0 mV) (Nabil et al. 2020). Also, the results of these two characterizations for the ZnONPs were observed to be approximately similar to another published report (Chai et al. 2018).

Earlier reports have evaluated the effect of foliar application of zinc nanoparticles on a variety of crops. However, use of ZnONPs as seed invigoration agents has attracted the attention of the researchers in recent decade due to use of substantially low concentrations and greater eco- or bio-safety relevance. The extent of interaction of the primed/coated components on the seed surface may vary for the respective treatments as seed coating ensures slow and sustained release of the active ingredient coated on the seed surface thereby resisting the loss to environment. In this study, the synthesized and characterized ZnONPs were compared with the bulk zinc salt by applying two different Zn-sources as seed priming and coating agents. These were observed to exhibit no significant effect on the vegetative growth, yield, and yield-related traits, but grain quality, soil nutrient, and microbiological characteristics in maize variety (PMH-1) crop cultivated under field conditions exhibited few significant variations. Germination which is an important phase that ensures optimal production and productivity of crops can be affected by use of seed invigoration agents. However, there was no improvement in the percent germination of the maize seeds in this study unlike the other published reports. However, the improved germination by Zn-source treatment was anticipated due to the possible role of zinc in elicitation of the hydrolytic enzymes for mobilization of the reserve food in the seed (Haider et al. 2020) besides in radicle and coleoptile development (Ozturk et al. 2006). Overall, it can be argued that as the maize grains used in this study possibly had substantial amount of native Zn-content, the application method and state of the Zn-source did not further enhance the germination percent. The number of plants per plot remained statistically at par among the various treatments with maximum count in the control treatment. Though the nanoparticle treatments were expected to improve the germination of the seeds and the plant stand, there was no eliciting effect recorded in this study. Similarly, the vegetative growth recorded as shoot height was also observed to be statistically at par with the control treatment in this study. Among the other vegetative parameters, estimation of the chlorophyll content provides useful positive or negative trends for applying the nanoparticles. Application of various zinc sources as seed priming and coating altered the chlorophyll content (Fig. 2). An increase in the chlorophyll content has been observed by application of ZnONPs (50 mg L^{-1}) in maize (*Zea mays*) (Adhikari et al. 2016). The enhanced chlorophyll content positively affected net photosynthesis and increased dry weight and improved yield. This might be due to the involvement of zinc in plant cell division and leaf membrane integrity (Gupta et al. 2016).

Zinc is considered a key element for maize as its deficiency, particularly during the grain filling stage, decreases plants' grain yield and efficiency. Seed coating and priming with Zn-salt and ZnONPs exhibited grain yield at par to the respective control treatments, and there was only small non-significant enhancement in the grain yield and yield-attributing traits as indicated in Table 2. This meagre enhancement in the yield and yield-contributing traits can be attributed to enhanced photosynthesis of the maize plants in ZnONPs seed priming/coating treatments compared to the control treatment. A significant increase in the yield-attributing parameters has been reported by other researchers. Estrada-Urbina et al. (2018) have recorded highest cob weight and enhanced number of rows per cob over the control treatment in maize on application of nano-ZnO particles (0.16 mg/seed). Likewise, higher number of kernels per row on nano-ZnO treatment have been reported. According to Uma et al. (Uma et al. 2019), nano-zinc oxide (800 ppm) significantly increased the grain yield (8750 kg ha^{-1}) in *Zea mays* compared to the control treatment. In a field experiment, Subbaiah et al. (2016) have reported 42 and 15% enhancement in the grain yield in ZnO nanoparticles (400 mg/L) treatment compared to the control and $ZnSO_4$ salt treatments, respectively. Mahdieh et al. (2018) have reported significant increase in the 1000-grain weight on treatment with 0.15% of nano-ZnO in pinto bean (*Phaseolus vulgaris*) crop.

Zinc altered the total starch content as both priming and coating treatments of the maize seeds with either of the Zn-sources resulted in significant improvement in the total starch content (Table 3). However, only seed coating treatment with Zn-sources improved the total soluble protein content as compared to the control treatment (Table 3). The positive impact of zinc on these seed quality traits may be due to the versatile role of zinc as cofactor of the enzymes related to starch synthesis and crystallization (Castillo-Gonzalez et al. 2018). The results of El-Metwally et al. (2018) support the findings of this study (Table 3). The researchers have reported that the application of nano-Zn fertilizer (30 ppm) resulted in the highest total carbohydrate (326.93 mg/g) content in the peanut seed compared to the control treatment. Likewise, Samreen et al. (2017) explained that the total protein content of mung bean seeds increased by 28.2 to 72.3 per cent. The FT-IR spectra of the harvested maize seeds also indicated the improved starch and protein contents through the specific intense peaks for the carbohydrates and peptides, which were observed to be more conspicuous in the coating treatment with the Zn-salt (20 mg L^{-1}) and ZnONPs (40 mg L^{-1}) treatments (Fig. 3). Similar FT-IR peaks in the maize flour have also been reported by Estrada-Urbina et al. (2019) obtained for the landraces of Mexican maize on ZnONPs treatment. Thus, application of various concentrations of Zn-source as coating and priming treatments significantly improved the grain nutrient contents in this study (Table 3). This improvement may be due to enhanced cation-exchange ability of the root tissue, which in turn increased the absorption of essential nutrients, especially nitrogen that is responsible for the higher protein content. Similarly, the P content in the cluster bean plant increased to 1023.27 mg kg^{-1} by application of ZnONPs (10 mg L^{-1}) over the control (923.19 mg kg^{-1}) treatment (Raliya and Tarafdar 2013).

Application of various zinc concentrations as coating and priming significantly improved the soil enzyme activities (Additional file 1: Table S2). A similar significant enhancement in the acid and alkaline phosphatase activities by applying nano-ZnO (10 mg L^{-1}) in the rhizosphere soil of cluster bean plant has been supported by Raliya and Tarafdar (2013). Another study by Nannipieri et al. (2011) reported that acid phosphatase activity was greatly reduced by exposure to ZnONPs (500 mg kg^{-1} dry soil) at 28 and 56 days after sowing soybean crop. Sri Sindhura et al. (2014) identified a similar significant improvement in soil bacteria and fungi population on ZnONPs treatment compared to control. Further, a dose-dependent effect of ZnONPs treatment on microbial population has been observed by Kumar et al .(2019). They have reported that ZnONPs (40 mg kg^{-1}) application resulted in higher pseudomonad counts than ZnONPs (10 mg kg^{-1}) treated soil.

The soil macronutrient and Zn contents also got altered by the application of ZnONPs (Table 4). Therefore, the possible increase in the zinc contents by the seed coating and priming treatments with ZnONPs might be due to improvement in the availability of Zn directly due to nanoscale dimensions of the applied ZnONPs. Further, the enhancement in the viable counts of the various plant growth promoting rhizosphere microbes (particularly the fungal population) can substantially affect the extent of improvement in the availability of insoluble or plant unavailable nutrients in the soil due to lowering of soil pH caused as a result of secretion of organic acids (Sharifi et al. 2016). In addition, the N-fixing microbes have a crucial role in fixation of the atmospheric nitrogen through process of biological nitrogen fixation (Bageshwar et al. 2017). The increased microbial population also indicated through high dehydrogenase activity in the ZnONPs treatments refer to organic forms of carbon and nitrogen as microbial cells (Additional file 1: Table S2). Another study in maize also supported the increased concentrations of Zn in soil under maize cultivation on application of ZnONPs in the soil (Liu et al. 2015).

Conclusions

Maize grain yield and quality is substantially affected by the zinc micronutrient. However, the external application of Zn will only be useful under Zn-deficient soil conditions. Also, the native Zn-content of the maize grain could be probable factor governing the benefits imparted by priming/coating of Zn-sources. The state of the zinc source bulk salt or nanoparticles can affect the benefit imparted to the crop. However, in this study

Zn-bulk salt and ZnONPs exhibited equivalent effects on percent germination, plant stand, and vegetative growth of the test plants. Though the seed coating treatment with ZnONPs (40 mg L^{-1}) improved the total and 1000-grain weight. Further, it also enhanced seed quality traits such as total starch, total soluble proteins, and nutrient content, including nitrogen, phosphorus, potassium, and zinc. Also, the seed invigoration treatments with Zn-sources elicited the soil available nutrients, microbial viable cell counts, and soil enzymatic activities. The same seed treatment, i.e. ZnONPs (40 mg L^{-1}), enhanced the soil available N, P, K, and Zn contents, viable cell counts of soil aerobic bacteria, fungus, actinobacteria, pseudomonads, and non-symbiotic N-fixers besides increasing the dehydrogenase and alkaline phosphatase enzyme activities. Therefore, the study results invariably indicate the differential positive effect of seed treatments with nano-zinc oxide particles in grain maize for only improvement in the quality traits of maize var. PMH-1 though the vegetative growth indicators were not statistically affected.

Materials and methods

Procurement of seeds

The seeds of maize (variety PMH-1) were obtained from ICAR-Indian Institute of Maize Research, PAU Campus, Ludhiana, Punjab. Uniformly sized fresh and disease-free seeds were screened and used for the experiment.

Synthesis and characterization of zinc oxide nanoparticles

The zinc oxide nanoparticles (ZnONPs) were prepared through wet chemical technique (Davis and Singh 2016) and were characterized for the SEM, SEM–EDS, TEM, and XRD analysis as depicted (Tondey et al. 2021). In addition, the hydrodynamic diameter and zeta potential of the nanoparticles were performed for the aqueous dispersions at room temperature (25 °C) on Delsa™ Nano HC Particle Analyzer (Beckman Coulter, USA).

Details of experimental conditions and treatments

The field experiment was conducted during March in 2019 at Experimental Research Farms, Ladhowal (Latitude: 30°58′36.8″ N, Longitude: 75°44′33.6″ E) of ICAR-Indian Institute for Maize Research, PAU Campus, Ludhiana, Punjab. The region exhibits sub-tropical climate conditions with a pattern of hot and dry summers followed by cold winters. During the maize growing season, the region received a maximum rainfall of 292.0 mm in June 2019. The temperature maxima and minima ranged from 18.5 to 44.28 °C and 6.4 to 32.0 °C, respectively (Fig. 4). The average sunshine hours during the crop growth period were 8.2 h. The soil of the experimental site was calcareous soil that exhibited the following properties: pH of 7.65, 4.01 mg kg^{-1} soil organic matter, 86.8 kg ha^{-1} available N, 8.43 kg ha^{-1} Olsen P, 111.4 kg ha^{-1} available K, and 0.34 mg kg^{-1} DTPA-Zn.

The experiment was laid out in a randomized block design for ten treatments with three replications. This experiment aimed to compare the effect of three different

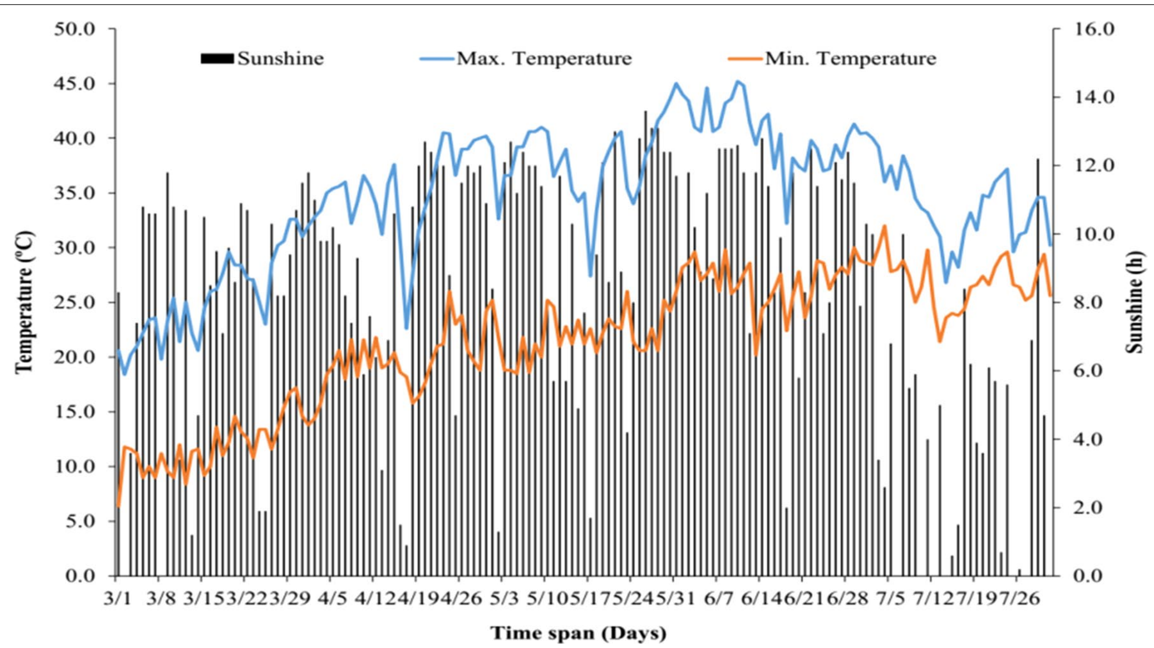

Fig. 4 Minimum and maximum temperatures during March to June months at experimental site (Ladowal 2019)

concentrations (0, 20, and 40 mg L^{-1}) of the zinc sources (conventional zinc sulphate fertilizer and nano-ZnO particles or ZnONPs) as seed priming and coating treatments on vegetative growth, grain yield, and seed quality attributes of maize (PMH-1) grown under field conditions. The details of the treatments are provided in Additional file 1: Table S1. The concentrations used were identified from the previously published studies (Freitas et al. 2020; Neto et al. 2020). The working concentrations were prepared from the stock solution (100 µg mL^{-1}) for ZnONPs and zinc sulphate solution by further diluting the stock with deionized water. For the seed priming treatment, maize seeds previously disinfected with sodium hypochlorite solution (2% v/v) were incubated with the working solutions (0.1 mL/seed) for half an hour. The seed coating treatment was performed by mixing the Zn-source solution(s) having different working concentrations with a natural polymer-plasticizer mix of pre-gelatinized corn starch (1.5% w/v starch + 1.0% v/v glycerol) (Tondey et al. 2021). The mixture (10 µL per seed) was then applied to the surface of the maize seeds and kept on rocking shaker for half an hour followed by air drying. Finally, the treated seeds were sown in the field and tested for vegetative growth and yield-attributing traits besides soil nutrient status, microbiological, and enzymatic studies. The field experiment was performed with the individual plot dimensions of $5 \times 5 = 25$ m^2. The maize seeds were sown with row-to-row distance of 60 cm, and the plant-to-plant distance was maintained at 20 cm.

Vegetative growth parameters

The seed germination (%) in the field was recorded for both the seed priming and coating treatments daily as per the equation

$$\%\text{Germination} = \frac{\text{Total number of seeds germinated at field}}{\text{Total number of seeds sown in field}} \times 100 \quad (1)$$

The number of plants per plot was recorded at V8 (late whorl stage designated as occurrence of 8 fully emerged leaves) stage (Vâtcă et al. 2021). The plant height and ear length were estimated for any three randomly selected plants at R5 (maturity) stage of the crop. For each treatment, the number of leaves were manually counted at V5 stage (15 DAS) and again at VT (tasseling/silking stage) stage (Shen and Liu 2015). The effect of different seed priming and coating treatments on plant chlorophyll content was measured with the help of a SPAD photometer (SPAD-502, Soil Plant Analysis Development (SPAD) Section, Minolta Camera Co, Osaka, Japan) at V8 and VT stages of growth in the standing maize crop. The SPAD readings have been converted to chlorophyll content (µg cm^{-2}) by use of equation (Cerovic et al. 2012);

$$\text{Chl}\left(\mu g\, cm^{-2}\right) = (99\ \text{SPAD})/(144 - \text{SPAD})$$

Yield and yield-attributing traits

Three plants were chosen randomly from each treatment, tagged, and the number of internodes per plant was counted at the VT stage. Before harvesting, these tagged plants were used to manually count the number of cobs per plant, rows of seeds per cob, total number of seeds per cob, and total grain weight. A random sample of 1000 seeds were taken and weighed to record 1000-seed weight for each treatment. The total grains were obtained after harvesting the crop and weighed on an electronic weighing balance. The data were represented in kg ha^{-1}.

Grain quality parameters

After harvesting seeds, the total starch content was determined using an alcohol extraction method and same samples were utilized to analyse soluble proteins (Lowry and Randall 1951). The total nitrogen content of the ground grain samples was determined through the Kjeldahl's method. Ammonium vanadate/molybdate reagent-based method was used to estimate the total phosphorus in tested samples with 2,4 dinitrophenol indicator (Piper 1944), and potassium content was measured through the flame photometer method (Jackson 1958).

Maize seeds (variety-PMH-1) were finely ground in a mixer grinder and sieved through the stainless steel sieve (100 µm) and then kept in ziplock bags in desiccator until further used (Achten et al. 2019). The dried test maize seed flour (25 mg of sample flour) samples were placed on zinc selenide crystal (flat-bed configuration) of attenuated total reflectance (ATR) assembly of FT-IR spectroscope (Model Thermo Nicolet 6700, USA), and the wavenumber (cm^{-1}) versus transmittance (a.u.) spectra were collected (Estrada-Urbina et al. 2018). The final spectra were obtained for the mid IR range (4000 to 650 cm^{-1}) by summation of 32 scans run at 4 cm^{-1} resolution through Omnic software.

Soil nutrient status, microbial population, and soil enzyme activities

The N, P, K content from the treated soil samples was determined through the alkaline permanganate method, ascorbic acid reduction (Olsen et al. 1954), and by a flame photometer using neutral ammonium acetate extractant. The available micronutrient (Zinc) contents in both grains and soil samples were estimated through DTPA (diethylenetriaminepentaacetic acid) method (Lindsay and Norvell 1978).

Viable cell counts of rhizospheric soil microorganisms were enumerated using the dilution spread plate technique (Throndsen 1978) at 0, 30, 60, and 90 days after sowing (DAS) and at harvesting.

Number of CFU/g soil = Number (average of 3 replicates) of colonies × Dilution factor.

The soil enzyme activities were determined at periodic intervals for each treatment to estimate soil alkaline and acid phosphatase activity (Tabatabai and Bremner 1969) and dehydrogenase activity (Casida et al. 1964).

Statistical analysis

The data for various parameters in the field experiment laid out in randomized block design with three replications were recorded. Ten treatments were compared and included two types of zinc sources (Zn-salt and ZnONPs) used at three different working concentrations (0, 20 and 40 mg L^{-1}). The data were subjected to analysis of variance (ANOVA) using PROC GLM procedure of SAS Software package (version 9.3, SAS Institute Inc., Cary, NC, USA). The normality of the data was evaluated through Shapiro–Wilk's test (Shapiro and Wilk 1965). Further, the average means of the seed treatments and Zn-source concentrations were compared based on the generalization of the least significant difference (LSD) test with ANOVA significance at $p \leq 0.05$.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s40543-022-00346-1.

Additional file 1. **Supplementary Table 1.** Details of the treatments evaluated in the field study. **Supplementary Table 2.** Mean effect of DAS, seed treatments and concentration of Zn-source on soil (a) enzyme and (b) rhizosphere soil microbial viable cell counts characteristics in maize crop (variety PMH-1) grown under field conditions. **Supplementary figure 1.** Effect of seed priming and coating with bulk salt and ZnONPs in maize variety PMH-1 cultivated under field conditions. **Supplementary figure 2.** Effect of ZnONPs and salt precursor ($ZnSO_4$) on cobs of *Zea mays* cultivated under field conditions. **Supplementary figure 3.** Effect of ZnONPs and salt precursor ($ZnSO_4$) on microbial counts of soil samples of *Zea mays* (var. PMH-1) cultivated under field conditions.

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Author contributions

A.K., A.S., and K.A.A.-E. contributed to conceptualization; A.K., A.S., and G.S.D. were involved in methodology; M.T., A.K., and A.S. contributed to validation; M.T. and A.S. were involved in formal analysis; A.K. and M.T. contributed to investigation; A.K. and K.A.A.-E. were involved in resources; M.T. and A.K. contributed to writing—original draft preparation; A.K., A.S., K.A.A.-E., and M.M.H. were involved in writing—review and editing; A.K. contributed to visualization; A.K. was involved in supervision; A.K. contributed to project administration; and A.K. were involved in funding acquisition. All authors have read and agreed to the published version of the manuscript.

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Availability of data and materials

Not applicable.

Declarations

Competing interests

The authors declare no conflict of interest.

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