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Effect of Nano Particles on Seed Longevity of Hybrid Maize (Hema) Under Ambient Condition

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ABSTRACT: A laboratory experiment was undertaken to estimate the effect of iron, zinc, thiram and sweet flag rhizome powder nano particles on seed longevity of hybrid maize, in the Department of Seed Science and Technology, UAS, Raichur, Karnataka. The experiment consisted of 12 treatments., T_1: Control, T_2: Only polymer, T_3: Nano Fe (250 ppm), T_4: Nano Zn (500 ppm), T_5: Nano thiram (500 ppm), T_6: Nano SFRP (1000 ppm), T_7: Nano Fe + Nano Zn (T_3+T_4), T_8: Nano thiram +Nano SFRP (T_5+T_6), T_9: Nano Fe + Nano Zn +Nano thiram (T_3+T_4+T_5), T_{10}: Nano Fe + Nano Zn +Nano SFRP (T_3+T_4+T_6), T_{11}: Nano Fe + Nano Zn + Nano thiram +Nano SFRP (T_3+T_4+T_5+T_6) and T_{12}: Nano Fe + Nano Zn + Nano thiram +Nano SFRP (50% of T_{11}). Among the different treatments imposed, seed polymer coating with nano Fe @ 250 ppm, nano Zn @ 500 ppm and nanothiram @ 500 ppm recorded significantly higher seed germination (88.25, 88.25 and 88.00%), seedling vigour index (3464, 3452 and 3423), dehydrogenase activity (0.620,0.631 and 0.619 OD values) and alpha amylase activity (16.50, 16.90 and 16.30 mm), along with lower electrical conductivity (1.086, 1.007 and 1.091 dSm^{-1}) respectively, as compared to control (84.00%, 2974,0.487 OD values,13.55mm and 1.262 dSm^{-1}, respectively, for the above parameters) at the end of storage period. However, the combination of different nano particles showed negative effect on all seed quality parameters during the storage period. From the present findings it can be concluded that the individual nano particles ie., either Fe @ 250 ppm or Zn @ 500 ppm or nanothiram @ 500 ppm can be used for improving the seed longevity of hybrid maize (Hema).

Key words: Maize, Polymer, Fe, Zn, Thiram, Sweet flag rhizome powder

INTRODUCTION

Quality seed is a basic input in agriculture for successful crop production. Thus, the seed producers hold a great responsibility of producing and distributing quality seeds to the end users. Since seed is a biological entity, ageing is an inevitable phenomenon. The ageing process cannot be stopped completely once commenced, but can be controlled to certain extent by adopting certain technologies. Storage of seeds is a serious problem in tropical and subtropical countries where high temperature and high relative humidity greatly accelerate seed ageing, resulting in deterioration and loss of viability [1]. Some physiological and biochemical changes during seed deterioration increases the lipid autoxidation and accumulation of toxic metabolites, free radical damage, breakdown in mechanism that triggers germination, changes in polar lipids, and ultra structural damage to cell and its organelles and reduces protein synthesis, respiration, glyco and phospholipids content, [2&3]. In order to alleviate the deterioration process in seeds, nanotechnological approaches may be a plausible solution.

Nanotechnology is a fascinating field of science dealing with atom by atom manipulation that yields processes and products which are likely to transfer traditional farming into precision agriculture through novel approaches [4&5].

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Nanotechnology advances the agricultural research such as reproductive science and technology, conversion of agricultural and food wastes to energy and other useful by-products through enzymatic nano bio-processing, disease prevention and treatment in plants using various nanocides [6]. Lot of work has been done to address a wide range of agricultural problems utilizing nanomaterials and nano-devices. Various nanotechnological approaches including nano-polymer for seed longevity, nano-sensors, nano-barcodes, use of nano-magnetic particles for aerial seeding *etc.*, have been reported [7]. However, the benefit of its utilization in seed hardening, seed invigoration and seed germination enhancement need to be explored. With this background an experiment was initiated with an objective to study the effect of nano particles on seed longevity of hybrid maize (Hema) under ambient condition.

MATERIALS AND METHODS

The experiment was conducted in the Department of Seed Science and Technology, College of Agriculture, Raichur, and Nano laboratory, Department of Processing and Food Engineering, College of Agricultural Engineering, Raichur, Karnataka, India (16° 15' N, 77° 20' E and 389 meter MSL Altitude of North-Eastern dry zone of Karnataka state) in 2015-16. The seeds were stored in cloth bags under ambient conditions for a period of nine months (September, 2015 - May, 2016) under ambient condition.

Description of the genotype

The single cross hybrid Hema (NAH-1137) is a long duration (120-130 days) hybrid popularized in southern transitional zone, central dry zone and eastern dry zone of Karnataka, suitable for temperate podzols to leached red soils of the tropics under rain fed and protective irrigation conditions. The hybrid yields 100-150 q ha^{-1} grain.

Source of seeds, nanoparticle and polymer

The seeds were procured from National Seed Project, UAS Bangalore. While, Fe and Zn nano particles were procured from Sisco Research Laboratories Pvt. Ltd., Mumbai, Maharashtra and the hydrophilic polymer (Disco Agro DC Red L-603) from Incotec Pvt., Ltd. Ahmadabad, Gujarat.

Synthesis and Characterization of nano particles

High-energy ball milling method was followed as it is a convenient way to produce nanosized powders [8]. Here, before starting mechanical milling, the powdered material of thiram and SFRP to be converted to nano form were poured in to a container along with several heavy balls (steel or tungsten carbide). Then at -196°C (liquid nitrogen induced temperature) the container was allowed for mechanical milling (at 25 Hz oscillating frequency) until the loaded material (ball to sample ratio of 4:1) was converted to nanosize. All the four nano particles were suspended directly in distilled water and dispersed by ultrasonic vibration (100W, 40 kHz) for 30 min using digital ultrasonicator. Small magnetic bars were placed in the suspension for proper stirring before their use in order to avoid aggregation of the nano particles. The size of the nano particles were characterized using zetasizer (Malvern Zetasizer, Nano-ZS, Malvern Instruments, UK) which works on the principle of dynamic light scattering. Zetasizer require as little as 12 micro litres of sample for measurement. Accurately 0.5 mg of sample was dispersed in 10 ml distilled water through ultra sonication and the measurements were taken. The particle size of 47.60, 43.82 nm, 113.1 nm and 185.8 nm were observed. The shape of Fe and Zn nano particles were characterized using scanning electron microscope which uses a focused beam of high energy electrons to generate a variety of signals at the surface of solid specimen. The scanning electron microscope (SEM) named ZEISS SMART SEM was used in the present investigation to characterize the morphology of nano particles. For taking images, 0.5 mg sample of each nano particles were dusted on one side of double sided adhesive carbon conducting tape. The tape was dusted off using blower to remove un-adhered nano particles and then mounted on 8 mm diameter aluminium stub. Then, the sample on the surface of stub was observed at different magnification and images were taken.

Experimental Design and Treatments

The laboratory experiment was conducted in the Department of Seed Science and Technology, University of Agricultural Sciences, Raichur to study the influence of inorganic and organic NPs on seed longevity of hybrid maize (*Zea mays* L.) Seeds with 8.2 per cent moisture were treated with only polymer (T_2); Nano Fe (250 ppm) (T_3); Nano Zn (500 ppm) (T_4): Nano thiram (500 ppm) (T_5); Nano SFRP (1000 ppm) (T_6); Nano Fe + Nano Zn (T_7), Nano thiram +Nano SFRP ((T_8); Nano Fe + Nano Zn +Nano thiram (T_9); Nano Fe + Nano Zn +Nano SFRP (T_{10}); Nano Fe + Nano Zn + Nano thiram +Nano SFRP (T_{11}) and Nano Fe + Nano Zn + Nano thiram +Nano SFRP (50% of T_{11}) (T_{12}). The experiment was planned in a Completely Randomized Block Design with four replications. For imposition of these treatments, the seeds were treated with the respective concentrations of the specific nano particles using 4 ml polymer kg^{-1} seed after dissolving in 40 ml distilled water (standardised from preliminary experiment). The treated seeds along with control (T_1), after thorough drying, were stored in cloth bags for nine months. The observations on germination percentage [9], seedling vigour index [10], electrical conductivity (dSm^{-1}) [11], dehydrogenase enzyme activity (OD value) [12] and alpha amylase activity (mm) [13] were recorded. The results obtained from the experiment were analysed [14] and presented in Table 1 and Table 2. The critical differences were calculated at one per cent level of probability wherever 'F' test was found significant for various seed quality parameters under the study.

RESULTS AND DISCUSSION

The germination percentage due to seed treatment with nanoparticles varied significantly during the entire nine months of storage period. At the initial month, significantly higher germination (97.50 %) was recorded in nano Fe @ 250 ppm (T_3) and it was on par with T_4 (97.25 %), T_5 (97.00 %), T_7 (96.50 %) and T_8 (97.00 %) whereas T_{11} registered the low value of 92.50% which had no significant difference with control (93.30%). Similarly, at ninth month of storage significantly higher germination (88.25 %) was recorded in both nano Fe @ 250 ppm (T_3) and nano Zn (T_4) (88.25 %), which were statistically on par with T_5 (88.00 %) and T_6 (87.50 %). T_{11} (T_3+T_4+T_5+T_6) registered the lowest germination (82.75 %) which was statistically on par with control T_1 (84.00 %). The probable reason for enhanced germination due to nano Zn and Fe over absolute control might be due to the nano size of particles that might have allowed them to penetrate easily through seed coat and thereby, ensuring better absorption and utilization of Fe and Zn by seeds [15]. The beneficial effect of nanoparticles in improving the germination can also be ascribed to higher precursor activity of nanoscale Zn and Fe in production of essential bio molecules vis-a-vis essential nutrients required for plant growth which are important components of various enzymes responsible for driving many metabolic reactions [16]. Further, nanoparticles would helps in quenching of free radicals from the germinating seeds [17]. In turn, oxygen produced during such process could also be used for respiration, which would further promote germination [17]. The probable reason for decreased seed germination at higher concentration could be due to increased absorption and accumulation of these nanoparticles both in extracellular space and within the cells resulting in reduction of cell division, cell elongation and inhibition of the hydrolytic enzymes involved in mobilization of food during the process of seed germination [18]. Similar results were also reported by [19] in *Raphanus sativus*, *Brassica napus*, *Lolium multiflorum*, *Lactuca sativa*, *Zea mays* and *Cucumis sativus* using multi walled carbon nanotubes, aluminium oxide and zinc oxide nano particles. Nano Fe @ 250 ppm (T_3) recorded the highest seedling vigour index (4667) and it was on par with T_4 (4633) followed by T_5 (4584) while, the lowest was recorded in T_{11} (3805) at initial month. However, at nine months after storage significantly higher seedling vigour index (3464) was recorded in T_3 (nano Fe @ 250 ppm) and the lowest (2788) in T_{11} (T_3+T_4+T_5+T_6). The same trend was followed during the storage period. The enhanced seedling vigour due to utilization of nanoparticles at optimum concentration might be due to better seed germination, seedling growth (root and shoot length) and seedling dry weight. Further, the enhanced physiological

Table 1. Effect of nano particles on seed germination and seedling vigour index of maize during storage

Treatments	Germination (%)			Seedling vigour Index		
	Initial	After 5 months	After 9 months	Initial	After 5 months	After 9 months
T_1: Control	93.00	88.50	84.00	3920	3475	2974
T_2: Only polymer(4 ml kg^{-1})	95.00	91.00	86.25	4119	3666	3121
T_3: Nano Fe @ 250 ppm	97.50	92.75	88.25	4667	4000	3464
T_4: Nano Zn @ 500 ppm	97.25	92.25	88.25	4633	3968	3452
T_5: Nano thiram @ 500 ppm	97.00	92.00	88.00	4584	3911	3423
T_6: Nano SFRP @ 1000 ppm	96.00	91.25	87.50	4463	3895	3374
T_7: (Nano Fe @ 250 ppm + Nano Zn @ 500 ppm)	96.50	91.75	86.75	4322	3831	3297
T_8: (Nano thiram @ 500 ppm + Nano SFRP @ 1000 ppm)	97.00	92.50	86.50	4286	3785	3287
T_9: (Nano Fe @ 250 ppm + Nano Zn @ 500 ppm + Nano thiram @ 500 ppm)	95.50	90.50	85.50	4098	3542	3051
T_{10}: (Nano Fe @ 250 ppm + Nano Zn @ 500 ppm + Nano SFRP @ 1000 ppm)	95.00	90.25	84.75	4051	3531	2998
T_{11}: (Nano Fe @ 250 ppm + Nano Zn @ 500 ppm + Nano thiram @ 500 ppm + Nano SFRP @ 1000 ppm)	92.50	87.50	82.75	3805	3302	2788
T_{12}: (Nano Fe @ 125 ppm + Nano Zn @ 250 ppm + Nano thiram @ 250 ppm + Nano SFRP @ 500 ppm)	95.25	89.75	84.25	4171	3562	2952
Mean	95.63	90.83	86.02	4260	3706	3180
SEm±	0.37	0.32	0.42	29	36	23
CD at 1%	1.17	1.02	1.29	85	102	66

Note: For all the treatments 4 ml polymer kg^{-1} seed after dissolving in 40 ml distilled water was used as a coating agent

performance of seeds due to nanoparticles treatment could be attributed to the quenching of free radicals by the nanoparticles [20]. Smaller size of the nanoparticles would have easily entered through cracks present on the outer seed surface, reacted with free radicals resulting in enhanced seed vigour. Sengupta and Mandal [21] proved that finely powdered pharmaceutical formulation and crude plant materials entered through cracks and crevices in onion seed coat

Table 2. Effect of nano particles on electrical conductivity (dSm^{-1}), dehydrogenase enzyme activity (OD value) and alpha amylase activity (mm) of maize during storage

Treatments	Electrical conductivity (dSm^{-1})			Dehydrogenase enzyme activity (OD value)			Alpha amylase activity (mm)		
	Initial	After 5 months	After 9 months	Initial	After 5 months	After 9 months	Initial	After 5 months	After 9 months
T_1: Control	0.726	0.979	1.262	0.881	0.649	0.487	19.61	16.40	13.55
T_2: Only polymer(4 ml kg^{-1})	0.639	0.891	1.189	0.994	0.727	0.520	21.45	18.98	14.12
T_3: Nano Fe @ 250 ppm	0.616	0.869	1.086	1.142	0.821	0.620	23.90	20.95	16.50
T_4: Nano Zn @ 500 ppm	0.603	0.859	1.007	1.150	0.832	0.631	24.29	21.27	16.90
T_5: Nano thiram @ 500 ppm	0.637	0.872	1.091	1.131	0.807	0.619	23.34	20.50	16.30
T_6: Nano SFRP @ 1000 ppm	0.645	0.893	1.132	1.089	0.802	0.608	22.75	19.47	16.90
T_7: (Nano Fe @ 250 ppm + Nano Zn @ 500 ppm)	0.660	0.892	1.159	1.067	0.799	0.559	21.78	19.49	14.20
T_8: (Nano thiram @ 500 ppm + Nano SFRP @ 1000 ppm)	0.654	0.897	1.163	1.005	0.761	0.549	22.08	18.75	12.80
T_9: (Nano Fe @ 250 ppm + Nano Zn @ 500 ppm + Nano thiram @ 500 ppm)	0.687	0.918	1.185	0.990	0.726	0.515	19.90	16.71	13.06
T_{10}: (Nano Fe @ 250 ppm + Nano Zn @ 500 ppm + Nano SFRP @ 1000 ppm)	0.700	0.923	1.179	0.975	0.711	0.540	20.24	16.57	12.65
T_{11}: (Nano Fe @ 250 ppm + Nano Zn @ 500 ppm + Nano thiram @ 500 ppm + Nano SFRP @ 1000 ppm)	0.765	1.007	1.293	0.875	0.638	0.468	17.00	14.75	10.65
T_{12}: (Nano Fe @ 125 ppm + Nano Zn @ 250 ppm + Nano thiram @ 250 ppm + Nano SFRP @ 500 ppm)	0.688	0.899	1.129	0.989	0.764	0.529	22.58	19.68	14.15
Mean	0.668	0.908	1.162	1.024	0.753	0.554	21.58	18.63	14.32
SEm±	0.014	0.010	0.013	0.015	0.008	0.008	0.32	0.30	0.31
CD at 1%	0.040	0.031	0.041	0.048	0.025	0.021	0.99	0.87	0.92

Note: For all the treatments 4 ml polymer kg^{-1} seed after dissolving in 40 ml distilled water was used as a coating agent resulting in enhanced seed vigour and viability. Similar results of increase in seedling vigour by the use of ZnO (0.2%) nanoparticles was reported in red gram [22] and black gram [20]. Further, Zero Valent Iron (ZVI) was reported to enhance seedling vigour in tomato [23]. Likewise, at the initial month of storage, significantly lower electrical conductivity of 0.603dSm^{-1} was recorded in nano Zn @ 500 ppm and it was on par with T_3 (0.616dSm^{-1}) and T_5 (0.637dSm^{-1}). While, the highest electrical conductivity was recorded in T_{11} (0.765dSm^{-1}) and it had no significant difference with T_1 (0.726). Similar trend was noticed up to 9 months of storage period. The result depicts that the thin polymer with optimum nanoparticle over seeds surface maintains the seed coat integrity, thereby reducing the leakage of solutes from the seeds. These results are in conformity with the earlier findings in cotton [24] and [25] in maize who treated polymer at 5ml kg^{-1}and

3 ml kg^{-1}, respectively. It was hypothesized that the nanoparticles at higher concentration may generate new pores on seed coat during penetration thereby influx and efflux of the nutrients and minerals from seed increases leading to higher electrical conductivity [26]. Similar results of lesser electrical conductivity at 0.5 g lit^{-1} of iron nanoparticles and higher electrical conductivity at 1 g lit^{-1} of iron nanoparticles treated seeds was observed in soybean [27]. However, dehydrogenase activity declined gradually with advancement in the storage period, irrespective of the treatment. Nano Zn @ 500 ppm recorded the highest dehydrogenase activity which was statistically on par with nano Fe @ 250 ppm and nanothiram @ 500 ppm. The lowest was recorded in combination treatment of nano Zn @ 500 ppm+ nano Fe @ 250 ppm+ nanothiram @ 500 ppm+ nano SFRP @ 1000 ppm which was statistically on par with control. Similar trend was followed over a period of nine months storage. Similarly, á- amylase activity differed significantly due to the nanoparticles seed treatment over a period of 9 months of storage. However, nano Zn @ 500 ppm recorded the highest á- amylase activity which was on par with nano Fe @ 250 ppm and nanothiram @ 500 ppm. While, the lowest á-amylase activity was recorded in the combination of nano Zn @ 500 ppm+ nano Fe @ 250 ppm+ nanothiram @ 500 ppm+ nano SFRP @ 1000 ppm. Reduction in glucose utilization occurs in the deteriorated seeds which are reflected through lower dehydrogenase and amylase activity. Thus, measured from the point of activity of these enzymes, nanoparticles treated seeds were found to have higher enzymatic activity, showing the positive role of nanoparticles in improving the seed vigour and viability. It is an established fact that, Zn and Fe (contain sulphur) acts as cofactors for the most of the enzyme complexes involved in respiration and food mobilization in seeds [28]. The increased availability of these micronutrients at nanoscale, with increased chemical reactivity, resulted in increase in synthesis and activity of the enzymes or could be due to repair of damaged vital cell organelles [29-31] and counteraction of lipid peroxidation and minimization of free radical reactions [32, 33].It might also be due to their capacity to maintain the cell endowments triggering the germination events [34]. The effect of Zn nanoparticle could be attributed to excellent proton radical scavenging property [23] and inactivation of free radicals by enzymes as a reflection of ageing and subsequent alleviation [20]. These beneficial effects of nano powders were also observed by [17] in aged seeds of spinach in improving seed vigour. Thus, the present investigation leads to the assumption that the deterioration in the enzymatic system of seed with ageing might have been restricted by the nanoparticles by quenching reactive oxygen species [17,35]. The increased availability of these micronutrients at nanoscale with increased chemical reactivity resulted in increase in synthesis and activity of the dehydrogenase enzyme. These results are in conformity with the findings of [36] with TiO_2 nanoparticles in maize. However, at higher concentration, coated seeds recorded lower dehydrogenase and á- amylase activity as heavy metal nanoparticles increased the activity of antioxidant enzymes like catalase and ascorbate peroxidase in plants [37]. Nanoparticles upon entering the cells, induce intracellular oxidative stress by disturbing the balance between oxidant and anti-oxidant processes. Excessive oxidative stress may also modify proteins, lipids, and nucleic acids, which further stimulates the anti-

oxidant defence system or even leads to cell death. Meanwhile, with increased reactive oxygen species production, nanoparticles can cause DNA damage and increase gene expression of the death receptor [38]. In addition, increased reactive oxygen species induced by nanoparticles in lysosomes can cause DNA point mutations or induce single or double strand breaks [1].

CONCLUSION

Seed treatment with Fe (250 ppm), Zn (500 ppm) and thiram (500 ppm) nanoparticles were superior in improving the longevity of maize seeds under ambient storage conditions. However, the combinations of nanoparticles resulted in negative effects. Among the various forms of active ingredients used for storage studies nano iron (250 ppm) could be recommended as it is cost effective.

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