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Influence of seed polymer coating with Zn and Fe nanoparticles on storage potential of pigeonpea seeds under ambient conditions

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Abstract: Present laboratory experiment was conducted with an objective to know the effect of seed polymer coating with Zn and Fe nanoparticles (NPs) in comparison to their bulk forms on storage potential of pigeonpea seeds. Results revealed that seed polymer coating with Zn and Fe NPs had significant effect on storability of pigeonpea seeds. Among the treatments Zn NPs at 750 ppm was found to be superior in all the studied parameters *viz*., seed germination (96.00, 88.67 and 81.67 %), seedling length (25.67, 22.57 and 18.60 cm), seedling dry weight (85, 81.45 and 78. 45 mg), field emergence (89.67, 77.67 and 63.33 %), seedling vigour index (2556, 2001 and 1519), alpha amylase and dehydrogenase enzymes activities at 0, 6 and 10 months, respectively and it was statistically on par with Zn NPs at 500 ppm and Fe NPs at 500 ppm. The nanoparticles treatment didn't affect the seed moisture content (%) and insect infestation (%), however the significant difference was observed between polymer coated and uncoated seeds. In over all, the results of the study demonstrated the possibility of application Nanotechnology in Seed Science Research.

Keywords: Nanoparticles, Pigeonpea, Polymer coating and Storability

INTRODUCTION

Seed storage is the integral part of the seed production programme. Loss of seed vigour and viability are said to be associated with the ageing phenomenon and results in poor stand and performance in the field. The storage potential of any seed depends on several factors like type of seed, period of storage, chemical composition, seed treatment, seed moisture and temperature and storage containers (Delouche and Baskin, 1973). Many researchers advocated hydration and dehydration techniques to improve vigour and viability of stored seeds, but these techniques are inconsistent and difficulties in handling bulk quantities of seeds. Hence, it would be very much appropriate if an invigouration treatment is given to fresh seeds to improve its vigour and viability during storage. In biological system ageing, senescence and deterioration leading to death are common phenomenon and seeds are no exception to this. The seed deterioration is inevitable, irreversible and inexorable but it can be delayed to some extent by proper management practices like preservation under controlled conditions and by some seed invigouration techniques (Basu, 1994). Preservation of seeds under controlled conditions is costlier and not feasible in the developing countries like India, under such situation seed invigouration techniques are best alternatives. Among these seed polymer coating with active ingredients is the promising one.

Seed polymer coating is a sophisticated process of applying precise amount of active ingredients along with a liquid material directly on to the seed surface without obscuring its shape while, total seed weight may increase up to 1 to 2 per cent. Since the coating is very thin, multiple coatings of various ingredients are also possible. The film forming formulation consists of a mixture of polymer, plasticizer and colourants. Advantages of polymer coating are the seed enhancement material can be placed directly on to the seed and smaller amount of chemicals are needed as compared to broadcasting or surface dressing on to the growing medium (Sherin Susan *et al*., 2005).

Nanotechnology has emerged as a new discipline and Nanoparticles (NPs) have become a centre of attraction for researchers because of its unique physico-chemical properties compared to their bulk particles (Monica and Cermonini, 2009). NPs of size below 100 nm fall in the transition zone between individual molecules and the corresponding bulk materials, which may gen-

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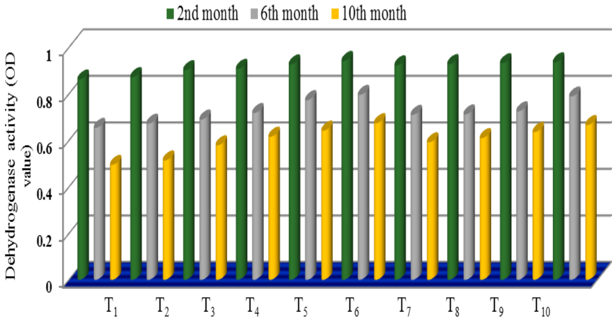

Fig. 1. *Influence of seed polymer coating with Zn and Fe nanoparticles on dehydrogenase activity (OD value) in pigeonpea during storage.*

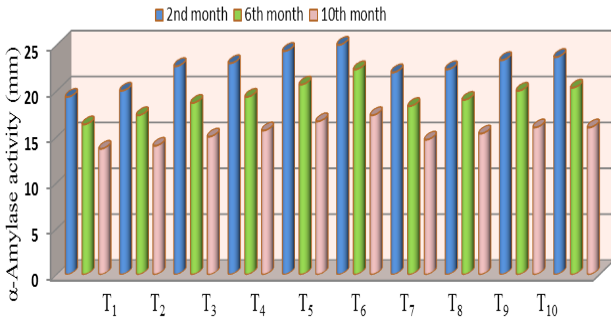

Fig. 2. *Influence of seed polymer coating with Zn and Fe nanoparticles on α-amylase activity (mm) in pigeonpea during storage (T_1- Control, T_2- Only polymer, T_3- Polymer + $ZnSO_4$ @ 1000 ppm, T_4- Polymer + $ZnSO_4$ @ 2000 ppm, T_5- Polymer + Zn nanoparticles @ 500 ppm, T_6- Polymer + Zn nanoparticles @ 750 ppm, T_7- Polymer + $FeSO_4$ @ 500 ppm, T_8- Polymer + $FeSO_4$ @ 1000 ppm, T_9- Polymer + Fe nanoparticles @ 250 ppm , T_{10}- Polymer + Fe nanoparticles @ 500 ppm).*

erate both positive and negative biological effects in living cell (Nel *et al.*, 2006). 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). The NPs are also known to donate electrons that pairs with the free radicals and thereby smother the impact of free radicals (Zheng *et al.*, 2005). 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 TiO_2 treated to groundnut seeds at dif-ferent 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, from our earlier study (Seed polymer coating with Zn and Fe nanoparticles: An innovative seed quality enhancement technique in pigeonpea (Pradeep Korishettar *et al.*, 2016)) the Zn and Fe NPs were found to have positive effect on quality in pigeonpea seeds. Hence, an effort was made in the present investigation to study the effect of seed polymer coating with Zn and Fe NPs on storability of the pigeonpea seeds under ambient conditions in non woven fabric bag (moisture pervious).

MATERIALS AND METHODS

The laboratory experiments of present investigation were carried out in the Department of Seed Science and Technology, College of Agriculture, University of Agricultural Sciences (UAS), Raichur during 2014-15. The seeds of pigeonpea cv. TS-3R and non woven fabric bags 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 required concentrations of nano Zn (@ 500 & 750 ppm) and nano Fe (@ 250 and 500 ppm); bulk $ZnSO_4$ (@ 1000 and 2000 ppm) and bulk $FeSO_4$ (@ 500 and 1000 ppm) solutions were prepared for the experiment by suspending particles directly in to the distilled water and further dispersed by ultrasonic vibration (100W, 40 kHz) for 30 min. Small magnetic bars were used for stirring the suspensions before use to avoid aggregation of the particles. Then the seeds were coated with polymer and suspensions as per the treatment imposition in 1: 5 ratio (6:30 ml / kg of seed) by using seed coating machine, subsequently seeds were air dried over night to the safe moisture content and packed in non-woven fabric bag (recommended moisture pervious packaging material) and stored under ambient conditions for the period of ten months (June, 2014 - April, 2015). Further, the stored seeds were subjected for various seed quality tests on bimonthly basis.

The germination percentage, seedling length (cm), seedling dry weight (mg), seed moisture content (%), insect infestation (%) were taken as per the procedures described by the ISTA (2013). The alpha amylase activity (mm) as per the procedure given by Simpson and Naylor (1962); dehydrogenase enzyme activity (OD value) as per the procedure outlined by Kittock and Law (1968) were recorded.

Field emergence (%) was recorded by planting 50 seeds in four replications on well prepared sand bed in plastic trays; the adequate moisture was maintained by regular watering. The final count was taken on 10^{th} day from planting taking into account emergence of normal seedlings and expressed in percentage. The seedling vigour index was calculated by using the formula given by Abdul-Baki and Anderson (1973).

Seedling Vigour Index = Germination (%) x Mean length of seedling (cm)

The mean data of the laboratory experiments were statistically analyzed by adopting completely random-

Table 1. Influence of seed polymer coating with Zn and Fe nanoparticles on seed germination (%) and field emergence (%) in pigeonpea during storage.

Treatment	Months after storage											
	0		2		4		6		8		10	
	G (%)	F e (%)	G (%)	F e (%)	G (%)	F e (%)	G (%)	F e (%)	G (%)	F e (%)	G (%)	F e (%)
T_1	90.70	85.00	88.67	81.67	85.33	75.67	81.67	65.33	74.00	54.00	63.00	42.67
T_2	92.30	86.33	89.33	82.00	86.67	76.33	83.33	69.33	80.33	61.67	75.33	53.33
T_3	94.00	88.00	90.67	84.67	88.00	78.00	84.67	70.67	80.67	64.67	77.67	56.67
T_4	94.30	88.00	91.67	85.33	89.00	81.00	85.33	73.33	81.33	66.67	78.33	58.33
T_5	95.30	89.00	93.33	86.67	91.67	82.00	87.33	75.67	83.33	68.33	80.00	60.67
T_6	96.00	89.67	94.00	87.33	92.33	83.33	88.67	77.67	84.67	70.67	81.67	63.33
T_7	93.70	87.67	91.00	84.33	88.67	79.33	85.00	71.33	81.00	65.00	78.00	57.33
T_8	94.00	88.00	91.67	85.00	89.00	80.33	85.00	73.00	81.33	66.33	77.33	58.00
T_9	94.70	88.67	92.33	86.33	90.67	81.67	86.00	74.33	82.67	67.67	78.67	59.00
T_{10}	95.00	89.33	93.67	87.00	92.00	82.67	87.67	76.67	84.33	69.67	80.33	61.33
Mean	94.00	88.00	91.63	85.00	89.33	80.00	85.47	72.70	81.37	65.50	77.00	57.00
S. Em±	0.44	0.53	0.39	0.65	0.41	0.42	0.42	0.64	0.35	0.43	0.42	0.87
C D @ 1 %	1.27	1.52	1.17	1.95	1.21	1.25	1.25	1.93	1.04	1.29	1.25	2.58

G: Germiation, F e: Field emergence, T_1- Control, T_2- Only polymer, T_3- Polymer + $ZnSO_4$ @ 1000 ppm, T_4- Polymer + $ZnSO_4$ @ 2000 ppm, T_5- Polymer + Zn nanoparticles @ 500 ppm, T_6- Polymer + Zn nanoparticles @ 750 ppm, T_7- Polymer + $FeSO_4$ @ 500 ppm, T_8- Polymer + $FeSO_4$ @ 1000 ppm, T_9- Polymer + Fe nanoparticles @ 250 ppm, T_{10}- Polymer + Fe nanoparticles @ 500 ppm, *Polymer @ 6 ml kg^{-1} of seed.

Table 2. Influence of seed polymer coating with Zn and Fe nanoparticles on seedling length (cm) and seedling dry weight (mg) in pigeonpea during storage.

Treatment	Months after storage											
	0		2		4		6		8		10	
	SL (cm)	SDW (mg)	SL (cm)	SD W (mg)	SL (cm)	SD W (mg)	SL (cm)	SDW mg)	SL (cm)	SDW (mg)	SL (cm)	SDW (mg)
T_1	22.37	80.50	21.93	79.60	18.80	78.25	16.60	76.40	14.53	75.25	12.17	74.30
T_2	22.84	81.15	22.87	80.90	19.80	79.25	17.93	77.15	15.87	75.80	13.83	74.80
T_3	25.10	83.25	24.40	81.85	21.73	80.40	19.73	78.00	17.57	76.35	15.47	75.35
T_4	25.36	83.40	24.63	82.15	22.63	81.30	21.13	78.60	18.57	77.40	16.53	76.10
T_5	25.70	84.35	25.33	83.10	23.97	81.60	21.87	79.30	19.43	78.40	17.63	77.15
T_6	26.63	85.00	25.67	84.15	24.63	82.70	22.57	81.45	20.37	79.95	18.60	78.45
T_7	24.07	83.35	23.47	82.10	21.80	80.90	19.70	78.35	17.53	76.70	16.03	75.60
T_8	24.97	83.45	24.07	82.40	22.00	81.25	20.43	78.50	17.77	77.30	16.30	75.95
T_9	25.74	84.15	25.17	83.15	22.90	81.75	21.47	79.20	18.70	78.10	17.03	76.75
T_{10}	26.24	84.65	25.47	83.60	24.17	82.40	22.30	80.50	19.77	79.05	18.07	78.00
Mean	24.90	83.30	24.30	82.30	22.24	80.95	20.37	78.75	18.01	77.45	16.17	76.25
S. Em±	0.26	0.16	0.20	0.32	0.67	0.29	0.38	0.44	0.35	0.43	0.45	0.38
C D @ 1 %	0.76	0.46	0.62	0.96	2.00	0.87	1.14	1.30	1.06	1.28	1.34	1.14

SL: Seedling length, SDW: Seedling dry weight, T_1- Control, T_2- Only polymer, T_3- Polymer + $ZnSO_4$ @ 1000 ppm, T_4- Polymer + $ZnSO_4$ @ 2000 ppm, T_5- Polymer + Zn nanoparticles @ 500 ppm, T_6- Polymer + Zn nanoparticles @ 750 ppm, T_7- Polymer + $FeSO_4$ @ 500 ppm, T_8- Polymer + $FeSO_4$ @ 1000 ppm, T_9- Polymer + Fe nanoparticles @ 250 ppm, T_{10}- Polymer + Fe nanoparticles @ 500 ppm, *Polymer @ 6 ml kg^{-1} of seed.

Table 3. Influence of seed polymer coating with Zn and Fe nanoparticles on seedling vigour index and seed moisture content (%) in pigeonpea during storage.

Treatment	Months after storage											
	0		2		4		6		8		10	
	S VI	S M (%)	S VI	S M (%)	S VI	S M (%)	S VI	S M (%)	S VI	S M (%)	S VI	S M (%)
T_1	2029	9.27	1945	10.00	1604	10.10	1356	10.13	1075	10.18	767	10.26
T_2	2221	9.20	2043	9.35	1716	9.40	1494	9.43	1274	9.48	1042	9.56
T_3	2359	9.30	2212	9.40	1913	9.43	1671	9.45	1417	9.53	1201	9.60
T_4	2402	9.20	2258	9.30	2015	9.33	1803	9.37	1510	9.43	1296	9.52
T_5	2440	9.10	2364	9.23	2197	9.35	1909	9.37	1620	9.40	1411	9.50
T_6	2556	9.13	2413	9.20	2275	9.33	2001	9.40	1724	9.41	1519	9.50
T_7	2218	9.20	2135	9.25	1933	9.37	1675	9.43	1420	9.45	1251	9.53
T_8	2347	9.27	2206	9.32	1959	9.40	1737	9.45	1445	9.47	1260	9.57
T_9	2438	9.30	2324	9.35	2076	9.40	1846	9.50	1546	9.50	1340	9.60
T_{10}	2493	9.20	2386	9.20	2223	9.35	1955	9.47	1667	9.48	1451	9.53
Mean	2350	9.22	2229	9.36	1991	9.45	1745	9.50	1470	9.53	1254	9.62
S. Em±	26	0.06	16	0.08	25	0.07	29	0.08	30	0.06	34	0.10
C D @ 1 %	75	NS	47	0.23	76	0.22	87	0.25	88	0.18	102	0.30

SVI: Seedling Vigour Index, SM: Seed Moisture content, T_1- Control, T_2- Only polymer, T_3- Polymer + $ZnSO_4$ @ 1000 ppm, T_4- Polymer + $ZnSO_4$ @ 2000 ppm, T_5- Polymer + Zn nanoparticles @ 500 ppm, T_6- Polymer + Zn nanoparticles @ 750 ppm, T_7- Polymer + $FeSO_4$ @ 500 ppm, T_8- Polymer + $FeSO_4$ @ 1000 ppm, T_9- Polymer + Fe nanoparticles @ 250 ppm, T_{10}- Polymer + Fe nanoparticles @ 500 ppm, *Polymer @ 6 ml kg^{-1} of seed.

ized 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

In the present investigation seed polymer coating with Zn and Fe NPs had significant effect on germination per cent from the initial month of storage and up to the end of ten months of storage (Table 1). At initial month of storage period significantly highest germination was recorded in Zn NPs at 750 ppm (96.00 %) and it was statistically on par with Zn NPs at 500 ppm (95.30 %) and Fe NPs at 500 ppm (95.00 %), while the lowest germination was recorded in control (90.70 %). Similar trend was noticed at all months of storage period. At the end of ten months of storage period, highest germination was recorded in Zn NPs at 750 ppm (81.67 %) followed by Fe NPs at 500 ppm (80.33 %), Zn NPs at 500 ppm (80.00 %) and lowest germination was recorded in control (63.00 %).

The Zn and Fe NPs consistently maintained higher germination over their bulk form and control during storage. The probable reason might be due to NPs would induce oxidation-reduction reactions via the superoxide ion radicals during germination, resulting the quenching of free radicals in the germinating seeds. In turn, oxygen produced in such process could also be used for respiration, which would further promote germination (Zheng *et al.,* 2005). The experiments carried out by Senthil kumar (2011) revealed that black gram seeds treated with ZnO nano rods and ZVI (Zero valent iron) NPs enhanced the physiological and biochemical properties resulting in improved vigour and viability of aged seeds. The reason attributed was the donation of electrons by the nanoparticles in scavenging the free radicals in the aged seeds. The lowest germination was recorded in control which was attributed to the impact of ageing, increased insect infestation with advancement in storage period.

Seedling growth characteristics in terms of seedling length (cm) and seedling dry weight (mg) were significantly influenced during ten months of storage. The seedling length and seedling dry weight were decreased gradually with advancement in storage period. However, seed polymer coating with Zn and Fe (@ 750 ppm and 500 ppm, respectively) was consistently promoted the seedling growth characteristics of pigeonpea at all months of storage period compared to their bulk form and control (Table 2). The probable reason could be that these NPs have positive effect on the reactivity of phytohormones especially Indole Acetic Acid (IAA) which is involved in the phytostimulatory actions. Zinc-rich ZnO NPs could increase the level of IAA in roots (sprouts) which in turn can increase growth rate of seedlings (Pandey *et al.,* 2010).

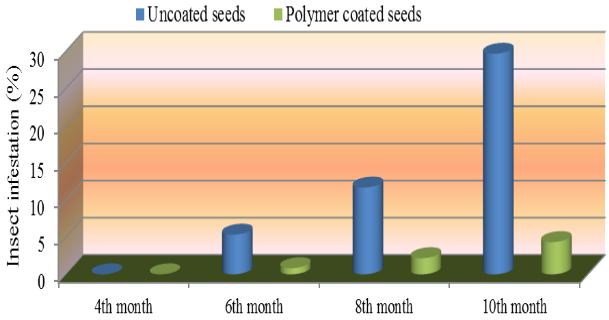

Fig. 3. *Influence of seed polymer coating on insect infestation (%) in pigeonpea seeds during storage.*

The seed vigour parameters like field emergence (%) (Table 1) and seedling vigour index (Table 3) also influenced by Zn and Fe NPs during the storage. Nano Zn at 750 ppm and nano Fe at 500 ppm recorded significantly higher values for seed vigour parameters consistently at all months of storage compared to other treatments. This could be ascribed to the beneficial effects of these NPs in repairing of damaged vital cell organelles, synthesis and activation of essential enzymes during germination and counteraction of lipid peroxidation and minimization of free radical reactions (Khanahmadi *et al*., 2010).

As observed in physiological parameters, NPs treated seeds experienced pronounced effect on the activity of dehydrogenase enzyme and α-amylase activity. Significant variation was observed for dehydrogenase activity in seeds due to seed polymerization with Zn and Fe NPs during ten months of storage period. However, dehydrogenase activity declined gradually with advancement in the storage period irrespective of the treatments. The results revealed that Zn and Fe NPs were found to increase the activity of the dehydrogenase enzyme at all months of the storage compared to their bulk form and control (Fig.1). So also, α-amylase activity differed significantly due to the application of Zn NPs compared to other treatments. Highest α-amylase activity was recorded with Zn NPs at 750 ppm at all months of the storage period (Fig. 2).

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, NPs treated seeds were found to have higher enzymatic activity, showing the positive role of NPs in improving the seed vigour and viability. It is an established fact that, Zn and Fe are the important metal micronutrients that act as cofactors for the most of the 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 enzymes or could be due to repair of damaged vital cell organelles and counteraction of lipid peroxidation and minimization of free radical reactions (Khanahmadi *et al*., 2010) or might be due to their capacity to maintain the cell endowments triggering the germination events as suggested by Bailly (2004).

The effect of Zn NPs could be attributed to excellent proton radical scavenging property as proposed by Sridhar (2012) and inactivation of enzymes by free radical as a reflection of ageing and subsequent alleviation by Senthilkumar (2011). These beneficial effects of nano size powders were also observed by Zheng *et al.* (2005) in aged seeds of spinach on improving the seed vigour. Thus, the present investigation leads to the assumption that the deterioration in the enzymatic system of seed with ageing might have been protected by the NPs by quenching reactive oxygen species.

The present investigation revealed that moisture content differed significantly throughout the storage period due to seed polymer coating (Table 3). The seeds being hygroscopic in nature, exhibited fluctuation in moisture content concomitantly with changes in atmospheric relative humidity and temperature. The moisture content of seeds increased as the storage period advanced. This may be due to gain or loss of moisture from the surrounding environmental conditions as the seeds were stored in the moisture pervious bag (non-woven fabric bag) and metabolic release of water during respiration. The fluctuation of moisture content was significantly higher in uncoated seeds compared to polymer coated seeds. This may be due to the polymer film acts as physical barrier, which is assumed to restrict the movement of water vapour in and out of the polymer coated seeds and hence, reduced the fluctuation of moisture content during the storage. However, moisture content of seeds was not significant among the polymer coated treatments throughout the storage period.

Insect infestation was not observed up to six months of storage. Irrespective of the treatments, infestation was increased with the advancement in storage period. Significantly lowest insect infestation was observed in polymer coated seeds. Whereas, uncoated seeds recorded highest infestation at all months of the storage period (5.33, 11.67 and 29.67 % @ 6, 8 and 10 months, respectively) (Fig. 3). The reason could be that, the incidence of insect depends on the moisture content of seed, atmospheric temperature and relative humidity. The less absorption of moisture by the polymer coated seeds compared to uncoated seeds did not favour the insect multiplication or may be the polymer formed an extra coat on seed surface which prevented the infestation and emergence of the insects (*Callosobruchus chinensis*).

Conclusion

Our investigations undoubtedly demonstrated that the seed polymer coating is the best and promising technology for applying active ingredients to the seeds, which improves the adherence and retention of applied active ingredients. Hence, improves the seed quality and storability considerably over the uncoated seeds. Zn NPs at 750 ppm or Fe NPs at 500 ppm found to be superior in enhancing storability of pigeonpea seeds under ambient condition. Since, the present work represents the preliminary study and first work of its kind in pigeonpea. Hence, further studies are required to confirm the results obtained in the present investigation.

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