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Bioefficacy of Fe_2O_3 quantum dots on enhancing seed germination and seedling vigour in black gram (*Vigna mungo*)

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Abstract
Quantum dots are zero dimensional nanostructures generally in the range off 1-10 nm. In this study, iron oxide (Fe_2O_3) QDs were synthesized, characterized and used for seed treatment on blackgram (VBN 8). Blackgram seed were treated wide spectrum of concentration ranging from 0-1000 ppm with iron oxide (Fe_2O_3) QDs. Iron oxide QDs at a concentration of 500 ppm with 2 hours treatment duration shows an improvement in Physiological parameters like root length, shoot length, germination %, seedling emergence, Vigour Index-I and dry matter production. However, Fe_2O_3 QDs, at higher concentrations of (1000 ppm) found to reduce the physiological activity of the seeds.

Keywords: Fe_2O_3, enhancing, germination, black gram, *Vigna mungo*

Introduction
Pulses are globally recognized as a staple dietary protein sources. Due to their wide climatic adaptation, drought & pest resistance and shorter duration enables pulses as universal potential nutritious food. It complements the diets with proteins, vitamins, minerals and essential amino acids. Bengal gram (*Cicer arietinum*), Lentil (*Lens culinaris*), Black gram (*Vigna mungo*), Pigeon pea or red gram (*Cajanus cajan*), Green gram (*Vigna radiata*), Moth bean (*Vigna aconitifolia*), Horse gram (*Dolichos uniflorus*), Lablab bean (*Lablab purpureus*), Pea (*Pisum sativum* var. *arvense*), Cowpea (*Vigna unguiculata*). (Banerjee, Mukherjee *et al*. 2021) [3] are accounted as a major pulses cultivated in India. Black gram (*Vigna mungo*) is known as urad bean. In India the blackgram is one of the important pulses grown in both Kharif and Rabi seasons. The nutrition composition of *Vigna mungo* per 100 g is of as follows; protein 25 g, potassium 983 g, calcium 138 g, iron 7.57 g, niacin 1.447 g, thiamine 0.27 g and riboflavin 0.25 g. Blackgram also contains high amount of folate (628 µg/100 g raw, 216 µg/100 g cooked). It is predominantly cultivated in South Asia, southern part of India, northern part of Bangladesh and Nepal.

Quality seeds are the most important assets in agriculture. Quality seeds are critical hence it ensures global food security by uniform germination, crop establishment and better yield. Yield of crops rely on multiple factors, but foremost critical factor is seed quality Currently, there are number of treatment methods available to enhance the germination parameters which includes seed coating, seed pelleting, seed priming, seed enrichment, seed infusion etc., (Korishettar, Vasudevan *et al*. 2016) [8].

Seed quality deterioration during long term storage is inevitable and has a negative impact on seed to lose viability due to spontaneous biochemical damage occurring at the cellular level, resulting in natural seed ageing, reduction in seed germination and ultimately leads to declination in crop productivity. Seed germination is reduced due to bio-molecular changes associated with accumulation of reactive oxygen species (ROS) and decline of cellular antioxidant capacity. Since, ancient times, seed priming has been used in several crops to improve germination, yield, quality seed production and stress management (Chen, Arora *et al*. 2013) [4].

Nanotechnological intervention in seed treatment was accomplished in agriculture by the use of engineered Nano particles (<100 nm) in seed treatment (Raja *et al*., 2020) [16], surface coating of seeds with nanoemulsions (Tamilarasan *et al*. 2019) [18], seed priming with metal oxide quantum dots (<10 nm) (Ashoknarayanan *et al*. 2021) [2], surface treatment of seeds using nanofibre (Mukiri *et al*. 2021) [12] etc., to improve the seed quality parameters in wide

spectrum of crops. Ultra smaller size, an increase in surface to volume ratio and quantum confinement property was observed with nanoparticles. The unique characteristics of nanoparticles, such as their size, shape, and dose, have an unusual direct or indirect effect on seed quality parameters such as germination percentage, seedling length, seedling dry weight and seed vigour. Seed priming treatment that can induce physiological changes in the seed that will help it germinate quickly. Nanotechnological intervention in seed treatment may improve the water uptake in the treated seeds and hence increase the hydrolysis of enzymes and triggers the germination process instantaneously. This treatments ensures reliable results even under difficult environmental condition such sodic soil, hill agriculture, drought condition etc., (Jisha, Vijayakumari *et al*. 2013) [6]; (Paparella, Araújo *et al*. 2015) [15]. Even though many results published in this area still the influence of OD nanomaterials on agricultural crops are found to be less reported. Hence, the efforts were made to understand the influence of iron oxide QD on black gram.

Materials and Methods

Materials

Bioefficacy study of Fe_2O_3 quantum dots on blackgram carried out in the Department of Nano Science and Technology in association with the Department of Seed Science and Technology, Tamil Nadu Agricultural University, Coimbatore. Both the physically and genetically pure seed variety for Blackgram (*Vigna mungo*) cv. VBN 8 was acquired from the Department of Pulses, Tamil Nadu Agricultural University, Coimbatore. Reagents such as Iron chloride (Cas no: 7705-08-0), Dodecanol (Cas no: 112-53-8), Oleic acid (Cas no: 1173097-41-0) used in the study were bought from Sigma-Aldrich in Bangalore, India and used for the study without any further processing.

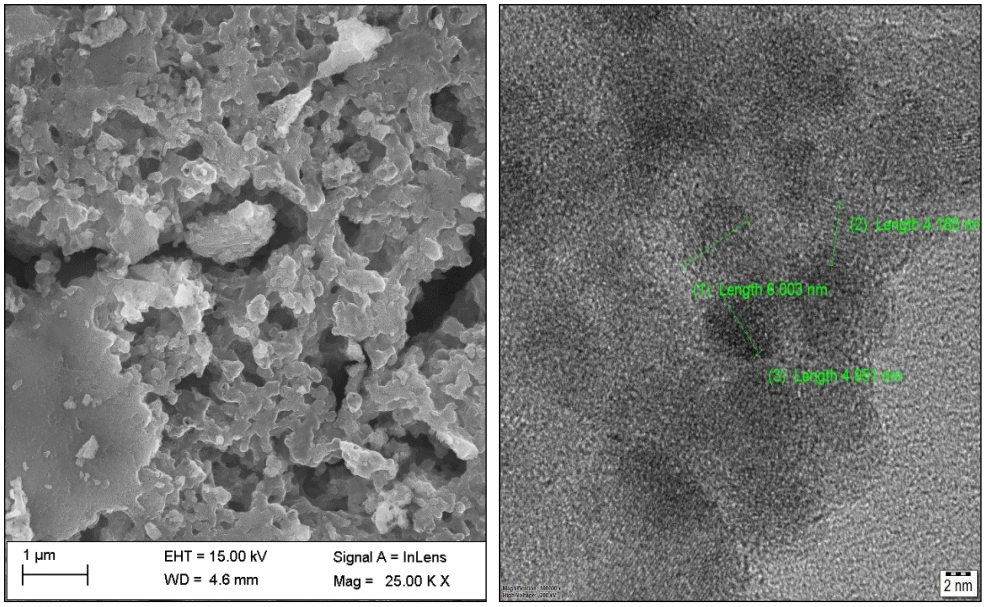
Methods

Synthesis of Fe_2O_3 quantum dots

Iron chloride anhydrous (0.162 g) was dissolved in oleic acid (1 mL) in a teflon coated autoclave (100 ml) under continuous stirring. Then, 40 mL of chemically pure dodecanol was added while continuous stirring. After that, the Teflon-lined autoclave was converted into a microwave autoclave reactor. In 30 minutes, the temperature was raised to 180 ^oC, after which it was maintained for 30 minutes. The resulting materials were centrifuged after cooling and then washed with acetone and 100% ethanol after which dried in vacuum at 60 ^oC for 8 hours.

Characterization of Fe_2O_3 quantum dots

Synthesized Fe_2O_3 quantum dots were characterized using SEM and TEM to determine their surface topography internal structures, shown in the figure 1.



SEM Image **TEM Image**

Fig 1: Synthesized Fe_2O_3 quantum dots were characterized using SEM and TEM to determine their surface topography internal structures

Seed priming with Fe_2O_3 quantum dots

Blackgram seeds were taken and washed with deionised water and dried. The seeds were sterilized with sodium hypochlorite (NaClO) solution and rinsed with deionised water. The conical flasks were labeled according to the prescribed treatments namely; T0-Absolute control, T1-Hydro priming, T2-0 ppm (treated with $D.H_2O$ and sonicated), T3-20 ppm, T4-40 ppm, T5-60 ppm, T6-80 ppm, T7-100 ppm, T8-150 ppm, T9-200 ppm, T10-300 ppm, T11-400 ppm, T12-500 ppm, T13-1000 ppm, of Fe_2O_3 QDs respectively. 100 ml of distilled water added respectively to the respective flasks. Appropriate volumes of quantum dots were introduced inside the appropriate prelabelled conical flask. Quantum dots were uniformly dispersed in the distilled water solution using bath Ultrasonicator [Citizon] for 10 minutes at the temperature of 32 °C. The disinfected seed were added to QD dispersed conical flasks and subjected to priming using orbital shaker incubator [Scigenics, Orbitek L-Mini] at 37 °C with 150rpm for 120 minutes durations, respectively. QD primed seeds were recovered after priming and shade dried overnight and stored for further use.

Germination percentage

According to ISTA (2015), the germination test was conducted using four replications of 100 seeds in a germination chamber maintained at a temperature of 25 ± 2

ºC and a relative humidity of 95 ± 2 per cent. The roll towel method for blackgram was used. At the end of the final count day, Blackgram (7 days) seedlings were evaluated; the seedlings were categorized by ISTA as normal seedlings, aberrant seedlings, hard seeds and dead seeds. After counting the number of healthy seedlings, the mean germination rate was determined and expressed as a percentage.

$$\text{Germination (\%)} = \frac{\text{Number of normal seedlings}}{\text{Total number of seeds sown}} \times 100$$

Root length (cm)

During the final count, ten healthy seedlings from each replication of the various treatments were randomly selected, and the root length-the distance from the primary root's collar to its tip-was measured. Ten healthy seedlings that were measured and expressed in centimeters were used to obtain the mean values.

Shoot length (cm)

The same ten normal, randomly selected seedlings that were used to measure the root length were also used to measure the shoot length, which is the distance between the collar area and the tips of the primary leaves. Ten healthy seedlings that were measured and expressed in centimeters were used to obtain the mean values.

Dry matter production (gram 10 seedlings^{-1})

After cotyledon and seed coat were removed, the ten normal seedlings that were chosen for seedling measures were folded and put inside a paper cover, dried in the shade for 24 hours, then dried at 80 ± 2 °C in a hot air oven [ESCO, OFA-32-8] for another 4 hours, and then cooled in a desiccators for 30 minutes. The dried seedlings were weighed using an electronic weighing balance and the average values were given in grams per 10 seedlings^{-1}.

Vigor index

Vigour index was calculated as per Abdul Baki and Anderson (1973) [1] formula. This method involves multiplying the germination percentage by the seedling length, which is the total of the root and shoot lengths, Vigour index is expressed as whole integers.

Vigor index = Germination (%) x Seedling length (cm)

Statistical analysis

The resultant data from the experiment were analyzed statistically using the techniques demonstrated by (Panse and Sukhatme, 1995) [14]. The experimental data was analyzed using (ANOVA) as a factorial combination and the experiment was designed in Completely Randomized Block Design. The critical differences (CD) were calculated at 5% probability level.

Results and Discussion

Nano seed priming with different concentrations of iron oxide (Fe_2O_3) QDs exhibited variations in germination percentage and growth parameters in black gram. The results showcased that nano priming of Fe_2O_3 QDs at 500 ppm as seed treatment significantly enhanced germination 93% which was 17% and 14% increase over untreated control and QD priming, respectively. The improvement on germination in nano priming seeds is due to creation of new pores on seed coat by the QDs which helps to increase the rate of water imbibition (Khodakovskaya, de Silva *et al*. 2011) [7]. It is also to note that the size of nano particles makes it easy for penetrating the seed surface which in turn enhances the absorption and utility by seeds. This also paves way for triggering ROS generation and starch degrading enzyme activity which accelerates the seed germination (Mahakham, Sarmah *et al*. 2017) [11]. Raja *et al*. (2020) [16] found that cu and ZnO nano particles seed priming enhanced the seed germination in black gram. The reduction in seed germination may be due to accumulation of nano particles within the cells which might reduce the rate of cell division, cell elongation (Lee and Kim 2000) [9].

The highest seedling length was recorded in seeds treated with Fe_2O_3 QDs at 500 ppm showcasing 42.9 cm. The Fe_2O_3 QDs at 400 ppm and 300 ppm were found to be on par in registering seedling length of 41.7 cm and 41.6 cm respectively. This is due to enhancement in synthesis of hydrolytic enzymes during early stages of growth which helps to utilize the food stores of seeds effectively. The quantum dots at higher concentrations may lead to arrest the cell elongation and division which affects the total growth of seedling. (Lin and Xing 2007) [10] Found that higher concentrations of quantum dots reduced the seedling biomass, induces the root tip shrinkage, epidermis collapse which ultimately reduced the seedling growth.

The treatment of Fe_2O_3 at 500 ppm for 120 minutes enhanced the seed vigor index up to 3990 which was followed by Fe_2O_3 at 400 ppm which showed vigor index of 3929. It is attributed to higher per cent and seedling growth increase is in seedling vigour. As the nano QD enters the permeable membrane which increases the IAA concentration and emergence rate which paves way to improve the vigor of seeds (Van Dongen, Ammerlaan *et al*. 2003) [19] (Table 1)

The shoot length and root length of black gram was found to be increased in treatment with 500 ppm for 120 minutes. The highest shoot length recorded was 25.4 cm followed by treatment with 400 ppm 24.9 cm. The lowest shoot length was recorded in treatment with 1000 ppm with 23.4 cm. The highest root length was recorded in 500 ppm concentration for 120 minutes showcasing 17.5 cm. The treatments 400 ppm and 300 ppm was found to be on par with each other. The lowest root length was recorded in 20 ppm with root length of 16.1 cm (Table 1).

The dry matter production was also found to vary within the seeds treated with different concentrations of QD. The highest dry matter was found in 500ppm concentration (0.46 g) while the lowest value of 0.3g was observed in control. This is because the quantum dots increased the level of IAA which in turn increase the growth rate of seedlings (Pandey, S. Sanjay *et al*. 2010) [13] The results showed that treatments 400 ppm and 300 ppm were found to be on par with each other. (Table 1)

Table 1: Effect of Fe_2O_3 quantum dots priming on seed quality in black gram

Priming Treatments	Seed Quality Parameters					
	Germination Percentage (%)	Root Length (cm)	Shoot Length (cm)	Seedling Length (cm)	Dry Matter Production (g/10seedlings)	Vigor Index
Control	76 (60.66)	14.9	20.3	35.2	0.32	2676
Hydropriming	79 (62.72)	15.7	20.5	36.2	0.33	2859
Hydropriming + sonication	79 (62.72)	16.1	23.7	39.8	0.34	3144
20 ppm	80 (63.43)	16.1	23.8	39.8	0.35	3190
40 ppm	83 (65.65)	16.3	23.8	40.1	0.36	3329
60 ppm	84 (66.42)	16.4	23.9	40.3	0.37	3384
80 ppm	85 (67.21)	16.5	24.3	40.9	0.38	3468
100 ppm	87 (68.86)	16.6	24.4	41.0	0.39	3566
150 ppm	88 (69.73)	16.6	24.5	41.1	0.40	3616
200 ppm	89 (70.63)	16.7	24.7	41.4	0.42	3685
300 ppm	91 (72.54)	16.8	24.8	41.6	0.43	3784
400 ppm	92 (73.57)	16.9	24.9	41.7	0.44	3929
500 ppm	93 (74.66)	17.5	25.4	42.9	0.46	3990
1000 ppm	83 (65.65)	17.1	23.4	40.5	0.43	3361
Mean	85 (67.21)	16.4	23.7	40.2	0.39	3427
S.Ed	2.0969	0.4413	0.5819	0.9659	0.0088	92.055
CD (0.05)	4.2953	0.9040	1.1919	1.9786	0.0181	188.572

Conclusion

QDs are capable of entering the seeds through pores present on the seed coat during water imbibition, induce water uptake ability of seeds, and enhance enzymatic activity. The present study demonstrates that application of Fe_2O_3 Quantum dots to improve Black gram seed quality. At the concentration of 500 ppm, Fe_2O_3 Quantum dots improve the seed germination, seedling length, seedling dry weight and seedling vigour. When the concentration of QD increases beyond 500 ppm, it shows antagonistic effect on the seed parameters.

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