



Seed polymer coating with Zn and Fe nanoparticles: An innovative seed quality enhancement technique in pigeonpea

Pradeep Korishettar^{1*}, S. N. Vasudevan¹, N. M. Shakuntala¹, S. R. Doddagoudar¹, Sharanagouda Hiregoudar² and B. Kisan³

¹Department of Seed Science and Technology, University of Agricultural Sciences, Raichur- 584104 (Karnataka), INDIA

²Department of Processing and Food Engineering, University of Agricultural Sciences, Raichur- 584104 (Karnataka), INDIA

³Department of Biotechnology, University of Agricultural Sciences, Raichur- 584104 (Karnataka), INDIA

*Corresponding author. E-mail: kpradeep5061@gmail.com

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Abstract: A laboratory study was undertaken to know the effect of seed polymer coating with Zn and Fe nanoparticles (NPs) at different concentration (10, 25, 50, 100, 250, 500, 750 and 1000 ppm) in pigeonpea at Department of Seed Science and Technology, UAS, Raichur. Among the treatments seed polymer coating with Zn NPs at 750 ppm recorded significantly higher seed germination (96.00 %), seedling length (26.63 cm), seedling dry weight (85.00 mg), speed of germination (32.95), field emergence (89.67 %), seedling vigour index (2556), dehydrogenase activity (0.975 OD value) and α -amylase activity (25.67 mm) and lowest abnormal seedlings (2.50 %) over their bulk forms and control followed by Fe and Zn NPs at 500 ppm. However, in contrast to beneficial effects, these NPs also shown inhibitory effect on germination and seedling growth at higher concentration (nano Zn >750 ppm and nano Fe > 500 ppm). Hence, from the results it is concluded that Zn NPs at 750 ppm can be used to enhance quality of the pigeonpea seeds.

Keywords: Fe, Nanoparticles, Pigeonpea, Seed polymer coating, Zn

INTRODUCTION

High quality seed is the basic and critical input that acts as key factor for successful agriculture. Modern agriculture with its bias for technology and precision, demands that each and every seed should readily germinate and produce a vigorous seedling ensuring higher yield. Many scientists all over the world have developed many new production techniques called "seed enhancement techniques" viz., seed polymer coating, seed colouring, seed pelleting, seed fortification, seed infusion, etc., among these seed polymer coating is the promising one. Seed polymer coating is the sophisticated process of applying precise amount of active ingredients along with a liquid polymer directly on to the seed surface without obscuring its shape. It is one of the most economic approaches for improving the performance of seed, which enables accurate and uniform sticking / coating of chemicals and reduces chemical wastage. It also paves way for including all the required ingredients like inoculants, protectants, nutrients, hydrophilic substances, herbicides, oxygen suppliers etc. (Sherin Susan *et al.*, 2005) Nanotechnology, the science of working with smallest possible particles, raises hopes for the future to overcome the difficulties encountered in agriculture. In

Seed Science Research, nanotechnology offers the tools like various nanoparticles for improvement of seed germination and related physiological parameters, nanomembranes and nanopolymer coating to enhance the storability of the seeds by incorporation of pesticides, nanosensors for better management of seed infestation during storage (Chinnamuthu and Murugesu Boopathi, 2009).

Nanoparticles (NPs) by virtue of their nano size (10^{-9} m) possess larger surface area resulting in increased catalytic activity and are highly reactive (Grassian, 2008). Khodakovskaya *et al.* (2009) reported that carbon nano tubes (CNTs) can penetrate thick seed coat and support water uptake by the seeds which could be responsible for the significantly faster germination and higher biomass production in tomato. It is also observed that nanoparticles of ZnO, Ag and TiO₂ treated to groundnut seeds at different concentrations viz., 500, 750, 1000 and 1250 mg kg⁻¹ outperformed the control significantly in terms of germination, shoot length, root length and vigour index (Krishna Shyla and Natarajan, 2014).

Zinc (Zn) and iron (Fe) being essential micronutrients, required for the normal plant growth and development and they are important components of various enzymes

that are responsible for driving many metabolic reactions in all crops. However, these micro elements are required in minute quantity for treating seeds. Recently use of these elements in the form of nanoparticles gaining importance especially for enhancing seed quality in few crops. In this context, an effort was made in the present investigation to find out the effect of seed polymerization with Zn and Fe nanoparticles along with its bulk forms on seed quality in pigeonpea.

MATERIALS AND METHODS

The research studies were carried out in the laboratory of Department of Seed Science and Technology, College of Agriculture, University of Agricultural Sciences, Raichur. The seeds of pigeonpea cv. TS-3R from Seed Unit, UAS Raichur, Zn and Fe nanoparticles from Sisco Research Laboratory Pvt. Ltd., Mumbai and liquid disco agro SP red polymer from Incotec Pvt. Ltd., Ahmadabad were used in the present investigation.

The different concentrations of Zn and Fe nanoparticles suspensions like 10, 25, 50, 100, 250, 500, 750, 1000 mg l⁻¹ were prepared for the experiment in distilled water. The nanoparticles were suspended directly in distilled water and dispersed by ultrasonic vibration (100W, 40 kHz) for 30 min. Small magnetic bars were placed in the suspension for stirring before use to avoid aggregation of the particles. The seeds were coated with polymer and nanoparticles suspensions in 1:5 ratio (6 ml: 30 ml / kg of seed) by using seed coating machine, subsequently seeds were air dried over night to safe moisture content. Observations on various seed quality parameters *viz.*, seed germination (%), abnormal seedlings (%) seedling length (cm), seedling dry weight (mg), speed of germination (Maguire, 1962), seedling vigour index (Abdul-Baki and Anderson, 1973), field emergence (%), dehydrogenase activity (OD value) (Kittock and Law, 1968) and α -amylase activity (mm) (Simpson and Naylor, 1962) were recorded as per the methods and procedures described by ISTA. The mean data of the laboratory experiments were statistically analyzed by adopting completely randomized design as outlined by Panse and Sukhatme (1985). 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

Results obtained on various seed quality parameters like seed germination (%), abnormal seedlings (%), seedling length (cm), seedling dry weight (mg), speed of germination, seedling vigour index, field emergence (%), dehydrogenase activity (OD value) and α -amylase activity (mm) are presented as follows.

Seed polymer coating with Zn NPs at 750 ppm recorded significantly higher germination (96.00 %) and was on par with Zn NPs at 500 ppm (95.30 %) and Fe

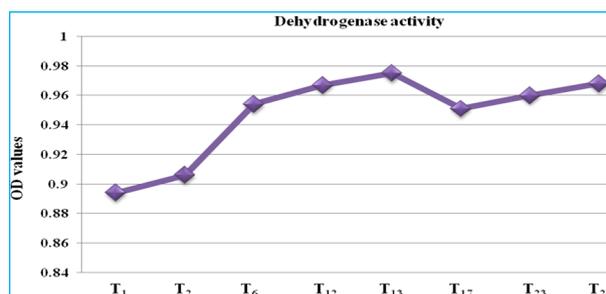


Fig.1. Comparison between the best treatments of seed polymer coating with Zn and Fe NPs on dehydrogenase activity (OD values) in pigeonpea.

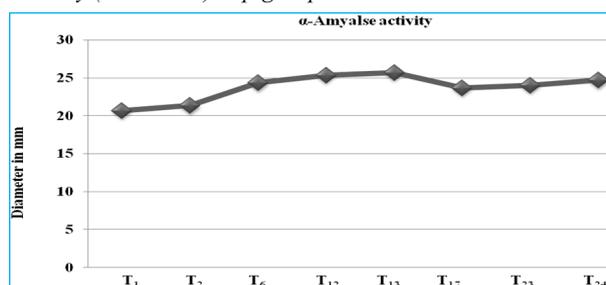


Fig.2. Comparison between the best treatments of seed polymer coating with Zn and Fe NPs on α -amylase activity (mm) in pigeonpea.

T₁-Control; T₂- Only polymer; T₆- Polymer + ZnSO₄ @ 2000 ppm; T₁₂- Polymer + Zn nanoparticles @ 500 ppm; T₁₃- Polymer + Zn nanoparticles @ 750 ppm; T₁₇- Polymer + FeSO₄ @ 1000 ppm; T₂₃- Polymer + Fe nanoparticles @ 250 ppm; T₂₄- Polymer + Fe nanoparticles @ 500 ppm

NPs at 500 ppm (95.00 %) (Table 1). Similarly, these treatments recorded lowest abnormal seedlings (2.50, 3.10 and 3.70 %, respectively). These treatments found to superior over their bulk form and absolute control. The probable reason for the enhanced germination due to nano Zn and Fe over their bulk form and absolute control might be due to the nano size of particles allow them to penetrate through seed coat easily and hence, provided better absorption and utilization of these particles by seeds. The beneficial effect of these NPs in improving the germination can also be ascribed to higher precursor activity of nanoscale Zn and Fe in production of essential biomolecules *vis-a-vis* essential nutrients required for plant growth and are important components of various enzymes which are responsible for driving many metabolic reactions in all crops. Further, NPs would induce oxidation-reduction reactions via the superoxide-ion-radical during germination, resulting the quenching of free radicals in the germinating seeds. Similar findings were reported earlier *viz.*, positive impact of ZnO and ZVI (Zero valent iron) nanoparticles upon wet and dry treatments on black gram seeds for physiological characters namely germination, seedling growth and vigour index and biochemical traits (Senthilkumar, 2011). Prasad *et al.* (2012) observed that ZnO nanoparticles at a concentration of 1000 ppm improved the germination, root growth, shoot growth dry weight and pod yield in

Table 1. Effect of seed polymer coating with Zn and Fe nanoparticles on seed physiological quality parameters in pigeonpea.

Treatments	Germination (%)	Abnormal seedlings (%)	Seedling length (cm)	Seedling dry weight (mg)
T ₁ - Control	90.70	5.70	22.37	80.50
T ₂ - Only polymer	92.30	4.70	23.24	81.15
T ₃ - Polymer + ZnSO ₄ @ 100 ppm	92.43	4.70	24.06	81.45
T ₄ - Polymer + ZnSO ₄ @ 500 ppm	92.70	4.00	24.47	81.30
T ₅ - Polymer + ZnSO ₄ @ 1000 ppm	94.00	3.70	25.10	83.25
T ₆ - Polymer + ZnSO ₄ @ 2000 ppm	94.30	4.03	25.36	83.40
T ₇ - Polymer + Zn nanoparticles @ 10 ppm	91.30	5.70	22.97	80.65
T ₈ - Polymer + Zn nanoparticles @ 25 ppm	91.70	5.00	23.07	81.70
T ₉ - Polymer + Zn nanoparticles @ 50 ppm	91.70	4.70	23.17	81.85
T ₁₀ - Polymer + Zn nanoparticles @ 100 ppm	92.70	4.00	24.97	83.50
T ₁₁ - Polymer + Zn nanoparticles @ 250 ppm	93.70	3.30	24.80	83.70
T ₁₂ - Polymer + Zn nanoparticles @ 500 ppm	95.30	3.10	25.70	84.35
T ₁₃ - Polymer + Zn nanoparticles @ 750 ppm	96.00	2.50	26.63	85.00
T ₁₄ - Polymer + Zn nanoparticles @ 1000 ppm	91.30	6.70	20.24	80.00
T ₁₅ - Polymer + FeSO ₄ @ 100 ppm	91.70	5.00	23.67	81.65
T ₁₆ - Polymer + FeSO ₄ @ 500 ppm	93.70	3.00	24.07	83.35
T ₁₇ - Polymer + FeSO ₄ @ 1000 ppm	94.00	3.30	24.97	83.45
T ₁₈ - Polymer + FeSO ₄ @ 2000 ppm	91.70	6.00	20.74	80.10
T ₁₉ - Polymer + Fe nanoparticles @ 10 ppm	91.00	4.70	23.70	81.50
T ₂₀ - Polymer + Fe nanoparticles @ 25 ppm	92.30	5.00	24.10	81.70
T ₂₁ - Polymer + Fe nanoparticles @ 50 ppm	93.30	4.30	24.60	82.15
T ₂₂ - Polymer + Fe nanoparticles @ 100 ppm	93.70	3.97	24.97	83.35
T ₂₃ - Polymer + Fe nanoparticles @ 250 ppm	94.70	3.70	25.74	84.15
T ₂₄ - Polymer + Fe nanoparticles @ 500 ppm	95.00	3.37	26.24	84.65
T ₂₅ - Polymer + Fe nanoparticles @ 750 ppm	91.30	6.37	19.60	79.15
T ₂₆ - Polymer + Fe nanoparticles @ 1000 ppm	89.70	7.00	18.57	79.00
Mean	92.78	4.52	24.11	82.15
S. Em±	0.44	0.54	0.31	0.16
C D @ 1 %	1.27	1.54	0.93	0.46

*Polymer @ 6 ml kg⁻¹ of seed

groundnut, significantly as compared to chelated ZnSO₄. Shailesh *et al.* (2013) observed the positive effect of nano ZnO, nano FeO and nano ZnCuFe oxide particles on growth of mung bean seedlings over the control. Krishna Shyla and Natarajan (2014) reported that nanoparticles of ZnO, Ag and TiO₂ treated to groundnut seeds at different concentrations *viz.*, 500, 750, 1000 and 1250 mg kg⁻¹ outperformed the control significantly in terms of germination, shoot length, root length and vigour index.

In the present investigation it was noticed that, NPs at higher concentration (Zn @ 1000 ppm; Fe @ 750 & 1000 ppm) decreased seed germination percentage, Fe NPs at 1000 ppm recorded lowest germination (89.70 %) among the treatments. The probable reason for decreased germination at higher concentration could be the increased absorption and accumulation of these NPs both in extracellular space and within the cells resulted in reduction in cell division, cell elongation and inhibition of the hydrolytic enzymes involved in food mobilization during the process of seed germination. Similar results were noticed *viz.*, Lee *et al.* (2010) observed inhibition of seed germination of *Arabidopsis* seeds treated with Al₂O₃ and ZnO nanoparticles above 4000 mg l⁻¹. Prasad *et al.* (2012) observed that ZnO nanoparticles at higher concentration (2000 ppm) had

inhibitory effect on growth and development in groundnut.

The overall experimental results depicted that Zn and Fe NPs promoted the seedling growth characteristics in terms of seedling length (cm) and seedling dry weight (mg). Zn NPs at 750 ppm has recorded significantly highest seedling length (26.63 cm) and seedling dry weight (85.00 mg) in comparison to their bulk form and was on par with Fe NPs 500 ppm (26.24 cm & 84.65 mg) (Table 1). This could be ascribed to the increased synthesis and activity of hydrolytic enzymes during the early phases of germination and effective mobilization of the available food reserves in the seeds resulted in the early emergence and growth of the seedlings. In proportional to increase in seedling growth, dry matter production was also increased. These results are in agreement with findings of Avinash *et al.* (2010) in *Cicer arietinum* *i.e.* ZnO NPs increased the level of IAA in the roots (sprouts) and thereby resulted in increase in the growth rate of the seedlings.

The Fe NPs at 1000 ppm recorded lowest seedling length (18.57 cm) and seedling dry weight (79.00 mg) compared to control and which was statistically on par with nano Fe at 750 ppm and nano Zn at 1000 ppm (Table 1). This reveals that in contrast to beneficial effect, these NPs also have inhibitory effect on seed-

Table 2. Effect of seed polymer coating with Zn and Fe nanoparticles on seed vigour parameters in pigeonpea.

Treatments	Speed of germination	Seedling vigour index	Field emergence (%)
T ₁ -Control	29.71	2029	85.00
T ₂ - Only polymer	31.34	2221	87.33
T ₃ - Polymer + ZnSO ₄ @ 100 ppm	31.72	2197	87.33
T ₄ - Polymer + ZnSO ₄ @ 500 ppm	31.90	2268	87.67
T ₅ - Polymer + ZnSO ₄ @ 1000 ppm	32.13	2359	88.00
T ₆ - Polymer + ZnSO ₄ @ 2000 ppm	32.27	2402	88.00
T ₇ - Polymer + Zn nanoparticles @ 10 ppm	31.43	2097	85.67
T ₈ - Polymer + Zn nanoparticles @ 25 ppm	31.90	2116	86.33
T ₉ - Polymer + Zn nanoparticles @ 50 ppm	31.22	2125	86.67
T ₁₀ - Polymer + Zn nanoparticles @ 100 ppm	32.17	2315	87.67
T ₁₁ - Polymer + Zn nanoparticles @ 250 ppm	32.50	2383	87.67
T ₁₂ - Polymer + Zn nanoparticles @ 500 ppm	32.70	2440	88.67
T ₁₃ - Polymer + Zn nanoparticles @ 750 ppm	32.95	2556	89.67
T ₁₄ - Polymer + Zn nanoparticles @ 1000 ppm	31.83	1848	86.33
T ₁₅ - Polymer + FeSO ₄ @ 100 ppm	30.08	2067	86.67
T ₁₆ - Polymer + FeSO ₄ @ 500 ppm	32.15	2218	87.67
T ₁₇ - Polymer + FeSO ₄ @ 1000 ppm	32.25	2347	88.00
T ₁₈ - Polymer + FeSO ₄ @ 2000 ppm	30.92	1902	86.67
T ₁₉ - Polymer + Fe nanoparticles @ 10 ppm	31.27	2157	86.00
T ₂₀ - Polymer + Fe nanoparticles @ 25 ppm	31.77	2224	86.33
T ₂₁ - Polymer + Fe nanoparticles @ 50 ppm	32.58	2295	87.67
T ₂₂ - Polymer + Fe nanoparticles @ 100 ppm	32.63	2340	88.33
T ₂₃ - Polymer + Fe nanoparticles @ 250 ppm	32.87	2438	89.00
T ₂₄ - Polymer + Fe nanoparticles @ 500 ppm	32.92	2493	89.33
T ₂₅ - Polymer + Fe nanoparticles @ 750 ppm	31.52	1789	85.67
T ₂₆ - Polymer + Fe nanoparticles @ 1000 ppm	31.97	1666	84.33
Mean	31.87	2203	87.21
S Em±	0.22	26	0.53
C D @ 1 %	0.63	75	1.52

*Polymer @ 6 ml kg⁻¹ of seed.

ling growth at higher concentration (nano Zn > 750 ppm and nano Fe > 500 ppm). The probable reason could be due to the excess absorption at higher concentration resulted from penetration of NPs in to cell wall and plasma membrane of epidermal layers in shoot and root and accumulation in vascular tissues thereby affected cell division and cell elongation. Hence, reduced the overall seedling growth of pigeonpea. These results were supported by the earlier findings *viz.*, ZnO nanoparticles at higher concentration (2000 ppm) had inhibitory effect on growth and development in groundnut (Prasad *et al.*, 2012). Abdel-Azeem and Elsayed (2013) reported inhibitory effect of silver nanoparticles (above 50 ppm) on root length, mitotic indices and chromosomal morphology in *Vicia faba*. Mahmoodzadeh *et al.* (2013) reported detrimental effect of TiO₂ nanoparticles on shoot and root biomass in wheat. Vinoth Kumar and Udaysoorian (2014) reported potential toxic effect of three different metal oxide nanoparticles *viz.*, Zinc oxide (nano ZnO), Aluminium oxide (nano Al₂O₃) and Titanium dioxide (nano TiO₂) in maize at 500, 1000 and 2000 ppm on seed germination, vigour index, shoot and root length. Among the tested concentration 2000 ppm was found highly toxic followed by 1000 ppm and among the nanoparticles ZnO was found to be more toxic followed by TiO₂ and Al₂O₃.

In the present investigation Zn and Fe NPs showed significant difference in speed of germination over their bulk form and control. Among different concentration, nano Zn at 750 ppm and nano Fe at 500 ppm recorded higher speed of germination (32.95 and 32.92 respectively), which was on par with the Fe NPs at 500 ppm (32.92), Fe NPs at 250 ppm (32.87), Fe NPs at 100 ppm (32.63), Zn NPs at 500 ppm (32.50) and Zn NPs at 250 ppm (32.50). The lowest was recorded in absolute control (29.71) (Table 2). The reason for rapid germination could be that the NPs may form new pores on seed coat during penetration facilitating the influx of water inside the seed or NPs may enter into the seed through the cracks present over the surface of the seed and activated the enzymes in early phase thereby enhanced the speed of germination (Sridhar, 2012).

The field emergence (%) also found to significantly increase due to the Zn and Fe NPs over the bulk form and control. Among the different concentrations, nano Zn at 750 ppm recorded highest field emergence (89.67 %), which was on par with nano Fe at 500 ppm (89.33 %), nano Zn at 500 ppm (89.00 %), nano Fe at 250 ppm (88.67 %) and lowest with 1000 ppm of Fe NPs (Table 2). It could be due to the overall beneficial effects of Zn and Fe NPs *viz.*, higher precursor activity of nanoscale Zn and Fe in production of essential biomolecules, increased cofactor activity in essential en-

zymatic systems and positive effect on reactivity of the phytohormones during the germination (Krishna Shyla and Natarajan, 2014). Higher concentration of NPs resulted in toxicity and hence, reduced the field emergence percentage (84.33).

The seedling vigour index also significantly increased due to Zn and Fe NPs. Among the treatments, nano Zn at 750 ppm recorded highest seedling vigour index (2556), which was on par with nano Fe at 500 ppm (2493), nano Zn at 500 ppm (2440), nano Fe at 250 ppm (2438). The increased vigour is mainly due to overall improvement in germination and seedling growth characteristics.

The enzymes dehydrogenase and α -amylase activities were significantly influenced by the NPs treatments. The highest OD value (0.975) for dehydrogenase activity was recorded by 750 ppm of Zn NPs followed by nano Fe at 500 ppm (0.968 OD value) (Fig. 1), nano Zn at 500 ppm (0.967 OD value) and nano Fe at 250 ppm (0.960 OD value). Zn and Fe are the important metal micronutrients that act as cofactors for the most of the dehydrogenase enzyme complexes involved in respiration and food mobilization in seeds. The increased availability of these micronutrients at nano-scale with increased chemical reactivity resulted in the increase in synthesis and activity of the dehydrogenase enzymes (Vijayalaxmi et al., 2013).

From the results it is evidenced that among the Zn and Fe, the Zn is the most critical micronutrient which influence the amylase activity. The α -amylase activity was significantly increased by Zn NPs at 750 and 500 ppm (25.67 and 25.33 mm, respectively) over other treatments (Fig. 2). This could be due to the increased availability of Zn at nanoscale with increased chemical reactivity resulted in the increase in synthesis and activity of the α -amylase enzyme.

It can be concluded that the Zn and Fe nanoparticles, which were applied along with the seed polymer are capable of entering into seeds utilizing the cracks and crevices available on the seed coat during the imbibitions into the seeds and would improve the enzymatic activity and free radical scavenging system by quenching the free radicals there by lowering the oxidative damages, eventually promoting viability and vigour of the seeds. Among the different concentrations 500 ppm of Zn NPs or Fe NPs can be used to enhance the seed quality in pigeonpea along with polymer coating. Since, present investigation represents the preliminary work and it is first work of its kind in pigeonpea, further intensive, in depth research is warranted to understand the mechanism of entry of nanoparticles and their mode of action in invigorating the seeds.

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