

Near Nano Size Powders of Chilli, Fenugreek, and Turmeric Improve Seed Physiological Performances in Oat (*Avena sativa*)

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(Received: September 2020, Revised: October 2020, Accepted: December 2020)

ABSTRACT: Various botanicals have been used traditionally for several purposes in agriculture including seed treatment for lengthening seed longevity while maintaining its planting quality. With recent advancements of nanotechnology in agriculture, use of input materials in nanoform has gained attention significantly. This experiment aimed to assess the influence of nano and near nano size powders of chilli, fenugreek and turmeric in three doses, viz. 1 g, 2 g, 3 g of botanical powder per kg of seed, on seed physiological performances in oat. In general, the botanical powders improved the seed germination, shoot and root length, seedling dry-weight, and seed vigour indices as compared to control. The botanicals also reduced the seed pathogen infection and improved seedling emergence and grain yield when treated seeds were planted in the field. However, the responses of seed to the botanical treatments were dose-dependent and varied across the botanicals. Results of this experiment reaffirms the traditional knowledge of beneficial effects of botanicals on plant seed performances and shows the importance of using them in nano and near nano forms.

Keywords: Nanoparticle, Botanicals, Seed treatment, Seed quality, Germination, Seed pathogen, Seed yield

Diverse use of crude botanicals, either in dry or liquid form, has been mentioned as traditional remedy to different animal and plant ailments since long back across the nations [1, 2]. Dry powder of herbs or botanicals have been traditionally used for seed treatment in order to improve their storability in a number of crops [3]. Although, antimicrobial activities of these powders have been claimed as the major mechanisms for improving seed storability [4], several reports claim that seed treatment with different herbs can enhance the seed quality traits such as germination, vigour, and field performances [5, 6]. Dry treatment with fine powders of different chemicals or pharmaceuticals such as aspirin, iodine, bleaching powder, etc. has been reported to influence seed germination traits by several researchers [7–9].

With the recent advancement of nanotechnological interventions in agricultural sectors, benefit of nanotechnology is being explored in different aspects of crop production, starting from nanofertilizers to

nanoformulations of pesticides to diseases detection with nanosensors [10]. Nanoparticle literally ranges from 1 to 100 nm and due to this small size, it acquires a wide array of advanced physical, chemical and electrical properties as compared to their bulk materials leading to some unprecedented advantages [11]. Different studies showed both positive and negative effects of nanoparticles on plants, animals and microbes [12–15]. Several promising results have been reported about enhancement of seed performances and yield in different crops due to nanoparticle application and use of agricultural inputs in nanoform [16].

Of late, the use of nanoparticles (NPs) for modulating seed germination and growth traits in various crops has received serious attention [13, 17–19]. It is evident that the base element of nanoparticle, concentration of NPs, and choice of crop play an important role in deciding their effects- whether positive or negative [14, 15, 17, 20]. Although, some putative mechanisms such as activation

of antioxidant system, photochemical reaction of chloroplasts, chloroplast aging, nitrogen photoreduction and photosynthetic carbon reaction have been suggested regarding how the NPs influence seed germination and plant growth traits [15, 21, 22], the exact mechanisms of actions are not well understood.

With the promising results of NPs on seeds, the use of micro or nano size powder of botanical origins is parallelly receiving substantial attention from the agricultural researchers for improvising crop production [6]. Though there are several literatures available on the effect of metallic or other engineered NPs on various seed quality parameters in a number of crops, information on the effects of micro or nano size powders of botanicals are scanty. The current study aimed to assess the role of nano and near nano size powders of botanicals (chilli, fenugreek, and turmeric) on the seed quality parameters as well as on the occurrence of fungal infection in oat seeds.

MATERIALS AND METHODS

Seed material: Nine months stored seeds of oat (cultivar JHO-822) were obtained from the Division of Seed Technology, Indian Grassland and Fodder Research Institute, Jhansi, Uttar Pradesh. Preliminary germination test was conducted to confirm the seed quality. Seeds were examined under scanning electron microscope (SEM) for any cracks developed during ambient storage at room temperature (Figure 1). Small cracks or openings on seed surfaces were assumed to act as the entry point of NPs into seeds [13].

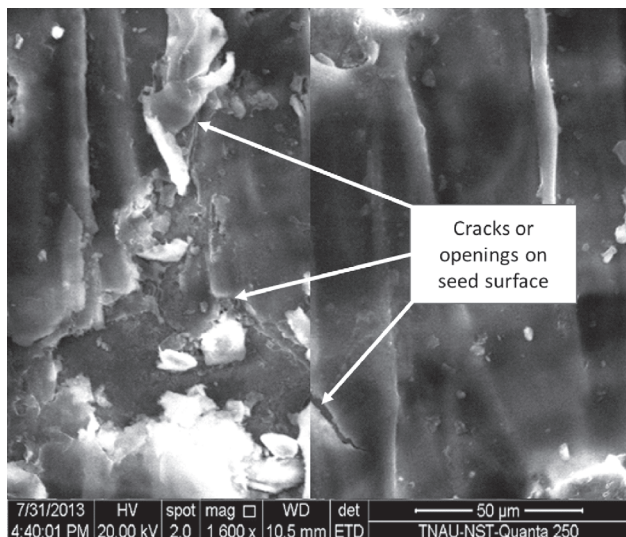


Figure 1. Oat seed surface under scanning electron microscope (SEM)

Nano and near nano size powder of botanicals:

Commercially available powders of chilli (*Capsicum annum*), fenugreek (*Trigonella foenum-graecum*) and turmeric (*Curcuma longa*) were procured from local market. Powder samples of 0.5 g were dispersed in 10 ml pure water and ultrasonicated for 5 minutes at 1500 rpm. The size was confirmed as the mixture of nano and near nano size particles by particle size analyzer technique (MALVERN, Zetasizer Ver.6.01 particle size analyzer) at the Department of Nanoscience and Technology, Tamil Nadu Agriculture University, Coimbatore, India. Since a large fraction of the powders were not dispersed in nanoform during dry ultrasonication (as a step during particle size estimation), they were considered as nano and near nano size powders to rule out any confusion about the particle size.

Seed treatment: The oat seeds were treated with dry powders of the three botanicals with the help of an electric shaker for 10 minutes at 500 rpm. Three different doses viz. 1g (D₁), 2g (D₂) and 3g (D₃) of each of the botanical powders were used for seed treatment, and all the results was compared with a control (D₀, no powder). The powders were ultrasonicated prior to treating the seeds so that they were in sufficiently dispersed condition at the time of seed treatment.

Germination percentage and shoot-root length and weight:

Three replicates of 100-seed each per treatment were placed on moist filter paper in 11 cm diameter petri dishes. Data were recorded after 7 days to calculate the germination (%). Shoot and root lengths and dry-weight were measured at the end of germination test (7 days).

Seedling vigour index: Shoot and root lengths were recorded for the calculation of Seed vigour index (SVI) in each treatment and the SVI was calculated as per the formula given below [23, 24].

Seedling vigour index-I = (average Seedling length) x % seed germination

Seedling vigour index-II = Seed ling dry Weight x % seed germination

Blotter test: Fifty seeds in each of three replications were placed on moistened filter paper in petri dishes for Blotter Test at 20±2°C in 12/12 h alternating light/dark cycles under UV light and darkness for 7 days to detect any visible pathogen infection. The result is reported on percent basis.

Field experiment: Treated seeds along with untreated control were stored for 5 months in room condition before sowing in October. Crop was raised with standard

agronomic package of practices at the research farm of Indian Grassland and Fodder Research Institute following a randomized block design with four replications. Seedling emergence rate was recorded in 2 m row in every replication. On maturity, seed yield (q/ha) was assessed on plot basis across the treatments.

Statistical analyses: Analysis of Variance (ANOVA) was conducted using JMP PRO 14 (SAS Institute Inc., Cary, NC). Prior to conducting ANOVA, all the data were checked for the normality by seeing the normal distribution curve visually, which was further confirmed by the Shapiro-Wilk W test using the JMP PRO 14. Means of the treatments were separated based on the Tukey's Honestly Significant Difference test with $\alpha=0.05$.

RESULTS AND DISCUSSION

Germination percentage

Nano and near nano size powders of all three botanicals- chilli, fenugreek and turmeric- improved germination (%) significantly over the control in oat (Table 1, Col I). The highest germination (100%) was observed with fenugreek powder at D₁ and it reduced subsequently with higher doses leading to the lowest germination (92.3%) at D₃. Among the three doses of chilli powder, D₁ (1g) showed highest germination improvement; however, it was at par with the other two doses. In case of turmeric powder, D₃ (3g) showed highest germination improvement over control, which was at par with D₂. So, in case of chilli and fenugreek, lower concentration of powders showed better performance in terms of germination (%).

Different nano and near nano size materials have been reported to influence seed germination in various species. Maity *et al.* [17] observed that four NPs viz. ZnO, TiO₂, CuO and Ag improved germination percentage significantly as compared to control in oat and berseem. Raskar and Laware [25] reported a decrease in onion (*Allium cepa* L.) germination with increase in the

concentration of nZnO above 20 μ g ml⁻¹. Likewise, Srinivasan [18] observed a reduction in cowpea germination with increase in the concentration of nCuO above 25 μ g ml⁻¹. The increase in germination with botanical treatments in the present study is in conformity with the findings of Albert [26] in tomato, Vijayan [27] in rice, Layek *et al.* [28] in bengal gram, Roopa [29] in muskmelon and Renugadevi [30] in clusterbean. Plant products are known to contain various antioxidants that would quench free reactive oxygen species (ROS) during seed ageing. ROS deteriorate the biochemical components of coat and other inner parts of the seed and a loss in such components leads to death of seeds [13]. The antioxidants present in the plant products play a major role in improving the performance of the seeds by reducing the ROS [31].

Shoot and root length

In Oat, the highest shoot length (6.8cm) was observed at D₃ of turmeric (Table 1, Col II). D₂ of turmeric, D₁, D₂ and D₃ of chilli and D₁ of fenugreek were statistically at par in terms of improvement of shoot length. D₃ of fenugreek showed no improvement of shoot length as compared to control. The chilli, fenugreek and turmeric powders didn't show a much variation in promotion of root length in oat in comparison to control (Table 2, Col I). Except D₁ of Chilli, all the doses of powders promoted root growth in oat. The highest root length was observed with D₃ of turmeric, which was at par with D₃ of chilli and D₂ of turmeric itself. The powder of plants parts is the potential source of natural antioxidants [32], which slowed down the deterioration of seeds and resulted in increased seedling growth.

Seedling dry weight

All the three doses of chilli powder were statistically at par with the control (Table 2, Col II). Interestingly, the higher doses of chilli powder treatments reduced the

Table 1. Effects of nano and near nano size powders of chilli, fenugreek and turmeric on seed germination (%) and shoot length (cm) of oat

Dose	Germination (%) (Col I)			Shoot Length (cm) (Col II)		
	Chilli	Fenugreek	Turmeric	Chilli	Fenugreek	Turmeric
0g (Control)	89.3 ^b	89.3 ^b	89.3 ^b	5.5 ^b	5.5 ^b	5.5 ^b
1 g	95.7 ^a	100.0 ^a	93.7 ^{ab}	6.2 ^a	6.3 ^a	6.0 ^b
2 g	94.3 ^a	96.3 ^{ab}	94.3 ^a	6.3 ^a	5.9 ^{ab}	6.6 ^a
3 g	94.7 ^a	92.3 ^{bc}	94.7 ^a	6.6 ^a	5.5 ^b	6.8 ^a

Different letters as superscripts within a column indicate significant differences ($p<0.05$) among doses of a particular botanical.

Table 2. Effects of nano and near nano size powders of chilli, fenugreek and turmeric on root length (cm) and seedling dry-weight (mg) of oat

Dose	Root length (cm) (Col I)			Seedling dry-weight (mg) (Col II)		
	Chilli	Fenugreek	Turmeric	Chilli	Fenugreek	Turmeric
0 g (Control)	21.8 ^b	21.8 ^c	21.8 ^c	3.46 ^a	3.46 ^a	3.46 ^b
1 g	22.0 ^b	22.5 ^{bc}	23.5 ^b	3.54 ^a	3.44 ^a	3.69 ^a
2 g	23.0 ^{ab}	23.3 ^{ab}	24.4 ^a	3.44 ^a	3.31 ^a	3.17 ^c
3 g	23.9 ^a	23.6 ^b	24.9 ^a	3.31 ^a	2.93 ^b	2.91 ^d

Different letters as superscripts within a column indicate significant differences ($p < 0.05$) among doses of a particular botanical.

seedling dry weight except at D_1 (1g). The similar trend was observed for fenugreek powder at D_3 that lowered the seedling dry weight significantly compared to other treatments and control. Whereas, the D_1 of turmeric powder improved the seedling dry weight significantly over control, but in higher doses it also reduced the seedling dry weight. The results are in conformity with Jegathambal [33] in sorghum and Kavitha [34] in blackgram. Sathish and Bhaskaran [35] reported that blackgram seeds treated with 3 g/kg of fenugreek seed powder with 1 h shaking showed an increased physiological performance in terms of dry matter production and vigour index.

Seed Vigour Index I (SVI) and II (SVII)

In oat, the turmeric and chilli powders at D_3 showed significant improvement in Seed Vigour Index I (SV-I) over control, which was at par with D_2 of turmeric and D_3 of chilli (Table 3, Col I). D_1 (1g) of all the three treatments were statistically nonsignificant among them in terms of SV-I improvement. The highest SV-I was observed with D_3 of turmeric powder and lowest was observed with D_1 of chilli powder. In case of Seed Vigour Index -II (SV-II), all the treatments with chilli powder significantly promoted SV-II over control but were statistically nonsignificant among them in terms of promoting SV-II (Table 3, Col II). The highest SV-II was observed with D_1 of turmeric powder and the lowest was observed with D_3 of fenugreek

powder. In general, the fenugreek and turmeric powders at higher doses (3g) reduced the SV-II significantly over control whereas lower dose, i.e. D_1 , significantly promoted the SV-II (Table. 3). Similar result was also found by Layek *et al.* [28] who reported that dry treatment with red chilli powder along with bleaching powder and aspirin, improved field performance and vigour of bengal gram seeds. The physiologically active substances present in the botanicals might have activated the embryo and other associated structures which resulted in the absorption of more water due to elasticity of cell wall and development and increased vigour index [36].

Seedling emergence

All the doses of botanical powders of chilli, fenugreek and turmeric promoted the rate of seedling emergence as compared to control (Figure 2). At day 3, the highest seedling emergence was observed with D_1 and D_3 of chilli powder followed by D_2 of chilli powder. At day 8 to 10, D_1 and D_2 of turmeric powder and chilli powder resulted in higher seedling emergence than the other treatments of fenugreek and turmeric. The D_3 of turmeric powder promoted highest seedling emergence at day 8 to 10. In general, the various doses of botanical powders showed diverse results in terms of increasing the seedling emergence rate. Maity *et al.* [13] reported a higher seedling emergence in oat with various NPs, whereas, Rajeendran *et al.* [6] found promotive roles of botanicals

Table 3. Effects of nano and near nano size powders of chilli, fenugreek and turmeric on Seed Vigour Index I and Seed Vigour Index II in oat

Dose	Seed Vigour Index I (SVI) (Col I)			Seed Vigour Index II (SVII) (Col II)		
	Chilli	Fenugreek	Turmeric	Chilli	Fenugreek	Turmeric
0 g (Control)	2437.89 ^c	2437.89 ^c	2429.70 ^c	308.98 ^b	308.98 ^b	307.94 ^b
1 g	2698.74 ^b	2880.00 ^b	2764.15 ^b	338.78 ^a	344.00 ^a	345.75 ^a
2 g	2762.99 ^{ab}	2811.96 ^b	2923.30 ^a	324.39 ^a	318.75 ^a	298.93 ^c
3 g	2888.35 ^a	2685.93 ^{bc}	3001.99 ^a	313.46 ^{ab}	270.44 ^d	275.58 ^d

Different letters as superscripts within a column indicate significant differences ($p < 0.05$) among doses of a particular botanical.

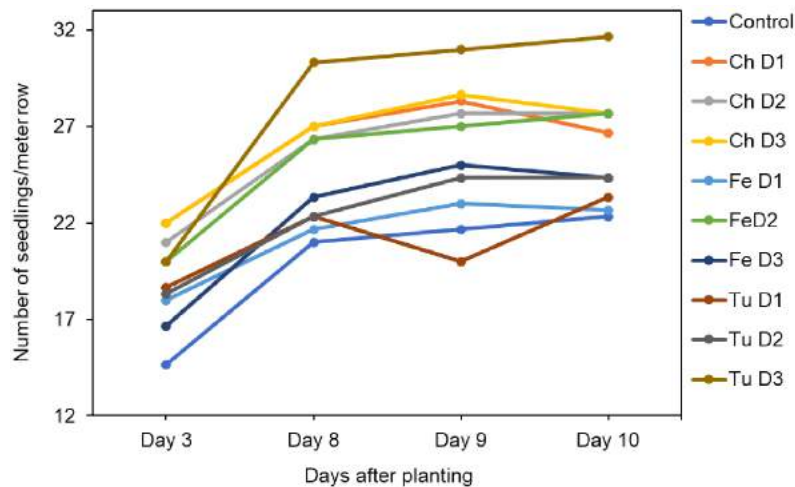


Figure 2. Effects of nano and near nano size powders of chilli, fenugreek and turmeric on seedling emergence (per meter row) of oat during field emergence of treated seed

Table 4. Effects of nano and near nano size powders of chilli, fenugreek and turmeric on fungal infection (%) and grain yield (q/ha) of treated oat seed

Dose	Fungal infection (%) (Col I)			Grain yield (q/ha) (Col II)		
	Chilli	Fenugreek	Turmeric	Chilli	Fenugreek	Turmeric
0g (Control)	94.3 ^a	94.3 ^a	94.3 ^a	16.3(±1.09)	16.3(±1.09)	16.3(±1.09)
1g	52.0 ^b	39.0 ^b	80.0 ^{ab}	14.2(±3.09)	15.3(±2.33)	14.8(±1.45)
2g	59.0 ^b	53.3 ^b	56.5 ^c	19.3(±5.4)	13.5(±1.61)	13.3(±0.67)
3g	43.7 ^b	80.7 ^b	61.7 ^{bc}	16(±2.08)	14.7(±1.13)	14.3(±1.01)
Significance	-	-	-	ns	ns	ns

Different letters as superscripts within a column indicate significant differences ($p < 0.05$) among doses of a particular botanical.

such as fenugreek and Moringa in improving seed performances in soybean.

Fungal infection (%)

In oat, all the three doses of powders of chilli, fenugreek and turmeric significantly reduced the fungal infection of seed over control (Table 4, Col I). The highest reduction in fungal infection was found with D₁ of fenugreek powder followed by D₃ of chilli powder. All the doses of chilli powder reduced the fungal infection significantly over control but were statistically at par among them. In case of fenugreek powder, lower doses (D₁) of treatment showed better performance in reducing the fungal infection. However, in case of chilli powder, lowest infection was observed with D₂ and increased subsequently with higher and lower doses (Table 4, Col I). Several reports suggest antifungal role of botanicals [37, 38] and nano and near nano size particles [17], which maintain the seed quality during extended storage.

Grain yield of treated seed

All the powders of chilli, fenugreek and turmeric failed to increase grain yield (q/ha) of oat in any of the doses applied, however, they did not show any toxic effect because of the nano and near nano sizes (Table 4, Col II). These powders promoted the seed quality parameters in different directions but could not promote the seed yield. Similar effects were reported by Maity *et al.* [13] in oat and Mahawer *et al.* [20] in barley treated with NPs. Dudai *et al.* [38] reported higher sorghum grain yield from seeds treated with different botanicals. Andresen *et al.* [40] confirmed the antimicrobial roles of *Agave sisalana* on sorghum seed, which improved the seed health and seedling growth by reducing the disease incidence and severity. Further research is needed in this direction to find the root cause behind this.

CONCLUSION

Nano and near nano size powders of chilli, fenugreek and turmeric improved physiological performances of oat

seed and also showed antifungal activity. This proves the potential of these botanicals, especially in microscopic size, in improving seed quality in short and long terms. While the world agriculture is facing a mammoth challenge of curtailing pesticide use for environmental safety and associated resistance development against the popular pesticides used in different purposes, revisiting the potential of botanicals in nano and near nano or micro sizes for improving seed and/or plant performances is highly recommended.

ACKNOWLEDGEMENTS

The authors are grateful to the Head, Department of nanoscience and Technology, TNAU and Head, Division of Seed Technology and Director, ICAR-IGFRI for support and guidance to conduct this research work.

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