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TIO_2 Nano particles on extending seed vigour and viability of naturally aged maize (*Zea mays* L.) Seeds

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Abstract
An investigation was carried out to now the effect of TiO_2 nano particles on extending seed viour and viability in naturally aged maize seeds. Aged seeds were dry dressed with (200 -, 800 mg kg $^{-1}$) titanium oxide nano particles and observed for seed germination and seedling vigour in terms of shoot and root length, dry matter production, electrical conductivity, dehydrogenase and peroxidase activity against untreated seeds. An significant increase was observed in all estimated parameters at lowest concentration(200 mg kg $^{-1}$) of titanium oxide (88%, 11.91cm, 19.45cm, 1.340g, 0.278 dSm^{-1} 0.037 OD, 0.044 OD 10 min $^{-1}$ respectively) for seed germination, shoot and root length, dry matter production, EC, DH, POD and the least values were recorded in untreated seeds (70%, 10.78 cm, 18.78cm 1.009 g, 0.361 dSm^{-1}, 0.010 OD and 0.013 OD 10 min^{-1} Respectively). It could be concluded that the positive effects are related with nano size and concentration of the TiO_2 nano particle and can be recommended for pre storage seed treatments.

Keywords: Maize, Titanium oxide, Nano particles, Aged seeds, Vigour

Introduction
Maize is the third largest cereal crop in the world next to wheat and rice grown almost in all the continents of tropics, sub-tropics and temperate regions. In India, maize occupies an area of 6.1 million hectares with an annual production of 10.5 million tonnes. In Tamil Nadu, it is cultivated in an area of 1.20 lakh ha with an estimated annual production of 1.97 lakh tonnes with productivity of 1680 kg/ha. It is used directly as food for human consumption as well as for poultry feed and also as raw material for number of industrial products. In recent years, new seed technologies have been developed that alter the nutrient composition of maize to improve grain quality for human consumption and livestock feed in this situation longevity of stored seed is affected by a number of environmental factors such as moisture content (mc), temperature and oxygen concentration in storage environment (Roberts 1972) [15]. Among the various factors responsible for seed deterioration, free radicals induced lipid peroxidation (Dadlani and Agarwal, 1983; Ramamoorthy and Basu, 1997) [3, 14] and loss of membrane integrity (Powell, 1981; Ramamoorthy and Basu, 1997) [12, 14] are the major causes for seed aging resulted in poor performance of seeds and seedlings finally dead. Several methods have been demonstrated for controlling seed deterioration suchas; hydration and dehydration (Rundrapal and Basu, 1982; Rundrapal and Nakamura, 1988) [17, 16]; halogenation (Mandal and Basu, 1986) [10], dry permeation (Anderson, 1973) [1], solvent infusion (Khan *et al.*, 1973) [7] *etc*., However, each treatments have its own limitation.
Owing to the smallest size and lager surface area (Gurunathan *et al.*, 2009), metal nanoparticles have an option to efficiently catalyse the chemical reactions especially in deteriorated seeds (Zheng *et al.*, 2008) [19]. Reports on the use of nano particles to overcome seed deterioration are very scanty. Hence studies were undertaken to know the effects of TiO_2 nano particles on extending seed vigour and viability of naturally aged maize seeds.

Materials and Methods
Seed material
Naturally aged breeder seed samples of COH (M) 5 maize hybrid with 70 % germination and 10 % moisture were obtained from Agricultural Research Station, Bhavanisagar used as base material for this investigation. The laboratory studies were carried out in the Department of Seed Science and Technology, Tamil Nadu Agricultural University, Coimbatore, India during 2011-2012.

Preparation of materials

TiO_2 nanoparticles (mixture of rutile and anatase, size\100 nm) were purchased from Sigma-Aldrich. Low vigour seed lot of maize seeds were dry dressed with TiO_2 nanoparticles @ 200, 400, 600 and 800 mg kg^{-1} in screw capped glass bottles at room temperature. The glass bottles containing seed and nanoparticles were shaken gently for 3 min., 5 times at an interval of 3h for a week. Seeds shaken without nanoparticles served as control.

Seed Germination and Growth

The seeds treated as above were subjected to germination test with four replicates of 100 seeds in paper towels. The test conditions were 25 ± 2°C and 95 ± 5 per cent RH, illuminated with fluorescent light of 750 – 1250 lux. After 7 days the seed quality parameters were observed as prescribed by ISTA 2009 [5].

Biochemical analysis

The aged treated seeds were observed for the biochemical attributes *viz.*, peroxidase (POD) activity (explained by Malik and Singh, 1980) [9], Dehydrogenase (DH) activity (explained by Kittock and Law, 1968) [8] and Electrical conductivity (explained by Presley, 1958) [13].

Statistical analysis

The experimental design was Completely Randomized Design (CRD) with four replicates of 25 seeds each. An analysis of variance (ANOVA) was made as suggested by Panse and Sukhatme (1999). Correlation was assessed by using Microsoft Excel software. Significance of mean difference of the variable means was separated using LSD at P = 0.05.

Results and Discussion

Results revealed that Nano – TiO_2 significantly reverse the seed deterioration process.

Among all those concentrations tried, aged seed dry dressed with Nano – TiO_2 at the lowest concentration (200 mg kg^{-1}) performed significantly and values recorded were for germination (88%), shoot length (11.91cm), root length (20.52cm) and dry matter production (1.340g) and the lowest values recorded in untreated control seeds (70%, 10.78 cm, 18.78cm 1.009 g) respectively (Fig1a. and 1b.).

Many biochemical investigations have proven that cellular membrane degradation by free radicals induced lipid peroxidation is the major cause for seed deterioration. As seed quality declined there was a concurrent increase in the levels of EC and concurrent decrease in DH and POD activities. In this investigation, Nano – TiO_2 @ 200 mg kg^{-1} treated seeds were, very effective in maintaining membrane integrity as well as lipid peroxidation as evidenced by lower EC (0.278 dSm^{-1}) and higher enzymatic activities of DH (0.037 OD) and POD (0.044 OD 10 min^{-1}). The higher EC (0.361 dSm^{-1}) and lower enzymatic activities were observed in untreated seeds (0.010 OD and 0.013 OD 10 min^{-1} respectively for DH and POD activity) (Fig2.).

Correlation studies also revealed that germination percent was significantly correlated with shoot length (0.959 **), root length (0.945**), dry matter production (0.956**), dehydrogenase activity (0.962**), peroxidase activity (0.942**) and negatively correlated with electrical conductivity (-0.956**) (Table1). The Results were in agreement with Zheng *et al.*, 2008 [19] in spinach; Castiglione *et al*., 2011; in *Vicia narbonensis and Zea mays*, they reported that Utilization of nano particles with proper concentration increased the vigour and viability of naturally aged seeds. The positive effect of nano TiO_2 on low vigour maize seed is may be attributed with metal nanoparticles are more efficient in scavenging superoxides (O_2^-) and the scavenging activity of metal nanoparticles are similar to free radical scavenging enzymes like superoxide dismutase (SOD) and catalase (CAT). In addition, metal nanoparticles with lowest concentration may reduce the accumulation of lipofuscin which is an endogenous autofluorescent marker generated by oxidative degeneration of cellular components that is accumulated with ageing (Kajitha *et al*., 2007; Watanabe *et al*., 2009) [6, 18].

It could be concluded that maize seeds dry dressed with TiO_2 nano particles @ 200 mg kg^{-1} can be recommended for pre storage or mid storage seed treatment to improving seed vigour and viability in maize seeds, but the mechanism by which the nano particles improves the vigour and viability needs still further study.

Table 1: Correlations between various vigor parameters of maize seedlings

	Germination %	**Shoot length (cm)**	**Root length (cm)**	**Dry matter production (mg)**	**Dehydrogenase (OD)**	**Peroxidase (OD10 min^{-1})**	**Electrical conductivity (dSm^{-1})**
Germination %	1						
Shoot length (cm)	0.959**	1.000					
Root length (cm)	0.945**	0.996**	1.000				
Dry matter production (mg)	0.956**	1.000**	0.996**	1.000			
Dehydrogenase (OD)	0.962**	0.997**	0.992**	0.996**	1.000		
Peroxidase (OD10 min^{-1})	0.942**	0.992**	0.995**	0.992**	0.990**	1.000	
Electrical conductivity (dSm^{-1})	-0.956**	-0.999**	-0.997**	-0.999**	-0.996**	-0.996**	1.000

*Significant at 5% level; **Significant at 1% level

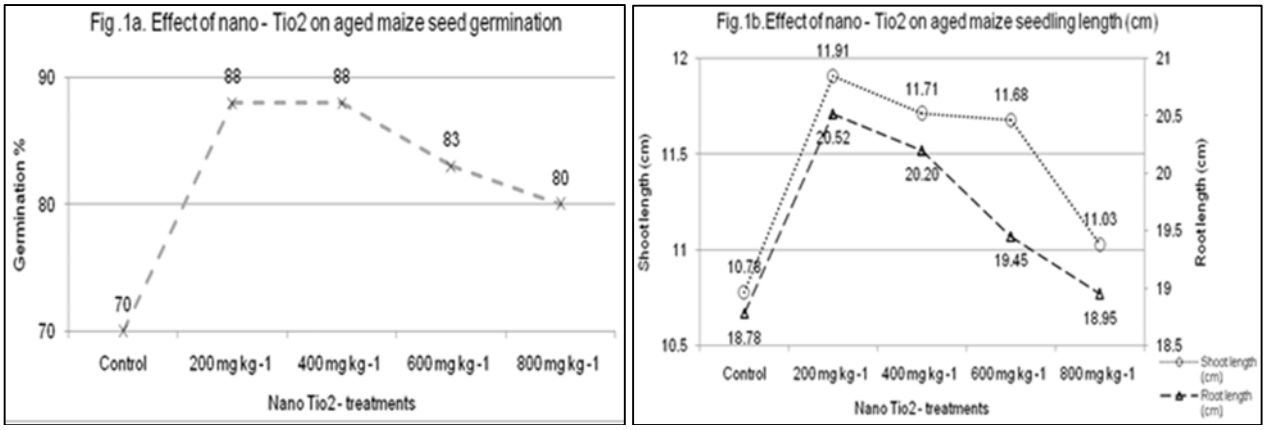

Fig 1: Effect of nano - TiO_2 on seed germination (%) and seedling length (cm) of naturally aged maize seeds

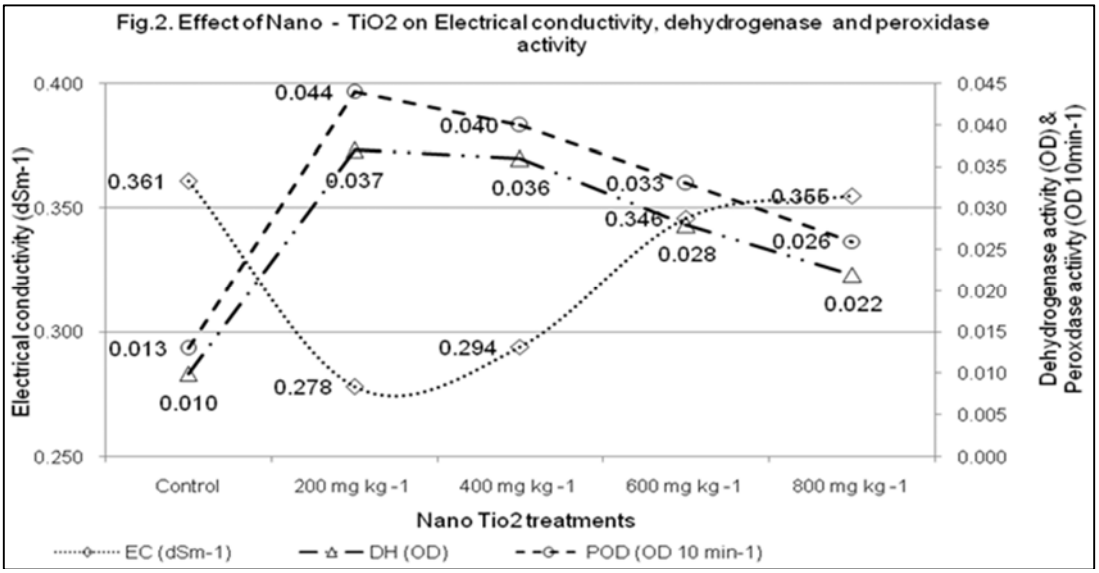

Fig 2: Effect of nano-TiO_2 on Electrical conductivity (EC), Dehydrogenase activity (DH) and Peroxidase activity (POD) of naturally aged maize seeds

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