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Seed Polymer Coating with ZnO, SiO_2 and TiO_2 Nanoparticles: An Innovative Seed Quality Enhancement Technique in Sweet Pepper

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Abstract: A laboratory study was undertaken to assess the effect of seed polymer coating with ZnO, SiO_2 and TiO_2 nanoparticles (NPs) at different concentrations (250 and 500 ppm) on different seeds of vigour lots in sweet pepper at Department of Seed Science and Technology, University of Agricultural Sciences, Bangalore. Among vigour level, high vigour seed lots differed significantly with highest seed germination (84.0%), speed of germination (9.54), mean seedling length (11.53 cm), mean seedling dry weight (4.07 mg), seedling vigour index – I (952), seedling vigour index – II (342), total dehydrogenase activity (1.654), total soluble seed protein (68.24 µg/g), amylase activity (85.47 µmol/min) and decreased electrical conductivity of seed leachates (35.91 µS/cm).Among treatments, seed polymer coating with ZnO NPs at 250 ppm recorded significantly higher seed germination (82.0%), speed of germination (9.22), mean seedling length (11.41 cm), mean seedling dry weight (4.24 mg), seedling vigour index – I (937)seedling vigour index - II (348), total dehydrogenase activity (1.506), protein content (72.80 µg/g) and á-amylase activity (76.30 µmol/min) and lowest electrical conductivity (30.64 µS/cm) over control, followed by SiO_2 and TiO_2 NPs at 250 ppm. However, higher concentrations of NPs reflected inhibitory effect on seed germination and seedling growth (nano ZnO, SiO_2 and TiO_2 at 500 ppm each).Based on the seed quality evaluation, it was concluded that seed coating with ZnO NPs at 250 ppm can be used to enhance of sweet pepper.

Keywords: Sweet pepper, Seed nano polymers, seed quality

INTRODUCTION

Sweet pepper (*Capsicum annuum* L.) is one of the most important vegetable crop in world. Sweet pepper is grown world-wide because of its adaptation to different agroclimatic regions and its wide variety of shapes, sizes, colours and pungencies of the fruit [1]. Bell pepper or sweet pepper belongs to the genus *Capsicum*, a member of the Solanaceae family that includes other members such as chilli, tomato, potato and eggplant. Cultivars of the plant produce fruits in different colours, including green, red, yellow and orange, but more exotic colours include purple, white and lime green. It is also known as bell pepper, capsicum and pepper. India produces an average of 563 thousand metric tonnes of capsicum annually from an area of 38 thousand hectares with a productivity of 14.82 MT per hectare accounting for one fourth of global production [2].

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 process of coating seeds with 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 [3]. 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 and nanopolymer coating to enhance seedling quality.

Zinc Oxide (ZnO), Silicon dioxide (SiO_2) and Titanium dioxide (TiO_2) being essential micronutrients are required for the normal plant growth and development and these are important components of various enzymes that are responsible for driving many metabolic reactions in plants. However, these micro elements are required in minute quantity for treating seeds. Recently use of these elements in the form of nanoparticles gained 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 ZnO, SiO_2 and TiO_2 nanoparticles on seed quality in sweet pepper.

MATERIAL AND METHODS

The research studies were carried out in the laboratory of Department of Seed Science and Technology, University of Agricultural Sciences, Bangalore. Three different vigour seed lots of Selection-3 sweet pepper having different germination levels, were procured from ORBI Seed International Private Limited Bangalore and used for the experiment. The seeds procured were dried to obtain uniform and safe level of seed moisture. ZnO, SiO_2 and TiO_2 nanoparticles obtained from AICRP on Seed (Crops), UAS, GKVK, Bangalore were used in the present investigation.

Polymer mediated coating was done by mixing 1.5 ml polymer with different nano chemicals like ZnO, SiO_2 and TiO_2 at 250 and 500 mg each. Later, seeds were air dried overnight. Further, three different seed lots of 85.0%, 80.0% and 71.0% as high, medium and low vigour seed lots, respectively were used for experiment. T_1: Control, T_2: Water Soaking, T_3: ZnO NPs at 250 ppm with water soaking, T_4: ZnO NPs at 500 ppm with water soaking, T_5: SiO_2 NPs at 250 ppm with water soaking, T_6: SiO_2 NPs at 500 ppm with water soaking, T_7: TiO_2 NPs at 250 ppm with water soaking, T_8: TiO_2 NPs at 500 ppm with water soaking.

Observations on various seed quality parameters *viz.*, seed germination (%), speed of germination (4), mean seedling length (cm), mean seedling dry weight (mg), seedling vigour index – I and II [5], electrical conductivity (µS/cm) total dehydrogenase activity (6), protein content (µg/g), á-amylase activity (µmol/min)were recorded as per the methods and procedures described by [7]. The mean data of the laboratory experiments were statistically analyzed by adopting factorial completely randomized design. 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.

RESULT AND DISCUSSION

Results obtained on various seed quality parameters like seed germination (%), speed of germination, mean seedling length (cm), mean seedling dry weight (mg), seedling vigour index – I and II, electrical conductivity (µS/cm) total dehydrogenase activity, protein content (µg/g), α-amylase activity (µmol/min) are presented as follows.

1. Seed germination (%)

The seed lot with highest (84.0%) germination was classified as high vigour, that with 75.0% germination as medium vigour seed lot (V_2) and the lot with lowest germination (68.0%) was taken as low vigour seed lot (V_3). Among polymer coating treatments, the highest (82.0%) seed germination was recorded in the polymer coated seeds with ZnO NPs at 250 ppm (T_3) followed by 80.0 and78.0% seed germination in SiO_2 NPs(T_5)and TiO_2 NPs (T_7)at 250 ppm each with polymer coating, respectively. Whereas, TiO_2 NPs at 500 ppm with polymer coating (T_8) showed the lowest (74.0%) seed germination [Table 1].

The probable reason for the enhanced germination due to nano Zn might be due to the nano size of particles which allowed them to penetrate through seed coat easily and hence, resulted in 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 production of essential biomolecules and nutrients required for plant growth and are important components of various enzymes which are responsible for driving many metabolic reactions in all crops [8, 9].

2. Speed of germination

Highest (9.54) speed of germination was recorded in high germination (vigour) seed lot (V_1), followed by in medium vigour (8.03)(V_2) and lowest (7.20) in low vigour seed lot (V_3). Among polymer coating treatments the highest (9.22) speed of germination was recorded in the polymer coated seeds with ZnO NPs at 250 ppm (T_3) followed by 8.69 and 8.27 in SiO_2 NPs (T_5)andTiO_2 NPs (T_7) at 250 ppm with polymer coating. Whereas, TiO_2 NPs at 500 ppm with polymer coating (T_8) showed the lowest (8.03) speed of germination (Table 1).

Table 1. Seed germination and speed of germination as influenced by nano priming with polymer coating on different vigour levels in sweet pepper

Treatments (T)	Seed germination (%)				Speed of germination			
	Vigour levels (V)			Mean	Vigour levels (V)			Mean
	V_1	V_2	V_3		V_1	V_2	V_3	
T_1	80.0	71.0	62.0	71.0	8.03	7.45	6.90	7.46
T_2	81.0	72.0	63.0	72.0	8.80	7.63	7.01	7.81
T_3	89.0	82.0	75.0	82.0	11.33	8.70	7.63	9.22
T_4	84.0	75.0	68.0	76.0	9.61	8.20	7.53	8.45
T_5	87.0	80.0	74.0	80.0	10.33	8.42	7.32	8.69
T_6	83.0	74.0	67.0	75.0	9.41	7.96	7.04	8.14
T_7	85.0	77.0	73.0	78.0	9.63	8.02	7.16	8.27
T_8	82.0	73.0	65.0	74.0	9.22	7.88	6.99	8.03
Mean	84.0	75.0	68.0	76.0	9.54	8.03	7.20	8.26
	S.Em±	CD(p=0.01)	CV (%)		S.Em±	CD(p=0.01)	CV (%)	
V	0.82	2.32	3.23		0.06	0.18	3.80	
T	0.50	1.42			0.10	0.30		
V×T	1.42	4.03			0.18	0.52		

Note: Temperature: 30°C; Soaking duration: 16 hrs for all priming treatment

Vigour levels: V_1: High vigour lot; V_2: Medium vigour lot; V_3: Low vigour lot

Treatments: T_1: Control; T_2:Hydropriming; T_3: ZnO NPs at 250 ppm with polymer coating; T_4: ZnO NPs at 500 ppm with polymer coating; T_5: SiO_2 NPs at 250 ppm with polymer coating; T_6: SiO_2 NPs at 500 ppm with polymer coating; T_7: TiO_2 NPs at 250 ppm with polymer coating; T_8: TiO_2 NPs at 500 ppm with polymer coating

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 [10].

3. Mean seedling length (cm)

Among seed vigour levels, the highest (11.33 cm) mean seedling length was recorded in high vigour seed lot (V_1), followed by 9.31 cm in medium vigour seed lot (V_2) and the lowest (8.84 cm) was observed in low vigour seed lot (V_3).Among polymer coating treatments, the highest (11.41 cm) mean seedling length was recorded in the polymer coated seeds with ZnO NPs at 250 ppm with polymer coating (T_3) followed by 10.90 and 10.48 cm in SiO_2 NPs (T_5)andTiO_2 NPs (T_7) at 250 ppm with polymer coating. Whereas, TiO_2 NPs at 500 ppm with polymer coating (T_8) showed the lowest (9.17 cm) mean seedling length which was significantly lower than other treatments (Table 2).

4. Mean seedling dry weight (mg)

Among seed vigour levels, the highest (4.07 mg) mean seedling dry weight was recorded in high vigour seed lot (V_1), followed by 3.84 mg in medium vigour seed lot (V_2) and lowest (3.30 mg) was observed in low vigour seed lot (V_3). Among polymer coating treatments, the highest (4.24 mg) mean seedling dry weight was recorded in the polymer coated seeds with ZnO NPs at 250 ppm (T_3) followed by 4.10 and 3.88 mg SiO_2 NPs (T_5) and TiO_2 NPs (T_7) at 250 ppm with polymer coating. Whereas, TiO_2 NPs at 500 ppm with polymer coating (T_8) observed the lowest (3.57 mg) mean seedling dry weight which was significantly lower than other treatments (Table 2).

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 [9, 10]

5. Seedling vigour index – I (SVI-I)

Among seed vigour levels, the highest (952) seedling vigour index - I was recorded in high vigour seed lot (V_1), followed by 706 in medium vigour seed lot (V_2) and lowest (608) was recorded in low vigour seed lot (V_3). Among priming treatments and showed highest SVI – I compared to control but differed in their magnitudes. The highest (937) seedling vigour index - I was recorded in the polymer coated seeds with ZnO NPs at 250 ppm (T_3) followed by 878 and 824 SiO_2 NPs (T_5) and TiO_2 NPs

Table 2. Influence of different vigour levels and polymer coating with nano particles on mean seedling length, mean seedling dry weight, seedling vigour index - I and seedling vigour index - II in sweet pepper

Treatments (T)	Mean seedling length (cm)				Mean seedling dry weight (mg)				SVI-I				SVI-II			
	Vigour levels (V)			Mean	Vigour levels (V)			Mean	Vigour levels (V)			Mean	Vigour levels (V)			Mean
	V_1	V_2	V_3		V_1	V_2	V_3		V_1	V_2	V_3		V_1	V_2	V_3	
T_1	9.94	7.76	7.47	8.39	3.45	3.19	2.90	3.18	795	549	462	602	276	225	180	227
T_2	10.60	8.04	7.82	8.82	3.80	3.39	3.00	3.40	862	579	490	644	309	244	188	247
T_3	12.28	11.30	10.64	11.41	4.56	4.30	3.86	4.24	1089	927	794	937	404	352	288	348
T_4	11.45	9.42	9.02	9.97	4.13	4.00	3.27	3.80	962	703	614	760	347	299	222	289
T_5	12.18	10.61	9.92	10.90	4.27	4.23	3.80	4.10	1055	845	734	878	370	337	281	329
T_6	11.33	8.87	8.21	9.47	4.10	3.90	3.20	3.73	944	657	547	716	342	288	213	281
T_7	11.77	10.14	9.51	10.48	4.20	4.10	3.33	3.88	996	781	694	824	355	316	243	305
T_8	11.07	8.33	8.11	9.17	4.03	3.63	3.03	3.57	908	610	530	682	331	266	198	265
Mean	11.33	9.31	8.84	9.83	4.07	3.84	3.30	3.74	952	706	608	755	342	291	227	287
	S.Em±	CD(p=0.01)	CV (%)		S.Em±	CD(p=0.01)	CV (%)		S.Em±	CD(p=0.01)	CV (%)		S.Em±	CD(p=0.01)	CV (%)	
V	0.07	0.19	3.39		0.03	0.07	3.40		5.69	16.18	3.63		3.29	9.37	3.41	
T	0.11	0.32			0.04	0.12			9.29	26.42			2.02	5.74		
V×T	0.19	0.55			0.07	0.21			16.09	45.75			5.71	16.23		

Note: Temperature: 30°C; Soaking duration: 16 hrs for all priming treatment

Vigour levels: V_1: High vigour lot; V_2: Medium vigour lot; V_3: Low vigour lot

Treatments: T_1: Control; T_2:Hydropriming; T_3: ZnO NPs at 250 ppm with polymer coating; T_4: ZnO NPs at 500 ppm with polymer coating; T_5: SiO_2 NPs at 250 ppm with polymer coating; T_6: SiO_2 NPs at 500 ppm with polymer coating; T_7: TiO_2 NPs at 250 ppm with polymer coating; T_8: TiO_2 NPs at 500 ppm with polymer coating

(T_7) at 250 ppm with polymer coating. Whereas, TiO_2 NPs at 500 ppm with polymer coating (T_8) recorded the lowest (682) seedling vigour index - I which was significantly lower than other treatments (Table 2).

6. Seedling vigour index - II

Among seed vigour levels, the highest (342) seedling vigour index - II was recorded in high vigour seed lot (V_1), followed by 291 in medium vigour seed lot (V_2) and lowest (227) was showed in low vigour seed lot (V_3). Among polymer coating treatments, the highest (348) seedling vigour index - II was recorded in the polymer coated seeds with ZnO NPs at 250 ppm (T_3)followed by 329 and 305 SiO_2 NPs (T_5) and TiO_2 NPs (T_7) at 250 ppm with polymer coating. Whereas, TiO_2 NPs at 500 ppm with polymer coating (T_8) recorded the lowest (265) seedling vigour index - II which was significantly lower than other treatments (Table 2).

The increased seedling vigour index- I is mainly due to overall improvement in germination and seedling growth characteristics and increased vigour index II is mainly due to 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 weight also increased. And overall improvement in germination and seedling growth characteristics improved the seed vigour [9].

7. Electrical conductivity of seed leachates (µS/cm)

Among seed vigour levels, the lowest (35.91 µS/cm) electrical conductivity was recorded in high vigour seed lot (V_1), followed by 37.99 µS/cm in medium vigour seed lot (V_2) and highest (49.73 µS/cm) was observed in low vigour seed lot (V_3). Among polymer coating treatments, the lowest (30.64 µS/cm) electrical conductivity was recorded in the polymer coated seeds with ZnO NPs at 250 ppm (T_3) followed by 32.45 and 36.48 µS/cm SiO_2 NPs (T_5) and TiO_2 NPs (T_7) at 250 ppm with polymer coating. Whereas, TiO_2 NPs at 500 ppm with polymer coating (T_8) recorded the highest (44.02 µS/cm) electrical conductivity which was significantly higher than other treatments (Table 3).

The EC of the seed leachate, a measure of membrane integrity is considered as a good index for seed viability. Minimum value of EC in the invigorated seeds is presumed to be because of the quenching of free radicals which in turn consequentially maintains the membrane integrity. Enhanced seed quality may be attributed to

Table 3. Electrical conductivity, total dehydrogenase activity (A_{480}), total soluble seed protein (µg/g) and amylase activity as influenced by polymer coating with nano particles and vigour levels in sweet pepper

Treatments	EC (µS/cm)				TDH (A_{480})				Total soluble seed protein (µg/g)				Amylase activity (µmol/min)			
(T)	Vigour levels (V)			Mean	Vigour levels (V)			Mean	Vigour levels (V)			Mean	Vigour levels (V)			Mean
	V_1	V_2	V_3		V_1	V_2	V_3		V_1	V_2	V_3		V_1	V_2	V_3	
T_1	48.10	54.84	69.10	57.35	1.098	1.020	0.614	0.911	39.98	34.78	30.75	35.17	76.28	51.26	42.60	56.71
T_2	41.11	42.92	55.28	46.43	1.418	1.240	0.640	1.099	44.23	37.74	34.55	38.84	79.76	59.26	45.56	61.53
T_3	26.06	28.38	37.49	30.64	2.192	2.007	1.729	1.972	94.8	67.11	56.49	72.80	96.40	71.08	61.42	76.30
T_4	35.79	36.13	48.65	40.19	1.645	1.437	0.763	1.281	75.75	59.60	43.24	59.53	82.82	65.42	52.01	66.75
T_5	28.73	29.57	39.05	32.45	2.001	1.958	1.531	1.83	88.57	65.91	52.34	68.94	94.34	66.83	58.70	73.29
T_6	37.48	38.59	50.36	42.14	1.531	1.435	0.764	1.243	69.21	53.01	38.75	53.66	81.64	63.25	51.10	65.33
T_7	31.66	33.16	44.61	36.48	1.876	1.752	0.997	1.541	80.02	64.56	44.55	63.04	91.43	65.04	55.90	70.79
T_8	38.34	40.37	53.35	44.02	1.473	1.332	0.753	1.186	53.32	43.04	37.42	44.59	81.05	62.34	49.67	64.35
Mean	35.91	37.99	49.73	41.21	1.654	1.523	0.974	1.383	68.24	53.22	42.26	54.57	85.47	63.06	52.12	66.88
	S.Em±	CD(p=0.01)	CV (%)	S.Em±	CD(p=0.01)	CV (%)	S.Em±	CD(p=0.01)	CV (%)	S.Em±	CD(p=0.01)	CV (%)				
V	0.34	0.98	4.1	0.01	0.03	4.3	0.6	1.69	5.34	0.76	2.16	3.41				
T	0.56	1.6		0.02	0.06		0.97	2.77		0.47	1.32					
V×T	0.98	2.77		0.03	0.1		1.68	4.79		1.32	3.75					

Note: Temperature: 30°C; Soaking duration: 16 hrs for all priming treatment

Vigour levels: V_1: High vigour lot; V_2: Medium vigour lot; V_3: Low vigour lot

Treatments: T_1: Control; T_2:Hydropriming; T_3: ZnO NPs at 250 ppm with polymer coating; T_4: ZnO NPs at 500 ppm with polymer coating; T_5: SiO_2 NPs at 250 ppm with polymer coating; T_6: SiO_2 NPs at 500 ppm with polymer coating; T_7: TiO_2 NPs at 250 ppm with polymer coating; T_8: TiO_2 NPs at 500 ppm with polymer coating

nanoparticles which could induce oxidation and reduction reactions *via* the superoxide ion radical during germination, resulting in quenching of free radicals in the germinating seeds. In turn, oxygen produced in this process could also be used for respiration, which would further promote germination.

8. Total dehydrogenase activity (TDH at A_{480})

Among seed vigour levels, the highest (1.654) total dehydrogenase activity was recorded in high vigour seed lot (V_1), followed by 1.523 in medium vigour seed lot (V_2) and lowest (0.974) was recorded in low vigour seed lot (V_3). Among polymer coating treatments, the highest (1.972) total dehydrogenase activity was recorded in the polymer coated seeds with ZnO NPs at 250 ppm (T_3)followed by 1.830 and 1.541 SiO_2 NPs (T_5)andTiO_2 NPs (T_7) at 250 ppm with polymer coating. Whereas, TiO_2 NPs at 500 ppm with polymer coating (T_8) observed the lowest (1.186) which was significantly lower than other treatments (Table 3).

Zn 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 nanoscale with increased chemical reactivity resulted in the increase in synthesis and activity of the dehydrogenase enzymes

9. Total soluble seed protein(µg/g)

Among seed vigour levels, the highest (68.24 µg/g) total soluble seed protein was recorded in high vigour seed lot (V_1), followed by 53.22 µg/g in medium vigour seed lot (V_2) and lowest (42.26 µg/g) was observed in low vigour seed lot (V_3). Among polymer coating treatments, the highest (72.80 µg/g) total soluble seed protein was recorded in the polymer coated seeds with ZnO NPs at 250 ppm (T_3)followed by 68.94 µg/g and 63.04 µg/g SiO_2 NPs (T_5)andTiO_2 NPs (T_7) at 250 ppm with polymer coating. Whereas, TiO_2 NPs at 500 ppm with polymer coating (T_8) recorded the lowest (44.59 µg/g) total soluble seed protein which was significantly lower than other treatments (Table 3).

The soluble seed protein content is an important biochemical parameter. The higher protein seed lots detected in ZnO NPs imbibed seeds suggests initiation of *de novo* synthesis of enzymes, during imbibition and early germination, to accommodate metabolic requirements in treated seeds. Highest protein content was recorded when ZnO NPs was used as nano -priming agents. On the other hand, the lowest TSS was recorded in the control.

10. Amylase activity (µmol/min)

Among seed vigour levels., the highest (85.47 µmol/min) amylase activity was recorded in high vigour seed lot (V_1), followed by 63.06 µmol/min in medium vigour seed lot (V_2) and lowest (52.12 µmol/min) was recorded in low vigour seed lot (V_3).

Among polymer coating treatments, the highest (76.30 µmol/min) amylase activity was recorded in the polymer coated seeds with ZnO NPs at 250 ppm (T_3) followed by 73.29 µmol/min in and 70.79 µmol/min SiO_2 NPs (T_5) and TiO_2 NPs (T_7) at 250 ppm with polymer coating. Whereas, TiO_2 NPs at 500 ppm with polymer coating (T_8) recorded the lowest (64.35 µmol/min) amylase activity which was significantly lower than other treatments (Table 3).

Zn is the most critical micronutrient which influence the amylase activity. 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.

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