

## Zn-seed Treatments to Enhance Seedling Vigour of Mungbean (*Vigna radiata* L.)

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**ABSTRACT:** Low productivity of mungbean (*Vigna radiata* (L.) Wilczek) is mainly attributed to poor crop establishment and its cultivation in Zn deficient soils of arid and semiarid regions. Zn-seed treatments have the potential to address the issues associated with poor crop establishment as well as soil Zn-deficiency. In the present study Zn-seed priming and Zn-seed coating treatments were standardized to enhance the early seedling vigour characters, which facilitate in better crop establishment. Among the Zn concentrations (300 ppm, 450 ppm, 600 ppm and 750 ppm) studied, priming seeds with 450 ppm Zn solution at 25 ± 2°C for 9 hours was found to be the best in enhancing the germination percentage (98.00), seedling length (37.76 cm), seedling dry weight (0.2786 g), seed vigour index I (3701.95) and II (26.28) when compared to the control and hydro-primed seeds. Among the fertilizers used (Zn-NCPC fertilizer, Amino acid chelated Zn fertilizer and EDTA chelated Zn fertilizer), seeds coated with mixture of Zn-NCPC fertilizer and chalk powder in 1:4 proportion was found to be the best seed coating treatment. Seed coating with Zn-NCPC (1:4) has manifested in significantly higher germination percentage (98.33), seedling length (38.18 cm), seedling dry weight (0.2864 g), seed vigour index I (3756.64) and II (28.17) when compared to the control. Coating seeds with either Amino acid chelated Zn fertilizer or EDTA chelated Zn fertilizer did not have any beneficial effects in comparison to the control.

**Keywords:** Mungbean, seedling vigour, germination, Zinc-priming, Zinc-seed coating, Zn-Nano Clay Polymer Composite (Zn-NCPC), Amino acid chelated Zn, EDTA chelated Zn.

### INTRODUCTION

Mungbean or green gram is an important pulse crop in cereal-based cropping systems of South Asia, East Asia and South-East Asia. In India, it is an important subsistence crop adding essential nutrients to the diets especially protein, iron, zinc, phosphorus and potassium. Though, India is the largest producer, accounting for 30 percentage of the global mungbean production (Nair and Schreinemachers 2020), it still imports mungbean from other countries (Myanmar, Kenya, Mozambique, Australia and Tanzania) to meet the huge consumption demand. Indian mungbean productivity (538.65 kg ha<sup>-1</sup> in 2020-21) is 1.34 times below the global average productivity (721 kg ha<sup>-1</sup>) (Nair and Schreinemachers 2020), which is mainly attributed to poor crop establishment (Naseem *et al.*, 1997; Bjelica, 2016; Rahmianna *et al.*, 2000; Ashraf

and Foolad 2005). Like any other pulse crop, mungbean is cultivated in marginal lands of arid and semiarid areas, where the crop suffers from water deficit due to erratic rainfall and soil nutrients deficiencies specially of zinc. Under such situations, inclusion of seeds with high initial seedling vigour in crop cultivation leads to better crop establishment (Kumar *et al.*, 2002).

Seed being a living entity, its quality is bound to deteriorate from harvesting to till its sowing in field, if not handled properly (Jacob *et al.*, 2016). Micronutrient seed treatments were found to be effective in enhancing early seedling vigour, crop establishment, crop growth and yield, besides overcoming the particular soil nutrient deficiency in crop (Farooq *et al.*, 2012). Zinc seed treatment has been found to enhance the early seedling vigour; thereby crop establishment and also could overcome soil Zn deficiency resulting in higher yields and grain Zn fortification in many crops

(Rehman *et al.*, 2015, 2018a, 2018b; Rehman and Farooq 2016; Farooq *et al.*, 2018; Ullah *et al.*, 2019a, 2019b, 2019c). In mungbean cultivation areas, as initial crop establishment problem is also associated with soil Zn deficiencies, the present study was undertaken to standardize a suitable Zn-seed treatments that could enhance the early seedling vigour of mungbean seeds.

## MATERIAL AND METHODS

**Plant material.** Seeds of mungbean variety PUSA Vishal were collected from Seed Production Unit, IARI, New Delhi for conducting the present investigation. Variety PUSA Vishal was selected because of its bold seeded nature and resistance to Mungbean Yellow Vein Mosaic Virus (MYMV).

**Zinc Fertilizers.** The required Zn fertilizer for seed priming ( $ZnSO_4 \cdot 7H_2O$ ) was procured from Sigma-Aldrich®, USA. Zn-loaded Nano Clay Polymer Composite (Zn-NCPC), which was used for seed coating experiment was obtained from Division of Soil Science and Agricultural Chemistry, ICAR-IARI, New Delhi. Amino acid chelated Zn fertilizer and EDTA chelated Zn fertilizer, which were used for seed coating experiment were purchased from local Delhi market. Zn content in all the Zn sources used differed. It was 22.74 per cent in  $ZnSO_4 \cdot 7H_2O$ , 10 per cent in Zn-NCPC fertilizer, 12 per cent each in Amino acid chelated Zn fertilizer and EDTA chelated Zn fertilizer.

**Treating seeds with Zn solution.** To identify the optimum Zn concentration for mungbean seed priming, batches of 100 seeds in 3 replicates were placed in the circular shaped boxes of size 16 cm diameter and 5 cm height, which contained two layers of filter papers that were submerged with 20 ml of  $ZnSO_4 \cdot 7H_2O$  solutions of different Zn concentration (300 ppm, 450 ppm, 600 ppm and 700 ppm) as per the treatment. Then each box was incubated in an incubator maintained at  $25 \pm 2^\circ C$  for 9 hours duration (duration of incubation as per the standardized hydropriming treatment of lab. Data not shown). At the end of incubation duration, three boxes representing three replicates of a particular Zn concentration was taken out and seeds were dried back to original seed moisture content (9 per cent) by spreading them on the blotter paper under the fan. For hydropriming, seeds were treated in same manner using only distilled water without any Zn supplementation in water. Seeds without any treatment (hydropriming and Zn-priming) were used as control. Thus, treated seeds along with control were subjected to germination test following the method recommended by ISTA (2018) and observations were recorded at the end of germination test. Germination percentage was calculated based on number of normal seedlings as follows:

$$\text{Germination percentage} = \frac{\text{Total number of normal seedlings}}{\text{Total number of seeds used for germination testing}} \times 100$$

Ten normal seedlings were randomly selected and each seedling's root length was measured on linearscale from the point of seed attachment to tip of the root. Similarly, shoot length of selected ten normal seedlings was measured on linear scale from the point of seed attachment to the tip of the plumule. Seedling length of the individual selected ten normal seedlings was obtained by adding the root length and shoot length of the respective seedling. The average shoot length, root length and seedling length of ten seedlings was used for the statistical analysis. After taking the individual seedling length of ten randomly selected normal seedlings, the fresh weight of all the seedlings together, was taken using the four digit analytical balance. Then, the seedlings were dried collectively overnight in an oven set at  $90^\circ C \pm 2^\circ C$  and the dry weight was measured using four digit analytical balance, which later was used for statistical analysis. Seed vigour index-I and seed vigour index-II were calculated using the following formula (Abdul-Baki and Anderson, 1973):

Vigour index I = Average seedling length of 10 seedlings (cm)  $\times$  germination percentage  
Vigour index II = Seedling dry weight of 10 seedlings (g)  $\times$  germination percentage

**Coating seeds with Zn-fertilizer.** In the present study we have attempted to explore the possibilities of delivering the Zn fertilizers (Zn-NCPC fertilizer, Amino acid chelated Zn fertilizer and EDTA chelated Zn fertilizer) as seed coats. Mungbean seeds were coated with respective Zn fertilizers in seed coating machine using gum Arabic as binder as per the manufacturer's instructions manual of seed coating equipment (model 'SATEC Concept ML 2000' of SATEC Equipment GmbH, Germany). Around 250 g of seeds were first coated with gum Arabic solution and then coated with dry powder of Zn-NCPC fertilizer and chalk powder mixture mixed in different proportions *viz.*, 1:1, 1:2, 1:3, 1:4, 1:5 and 1:6 on volume basis. Similarly, another batch of 250 g seeds each, were coated using either Amino acid chelated Zn-fertilizer or EDTA chelated Zn-fertilizer and chalk powder mixture mixed in different proportions (1:1, 1:2, 1:3, 1:4, 1:5, 1:6 and 1:7 on volume basis) separately. After coating with the respective fertilizers, seeds were dried overnight under the fan and subjected to germination test as per ISTA (2018) and observations on germination percentage, seedling root length, seedling shoot length, seedling length, seedling dry weight, Seed vigour index-I and seed vigour index-II were taken as detailed above. Seeds without any coatings were used as control.

**Statistical analysis.** All the experimental analysis were subjected to single factor ANOVA analysis of completely Randomized Design using SPSS 13 and Critical Difference (CD) values was calculated at  $p=0.05$  to compare the difference between the treatments of the respective experiments.

## RESULTS AND DISCUSSION

During the financial year 2018-19, India imported 0.57 million tonnes of mungbean, which accounted for 22.71 per cent of the total pulses imported (Anonymous, 2019), indicating that the mungbean domestic demand is around 24.44 per cent more than the quantity being produced. Thus, productivity deficit and consumption demand, warrants immediate strategies to increase the mungbean productivity, which is lower than global average productivity. In majority of the mungbean cultivation areas, limited crop establishment and soil Zn deficiency are the major constraints for the lower productivity. To address this, enhancing the mung bean seed quality through Zn-seed treatment is the better alternative, which addresses the problems associated with both crop establishment as well as soil Zn deficiency. Hence, in the present investigation, we standardized the Zn-seed treatments *viz.*, Zn- priming and seed coating with different Zn-fertilizers, to identify the best Zn-seed treatment, which could be used during mungbean cultivation. The obtained results during the process are detailed below:

**Zn-priming.** Priming of mungbean seeds with ZnSO<sub>4</sub> had a significant effect on the seed quality parameters compared to control (Table 1). Seed priming with 450 ppm Zn at 25 ± 2 °C for 9 hours has manifested in significantly higher germination percentage (98.00), seedling length (37.76 cm), seedling dry weight (0.2786 g), seed vigour index I (3701.95) and II (26.28) when compared to the control and hydro- primed seeds. Better seedling vigour parameters in 450ppm Zn-primed seeds in comparison to control and hydro-primed seeds is attributed to the involvement of Zn in cell division, cell proliferation, protein synthesis and retaining membrane structure (Sarwar, 2011). Similar results enhanced seedling vigour parameters were reported in mungbean for Zn-seed priming (Haider *et al.*, 2020) and Phosphorus-seed priming (Al-Salhy and Rasheed, 2020). With the increase in concentration of Zn (600 ppm and 750 ppm), there was a decrease in seedling vigour parameters, which could be attributed to the possible Zn toxicity at 600 and 750 ppm as reported in case of boron seed priming and Zn-priming (Farooq *et al.*, 2011; Rehman *et al.*, 2013; Haider *et al.*, 2020). Priming the mungbean seeds at Zn concentration affects cellular antioxidant enzymes system leading to decreased seedling vigour parameters and failure of seed to germinate at extreme higher Zn concentration (Khan *et al.*, 2021).

As SVI-I and SVI-II values account for the germination as well as seedling growth potential that directly influence the crop stand establishment under field conditions, seed priming with 450 ppm Zn solution for 9 hrs. at 25 ± 2°C is best suited for getting the better crop

establishment in mungbean.

**Seed coating.** Coating of mungbean seeds with Zn-NCPC had a significant effect on the seed quality parameters compared to control (Table 2). Seed coating with Zn-NCPC (1:4) has manifested in significantly higher germination percentage (98.33), seedling length (38.18 cm), seedling dry weight (0.2864 g), seed vigour index I (3756.64) and II (28.17) when compared to the control. Germination percentage of seeds coated with Zn-NCPC (1:1) has decreased in comparison to control as number of abnormal seedlings has increased. Whereas all other seedling vigour parameters (seedling length, seedling dry weight, vigour index I and II) were found on par with the control. Similarly, seeds coated with amino acid chelated Zn fertilizer (1:7) has resulted in germination percentage (95.67), seedling length (31.96 cm), seedling dry weight (0.2483 g), seed vigour index I (3060.86) and II (23.77) that were on par to the control (Table 3). The seeds coated with higher dose of Amino acid chelated Zn fertilizer (1:1, 1:2 and 1:3) has significantly reduced seed germination in comparison to the control as the number of abnormal seedlings noticed were more. This could be because of the excessive Zn concentration as well as amino acids present in the fertilizer formulation. All the treatments combinations in case of seeds coated with EDTA chelated Zn fertilizer has significantly reduced all the seedling vigour parameters in comparison to control (Table 4). Among the three fertilizers used, coating seeds with Zn-NCPC fertilizer (1:4) found to be the best as significant enhancement of early seedling vigour parameters were observed in comparison to control. Enhanced seedling vigour parameters in case of seeds coated with optimum dose of Zn-NCPC fertilizer could be attributed to the biological role played by the Zn element. At the time of seed germination and subsequent seedling growth, cell cycle, cell division, cell proliferation, cell elongation and seed reserve mobilization should happen at optimum phase and time, so that the resulting seedling will be of high vigour to surpass the suboptimal abiotic and biotic stresses. Zn being an essential structural entity of numerous enzymes; plays a crucial role in cell cycle, cell division, cell proliferation, cell metabolism, cell elongation, protein synthesis, structural integrity of cell, chlorophyll synthesis, water use efficiency, shoot and root structure, abiotic and biotic stress tolerance through suppression of Reactive Oxygen Species (ROS) and nitrogen fixation in legumes (Hassan *et al.*, 2020; Hacisalihoglu, 2020) and seeds with higher intrinsic Zn concentration produce seedlings of high vigour than seeds of lower intrinsic Zn concentration, which facilitates in better crop establishment and in realization of better crop yields (Boonchuay *et al.*, 2012; Cakmak, 2008).

**Table 1: Effects of Zn-seed priming on seedling vigour parameter of mungbean seeds.**

Treatments	Ger (%)	ShL (cm)	RoL (cm)	SdL (cm)	SdDW (g)	SVI	SVII
Control	94.00	19.89	11.75	31.64	0.2479	2975.62	23.30
Hydroprimed	97.67	22.83	12.93	35.77	0.2671	3493.87	26.09
300PPM-Zn primed	97.67	23.04	13.52	36.56	0.2690	3570.90	26.28
450PPM-Zn primed	98.00	23.81	13.95	37.76	0.2786	3701.95	27.31
600PPM-Zn primed	95.00	23.47	12.42	35.89	0.2630	3411.71	25.00
750PPM-Zn primed	90.33	21.27	10.52	31.79	0.2417	2873.26	21.84
<b>Mean</b>	<b>95.44</b>	<b>22.39</b>	<b>12.52</b>	<b>34.90</b>	<b>0.2612</b>	<b>3337.89</b>	<b>24.97</b>
<b>CD @ 0.05 P</b>	<b>2.94*</b>	<b>2.78*</b>	<b>1.57*</b>	<b>4.18*</b>	<b>0.0175*</b>	<b>477.74*</b>	<b>2.36*</b>

Ger: Germination %

ShL: Average shoot length of 10 seedlings

RoL:

Average root length of 10 seedlings SdL: Average length of 10 seedlings

SdDw:

Dry weight of 10 seedlings SV-I: Seed Vigour Index – I

SV-II: Seed Vigour Index – II

CD - Critical difference obtained from ANOVA of CRD

\* indicates that treatment effects were significant at 0.05 probability in the respective analysis

**Table 2: Effects of Zn-NCPC fertilizer seed coating on seedling vigour parameter of mungbean seeds.**

Treatments	Ger (%)	ShL (cm)	RoL (cm)	SdL (cm)	SdDW (g)	SVI	SVII
Control	94.00	19.71	11.97	31.68	0.2439	2978.48	22.93
Zn-NCPC (1:1)	90.67	22.82	9.39	32.21	0.2461	2922.54	22.32
Zn-NCPC (1:2)	95.33	23.12	12.20	35.32	0.2698	3369.10	25.73
Zn-NCPC (1:3)	97.67	23.78	13.49	37.27	0.2758	3641.50	26.95
Zn-NCPC (1:4)	98.33	24.13	14.05	38.18	0.2864	3756.64	28.17
Zn-NCPC (1:5)	98.33	23.56	13.74	37.30	0.2712	3669.74	26.69
Zn-NCPC (1:6)	98.33	23.31	12.97	36.29	0.2671	3569.71	26.29
<b>Mean</b>	<b>96.10</b>	<b>22.92</b>	<b>12.54</b>	<b>35.46</b>	<b>0.2658</b>	<b>3415.39</b>	<b>25.58</b>
<b>CD @ 0.05 P</b>	<b>3.33*</b>	<b>2.06*</b>	<b>1.73*</b>	<b>3.73*</b>	<b>0.0261*</b>	<b>474.45*</b>	<b>3.34*</b>

Ger: Germination %

ShL: Average shoot length of 10 seedlings

RoL:

Average root length of 10 seedlings SdL: Average length of 10 seedlings

SdDw:

Dry weight of 10 seedlings SV-I: Seed Vigour Index – I

SV-II: Seed Vigour Index – II

CD - Critical difference obtained from ANOVA of CRD

\* indicates that treatment effects were significant at 0.05 probability in the respective analysis

**Table 3: Effects of Amino acid chelated Zn fertilizer seed coating on seedling vigour parameter of mungbean seeds.**

Treatments	Ger (%)	ShL (cm)	RoL (cm)	SdL (cm)	SdDW (g)	SVI	SVII
Control	93.67	19.95	11.60	31.55	0.2426	2957.59	22.73
AmZn (1:1)	70.67	13.23	8.13	21.36	0.1569	1513.23	11.09
AmZn (1:2)	77.67	17.33	9.33	26.67	0.1883	2073.00	14.63
AmZn (1:3)	80.33	17.52	10.25	27.77	0.1983	2233.70	15.95
AmZn (1:4)	93.67	17.76	10.87	28.63	0.2044	2683.43	19.15
AmZn (1:5)	94.67	17.88	10.95	28.83	0.2087	2731.94	19.77
AmZn (1:6)	95.33	18.73	11.56	30.30	0.2336	2891.34	22.29
AmZn (1:7)	95.67	19.73	12.23	31.96	0.2483	3060.86	23.77
<b>Mean</b>	<b>87.71</b>	<b>17.77</b>	<b>10.62</b>	<b>28.38</b>	<b>0.2101</b>	<b>2518.14</b>	<b>18.67</b>
<b>CD @ 0.05 P</b>	<b>4.66</b>	<b>2.33</b>	<b>2.29</b>	<b>4.60</b>	<b>0.0193</b>	<b>502.71</b>	<b>2.67</b>

Ger: Germination %

ShL: Average shoot length of 10 seedlings

RoL:

Average root length of 10 seedlings SdL: Average length of 10 seedlings

SdDw:

Dry weight of 10 seedlings SV-I: Seed Vigour Index – I

SV-II: Seed Vigour Index – II

CD - Critical difference obtained from ANOVA of CRD

\* indicates that treatment effects were significant at 0.05 probability in the respective analysis

**Table 4: Effects of EDTA chelated Zn fertilizer seed coating on seedling vigour parameter of mungbean seeds.**

Treatments	Ger (%)	ShL (cm)	RoL (cm)	SdL (cm)	SdDW (g)	SVI	SVII
Control	94.33	19.43	12.12	31.55	0.2461	2978.05	23.22
EDTAZn (1:1)	55.67	7.36	6.31	13.67	0.1504	764.39	8.39
EDTAZn (1:2)	61.67	7.49	6.52	14.01	0.1535	866.61	9.48
EDTAZn (1:3)	64.67	9.18	8.25	17.43	0.1728	1130.57	11.19
EDTAZn (1:4)	65.33	10.09	8.84	18.93	0.1796	1238.75	11.74
EDTAZn (1:5)	67.33	12.18	9.25	21.43	0.1906	1445.79	12.85
EDTAZn (1:6)	69.67	13.25	9.38	22.63	0.1948	1576.90	13.57
EDTAZn (1:7)	70.67	13.68	9.89	23.57	0.1962	1668.78	13.88
<b>Mean</b>	<b>68.67</b>	<b>11.58</b>	<b>8.82</b>	<b>20.40</b>	<b>0.1855</b>	<b>1458.73</b>	<b>13.04</b>
<b>CD @ 0.05 P</b>	<b>5.00</b>	<b>2.10</b>	<b>2.08</b>	<b>4.16</b>	<b>0.0224</b>	<b>379.50</b>	<b>2.38</b>

Ger: Germination %

ShL: Average shoot length of 10 seedlings

RoL:

Average root length of 10 seedlings SdL: Average length of 10 seedlings

SdDw:

Dry weight of 10 seedlings SV-I: Seed Vigour Index – I

SV-II: Seed Vigour Index – II

CD - Critical difference obtained from ANOVA of CRD

\* indicates that treatment effects were significant at 0.05 probability in the respective analysis

## CONCLUSION

Lower productivity of mungbean is attributed to poor crop establishment as well as its cultivation in Zn deficient soils. Seed treatments that enhance the seedling vigour parameters as well as supplement the Zn like Zn-seed priming and seed coating with Zn fertilizers could address the said problem. Hence, the present study was undertaken to standardize the Zn-seed treatments in Mungbean. It was found that priming seeds with 450 ppm Zn solution for 9 hrs. at  $25 \pm 2^\circ\text{C}$  has significantly enhanced the seedling vigour characters in comparison to control and hydro-primed seeds. Among the Zn-fertilizers used, Zn-NCPC fertilizer was found to highly suitable for seed coating (in 1:4 combination) with significant positive results. Where as Amino acid chelated Zn fertilizer and EDTA chelated Zn fertilizer were not found suitable for seed coating to enhance the seedling vigour characters.

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**Conflict of Interest.** None.

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