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B Bala Raju
Department of Genetics and Plant breeding, Sam Higginbottom University of Agriculture Technology and Sciences, Allahabad, Uttar Pradesh, India

Prashant Kumar Rai
Department of Biotechnology, Sam Higginbottom University of Agriculture Technology and Sciences, Allahabad, Uttar Pradesh, India

Correspondence
Prashant Kumar Rai
Department of Biotechnology, Sam Higginbottom University of Agriculture Technology and Sciences, Allahabad, Uttar Pradesh, India

Studies on effect of polymer seed coating, nanoparticles and hydro priming on seedling characters of Pigeonpea (*Cajanus cajan* L.) seed

B Bala Raju and Prashant Kumar Rai

Abstract
A laboratory study was undertaken to know the effect of seed polymer coating, nanoparticles (NPs) Zn, Fe at different concentration (10 and 25 ppm), $Znso_4$, $Feso_4$ (100 and 500 ppm) and hydropriming with different durations (6 and 12 hrs.) on seedling characters of pigeonpea seed. At the Department of Genetics and Plant Breeding, Naini Agricultural Institute, Sam Higginbottom University of Agriculture, Technology and Sciences. Among the treatments seed polymer coating with Fe NPs at 25 ppm recorded significantly higher seed germination (94.75 %), speed of germination (94.75 %), seedling root length (12.58), seedling shoot length (19.58), seedling length (32.15 cm), seedling dry weight (0.43g), seedling fresh weight (0.74), seedling vigour index I (2953.70), seedling vigour index II (407.00) and lowest abnormal seedlings (2.50 %) over their bulk forms and control followed by Fe and Zn NPs at 25 ppm. The hydropriming have positive influence on seed quality parameters of pegionpea individually but the effect of priming method was found significant. Hence, from the results it is concluded that Fe and Zn NPs at 25 ppm can be used to enhance quality of the pigeonpea seed.

Keywords: Fe, Nanoparticles, Pigeonpea seed, Seed polymer coating, Zn, Hydropriming, $Znso_4$, $Feso_4$

Introduction

High quality seed is the basic and critical input that acts as key factor for successful agriculture. The pigeon pea (*Cajanus cajan* L.) is a perennial legume from the family Fabaceae. Since its domestication in India at least 3,500 years ago, its seeds have become a common food grain in Asia, Africa, and Latin America. It is consumed on a large scale mainly in south Asia and is a major source of protein for the population of that subcontinent. The cultivation of the pigeon pea goes back at least 3,500 years. The center of origin is probably peninsular India, where the closest wild relatives (*Cajanus cajanifolia*) occur in tropical deciduous woodlands. Pulses constitute an important ingredient in predominantly vegetarian diet and are important source of protein that nutritionally balances the protein requirement of vegetarian population. They supply minerals and vitamins and provide an abundance of food energy. Pulses provide a cheaper source of nutrients/ proteins as they generally contain nearly twice as much as protein as that of cereals and hence correctly called poor man’s meat. Pulses are also important for sustainable agriculture enriching the soil through biological nitrogen fixation, fixes about 40-50 kg of N/ha (Hariprasanna and Bhatt, 2002) [10]. Pigeonpea is an important and old crop of this country. It is the second most important pulse crop only after chickpea. India is producing 14.76 million tons of pulses from an area of 23.63 million hectare, which is one of the largest pulses producing countries in the world. However, about 2-3 million tons of pulses are imported annually to meet the domestic consumption requirement. Thus, there is need to increase production and productivity of pulses in the country by more intensive interventions.

Pigeonpea seed is composed of cotyledons (85%), embryo (1%), and seed coat (4%) (Faeis and Singh, 1990) pigeon pea contains 19-23% proteins, 1-2% fat, 45-55% carbohydrates, 1-5% fibers, soluble sugars 3-5% water 1.5% and energy 16-18%. Pulses have a special role in meeting protein requirement of predominantly vegetarian population of India. Pulses contain 20 to 25% protein on dry seed weight basis, which is nearly 2-3 times higher than that in cereals (Prasad, 2004).

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 coloring, 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. It also paves way for including all the required ingredients like inoculants, protectants, nutrients, hydrophilic substances, herbicides, oxygen suppliers *etc*. Nanotechnology, the science of working with smallest possible particles, raises hopes for the future to overcome the difficulties encountered in agriculture. Nanoparticles (NPs) by virtue of their nano size (10^{-9}m) possess larger surface area resulting in increased catalytic activity and are highly reactive.

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 on seedling characters of pegionpea seed.

Materials and Methods

The research studies were carried out in the Seed science post Graduate Laboratory, Department of Genetics and Plant Breeding, Sam Higginbottom University of Agriculture Technology and Sciences, Allahabad, 211007, Uttar Pradesh, India. The seed of pigeonpea cultivated variety is Abhaya ICPL 332.

The different concentrations of Zn and Fe nanoparticles suspensions like 10 and 25 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 are suspended in prepared solution for about 6 hrs. Subsequently seeds were air dried over night to safe moisture content. Observations on various seed quality parameters *viz*., seed germination (%), speed of germination seedling root length (cm), seedling shoot length (cm), seedling length (cm), seedling fresh weight (g), seedling dry weight (g), seedling vigour index I (Abdul-Baki and Anderson, 1973) [2], seedling vigour index II, 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) [3]. 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 (%), speed of germination, seedling root length (cm), seedling shoot length (cm), seedling length (cm), seedling fresh weight (g), seedling dry weight (g), seedling vigour index I, seedling vigour index II are presented as follows.

1. Germination %

Seed polymer coating with Fe NPs at 25 ppm recorded significantly higher germination (94.75 %) and was on par with Fe NPs at 10 ppm (90.25 %) and Zn NPs at 25 ppm (91.25 %). Similarly, these treatments recorded lowest abnormal seedlings (3.50, 4.10 and 3.70 %, respectively). When regarding the data the germination percentage found best in Polymer + Fe nanoparticles @ 25 ppm among all the treatments.

The probable reason for the enhanced germination due to the Nano size of particles allow them to penetrate t hrough seed coat easily and hence, provided better absorption and utilization of these particles by seeds. The beneficial effect of the these NPs in improving the germination and production of essential biomolecules as well as essential nutrients required for plant growth and are important components of various enzymes which are responsible for driving many metabolic reaction (Senthilkumar, 2011) [5].

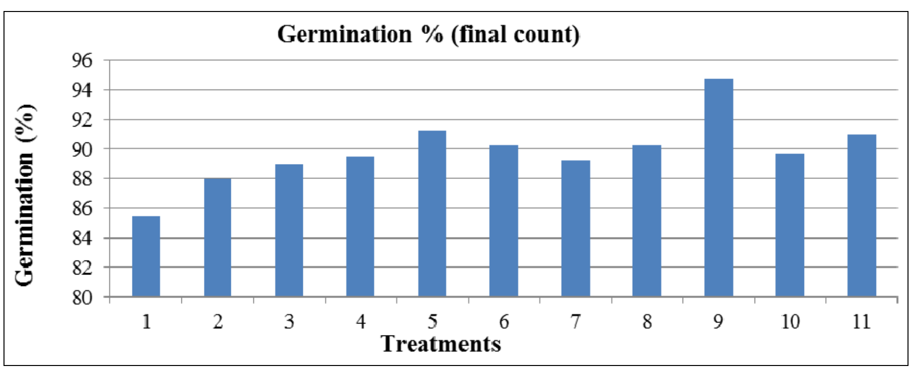

Fig 1: Germination percentage as influenced by different priming methods on Pegionpea seeds

2. Speed of germination

In case of polymer seed coating speed of germination was significantly higher in Fe nanoparticle at 25 ppm (94.75) followed by Zn nanoparticles at 25 ppm (91.25) when compared with control (85.50). Regarding the data the speed of germination found best in Fe nanoparticles Polymer + Fe nanoparticles @ 25 ppm among all the treatments.

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.

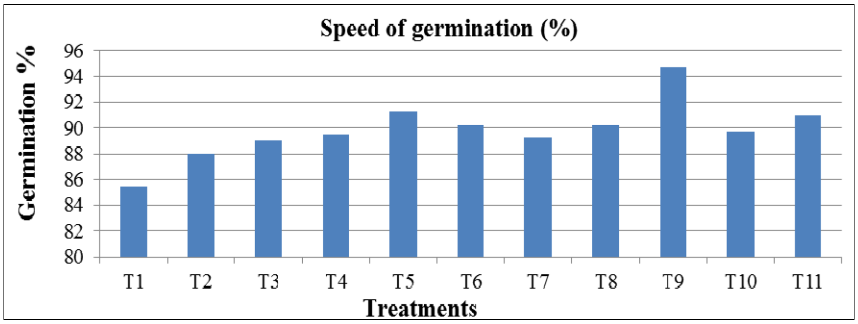

Fig 2: Speed of germination as influenced by different priming methods on pegionpea

3. Seedling root length (cm)

In case of seedling root length was significantly higher in T_9 treatment (12.58 cm) (Polymer + Fe nanoparticles @ 25 ppm) followed by T_5 treatment (11.30 cm) (Polymer + Zn nanoparticles @ 25 ppm) compared with control (8.90 cm), among these treatments Fe NPs (Polymer + Fe nanoparticles @ 25 ppm) found to be the best.

Reported that nanoparticles of ZnO, Ag and TiO2 treated to groundnut seeds outperformed the control significantly in terms of germination, shoot length, root length and vigour index.

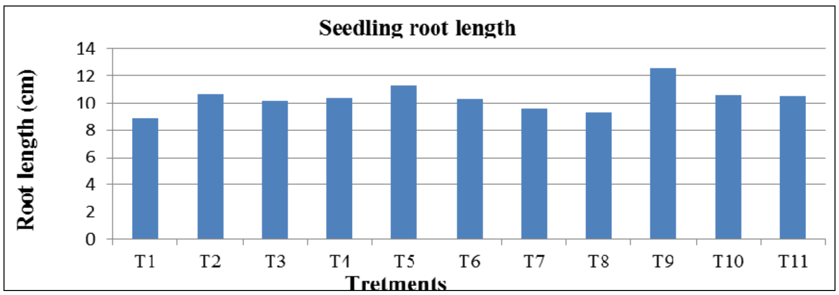

Fig 3: Seedling root length as influenced by different priming methods on pegionpea

4. Seedling shoot length (cm)

In case of seedling shoot length was significantly higher in Fe NPs @ 25 ppm (19.58 cm) (Polymer + Fe nanoparticles @ 25 ppm) followed by Zn NPs @ 25 ppm (18.48 cm) (Polymer + Zn nanoparticles @ 25 ppm) when compared with control (15.45 cm). Regarding the data the seedling shoot length treated with Fe NPs found (Polymer + Fe nanoparticles @ 25 ppm) best in among all the treatments.

Prasad *et al.* (2012) [4] observed that ZnO nanoparticles improved the germination, root growth, shoot growth dry weight groundnut, significantly as compared to chelated ZnSO4. observed the positive effect of nano ZnO, nano FeO and nano ZnCuFe oxide particles on growth of mung bean seedlings over the control. 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.

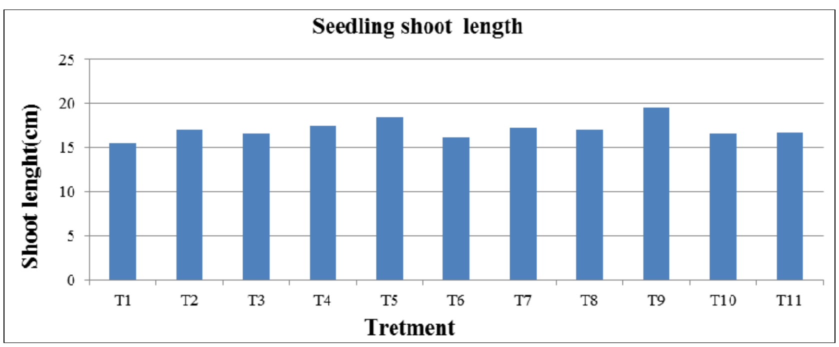

Fig 4: Seedling shoot length as influenced by different priming methods on pegionpea

5. Seedling length (cm)

In case of seedling length was significantly higher in treatment Fe NPs @ 25 ppm (32.15 cm) (Polymer + Fe nanoparticles @ 25 ppm), followed by Zn NPs @ 25 ppm (29.78 cm) (Polymer + Zn nanoparticles @ 25 ppm). When compared with control (24.35 cm). Regarding the data the seedling length found best in Fe NPs @ 25 ppm among all the treatments.

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 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.

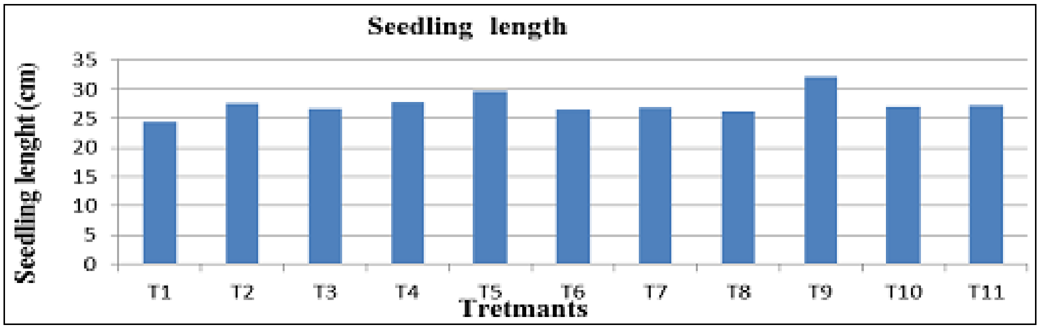

Fig 5: Seedling length as influenced by different priming methods on pegionpea seed Seedling

6. Seedling fresh weight (g)

In case of priming seedling fresh weight was significantly higher in Polymer + Fe nanoparticles @ 25 ppm (0.74 g) followed by Polymer + Zn nanoparticles @ 25 ppm (0.66 g) when compared with control (0.58 g). When the data regarding the seedling fresh weight found Polymer + Fe nanoparticles @ 25 ppm best in among all the treatments.

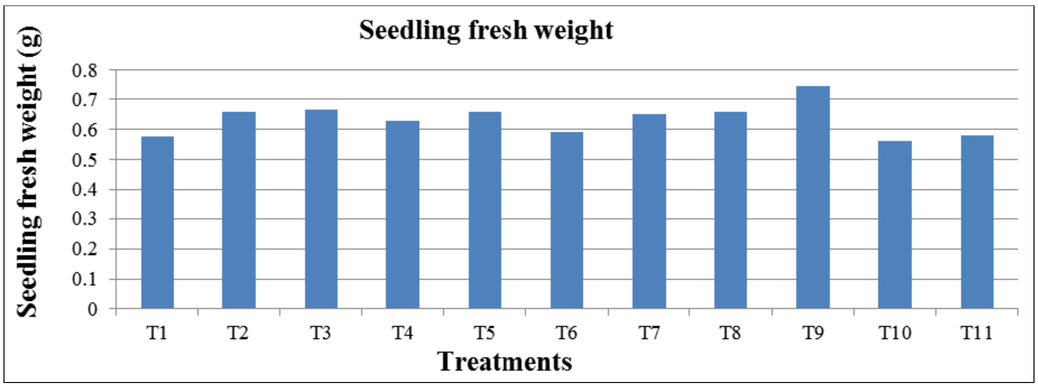

Fig 6: Seedling fresh weight as influenced by different priming methods on pegionpea seed

7. Seedling dry weight (g)

In case of seedling dry weight was significantly higher in Polymer + Fe nanoparticles @ 25 ppm (0.43 g) followed by Polymer + Zn nanoparticles @ 25 ppm (0.38 g) when compared with control (0.34 g). Regarding the data the seedling dry weight found best in Polymer + Fe nanoparticles @ 25 ppm among all the treatments.

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.

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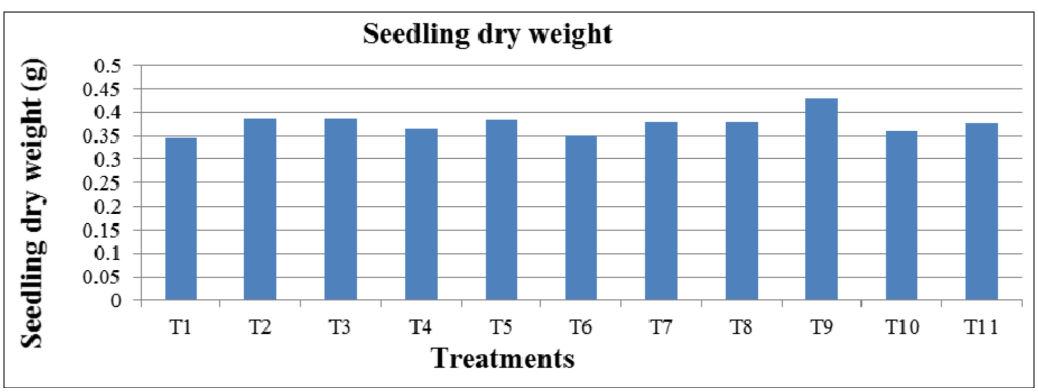

Fig 7: Seedling dry weight as influenced by different priming methods on pegionpea

8. Seed vigour index I

In case of priming seed vigour index I was significantly higher in Polymer + Fe nanoparticles @ 25 ppm (2953.70) seeds followed by Polymer + Zn nanoparticles @ 25 ppm (2714.40), when compared with control (2299.10). Regarding the data the seed vigour index length found best in Polymer + Fe nanoparticles @ 25 ppm among all the treatments

The increased vigour index I is mainly due to overall improvement in germination and seedling growth characteristics.

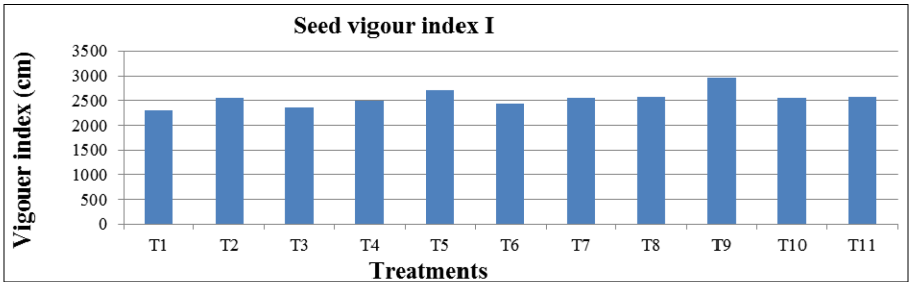

Fig 8: Seed vigour index I as influenced by different priming methods on Pegionpea seed

9. Seed vigour index II

In case of priming seed vigour index II was significantly higher in Polymer + Fe nanoparticles @ 25 ppm (407.00) seeds of pegionpea followed by Polymer + Zn nanoparticles @ 25 ppm (350.60) when compared with control (294.40). When the data regarding the seed vigour index mass found Polymer + Fe nanoparticles @ 25 ppm best in among all the treatments.

The increased vigour index II is mainly due 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 and dry matter production increased. And overall improvement in germination and seedling growth characteristics improved the seed vigour.

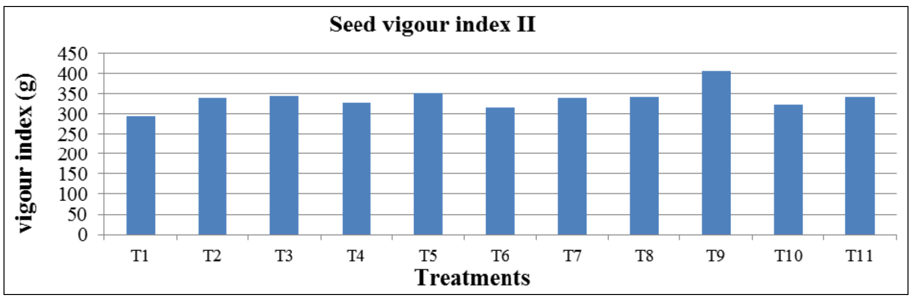

Fig 9: Seed vigour index II as influenced by different priming methods on pegionpea

Table 1: Mean performance of pegionpea seeds for 9 seedling characters

S. No	Treatments	Germination (%)	Speed of germination (%)	Root length (cm)	Shoot length (cm)	Seedling length (cm)	Fresh weight of seedling (g)	Dry weight of seedling(g)	Seed vigour I	Seed vigour II
1	T1	85.50	85.50	8.90	15.45	24.35	0.58	0.34	2299.10	294.40
2	T2	88.00	88.00	10.65	16.98	27.63	0.66	0.39	2560.00	339.30
3	T3	89.00	89.00	10.15	16.53	26.68	0.66	0.39	2367.80	344.20
4	T4	89.50	89.50	10.35	17.35	27.70	0.63	0.37	2493.40	328.00
5	T5	91.25	91.25	11.30	18.48	29.78	0.66	0.38	2714.40	350.60
6	T6	90.25	90.25	10.33	16.20	26.53	0.59	0.35	2440.60	316.50
7	T7	89.25	89.25	9.60	17.23	26.83	0.65	0.38	2550.10	340.20
8	T8	90.25	90.25	9.28	16.95	26.23	0.66	0.38	2570.80	342.40
9	T9	94.75	94.75	12.58	19.58	32.15	0.74	0.43	2953.70	407.00
10	T10	89.75	89.75	10.58	16.58	27.15	0.56	0.36	2546.60	323.10
11	T11	91.00	91.00	10.50	16.73	27.23	0.58	0.38	2581.80	343.30
	Grand Mean	89.86	85.50	10.38	17.09	27.48	0.63	0.38	2552.60	339.00
	F Test	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**S**
	C.D. (5%)	0.654	0.654	0.413	0.518	0.628	0.031	0.014	52.833	12.91
	SE(m)	1.882	1.882	1.187	1.49	1.807	0.09	0.04	152.01	37.16

Table 2: Analysis of variance for 9 seedling characters in Pegionpea seeds

S. No	Characters	Mean sum of squares		
		Replication (d. f. = 03)	Treatments (df=6)	Error (df=21)
1	Germination percentage	6.33	20.46**	1.71
2	Speed of germination	6.33	20.46**	1.71
3	Root length	0.81	3.94**	0.68
4	Shoot length	7.59	4.96**	1.07
5	Seedling length	6.03	16.27**	1.57
6	Seedling fresh weight	0.00	0.01**	0.00
7	Seedling dry weight	0.03	0.20**	0.07
8	Seed vigour I	21964.84	121129.85**	11165.46
9	Seed vigour II	182.85	3073.48**	667.12

Conclusion

It can be concluded that the seeds are treated with different NPs with different concentrations are effective on pegionpea seed, but the treatment T_9 shows more effective polymer + Fe NPs @ 25 ppm on germination percentage (94.75 %) and all other physiological parameters. And Zn NPs also have effective result on germination (91.25%), and hydropriming showed better result. Finally it's concluded that among the different concentrations 25 ppm of Zn NPs or Fe NPs can be used to enhance the seed quality in pigeonpea along with polymer coating.

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