Mysore J. Agric. Sci., 57 (2) : 310-316 (2023)

Effect of Green Synthesized and Chemical Nanoparticles on Seed Quality Parameters in Pigeonpea [*Cajanus cajan* (L.) Millsp.]

Sampathi Sowjanya and S. Rajendra Prasad
Department of Seed Science and Technology, College of Agriculture, UAS, GKVK, Bengaluru - 560 065
e-Mail : saisowji1107@gmail.com

Authors Contribution

Sampathi Sowjanya : Conceptualization, formal analysis, investigation, methodology and written original draft;
S. Rajendra Prasad : Conceptualization, supervision of the project, resource mobilasation, review and editing

Corresponding Author :
Sampathi Sowjanya
Department of Seed Science and Technology, College of Agriculture, UAS, GKVK, Bengaluru

Received : August 2022
Accepted : January 2023

Abstract

An experiment was conducted to standardize green and chemical nanoparticle concentrations and their effect on seed quality in pigeonpea. Among the green nanoparticles, green zinc oxide @ 1250 ppm recorded maximum seed germination (96.25%), speed of germination (28.64), mean shoot length (29.23 cm), mean root length (24.32 cm), mean seedling length (53.55 cm), seedling dry weight (60.5 mg), seedling vigour index-I (5153), seedling vigour index-II (5823), field emergence (94.50%), total dehydrogenase activity (2.353 A_{480}nm) and lowest electrical conductivity (26.49 µS/cm/g). Among chemical nanoparticles, zinc oxide @ 500 ppm recorded maximum seed germination (95.50%) speed of germination (24.28), mean shoot length (23.48 cm), mean root length (20.24 cm), mean seedling length (43.71 cm), seedling dry weight (56.3 mg/seedling), seedling vigour index-I (4174), seedling vigour index-II (5371), field emergence (92.5%), total dehydrogenase activity (2.021 A_{480}nm) and lowest electrical conductivity (31.88 µS/cm/g), whereas in control lowest seed germination (92.75%), speed of germination (21.63), mean shoot length (19.72 cm), mean root length (19.14 cm), mean seedling length (38.86 cm), seedling dry weight (47.0 mg/seedling), seedling vigour index-I (3575), seedling vigour index-II (4324), field emergence (90%), total dehydrogenase activity (1.773 A_{480}nm) and highest electrical conductivity (35.87 µS/cm/g) was observed. These findings suggest that, seed treatment with green zinc oxide @ 1250 ppm and chemical zinc oxide @ 500 ppm nanoparticles will be helpful to maintain seed quality in pigeonpea.

Keywords : Pigeonpea, Nanoparticle, Seed quality

Pigeonpea [*Cajanus cajan* (L.) Millsp.] is an important pulse crop in India and it belongs to the family *Fabaceae*, which is the major source of dietary protein for most of the vegetarian population and it is backbone of nutritional security of our country. Seed quality is affected by both biotic and abiotic factors, which influence germination, as well as other measures of seed quality which affect the ability of seeds to produce seedlings which can emerge and establish. Pulses are highly susceptible to infestation by bruchids, thus causing severe deterioration in seed quality other than storage losses. Generally, management of stored product pest is done through fumigation and also controlled by synthetic insecticides, which have many limitations and undesirable side effects. In this regard, nanotechnology has contributed maximum to agro-technological revolution. Treating seeds with green nanoparticles act as a best seed quality enhancement technique, where we can observe faster gemination, healthy and vigorous seedlings.

In the 21st century green nanotechnology regarded as the upcoming industrial revolution and 4th generation seed treatment for improving seed quality traits. Green nanotechnology is use of biological routes such as involving microorganisms and plants for the synthesis of nanoparticles (NPs).

Biosynthesis of plant based support materials has gained much importance as compared to conventional adsorbents due to their plentiful existence, low cost, nontoxic nature, high efficiency as well as environmental friendly in nature, which are considered as green nanoparticles. Green nanoparticles are effectively utilized to control pests and diseases, to promote plant growth and seed storage (Balogun *et al.*, 2020).

The problem of various biosafety issues with respect to the environment, plants, animals and human beings can be minimized to a great extent by utilizing the nano particles derived from biological sources such as proteins and carbohydrates which have low impact on the same system. Several green NPs (ZnO, Ag, MgO, Fe and CuO) have been applied as seed pre-treatment agents. It can internalize the seed coat and support water uptake inside the seeds, could possibly interact with a-amylase enzyme or act as nanocatalyst; thereby enhancing seed starch degradation for seed germination and seedling growth, also mitigating the detrimental effects of seed ageing and in helps elevated levels of antioxidant enzymes (Khan *et al.,* 2020). Metal NPs have an important role in potentiality to affect the physiological condition and also it can modulate the innate immune system (Sarkar *et al.,* 2020). In view of the above, current research has been undertaken with the objective to evaluate the effect of green synthesized and chemical nanoparticles on seed quality parameters in pigeonpea.

Material and Methods

The current experiment was conducted by using the freshly harvested seeds from NSP Bangalore during 2020-2021. The seeds are used to study the seed storability experiment by using green synthesized and chemical NP *i.e.,* green zinc oxide (500, 750, 1000 & 1250 ppm), green silica (250, 500, 750 & 1000 ppm), chemical zinc (250 & 500 ppm), chemical silica (250 & 500 ppm) along with spinosad as (4.4 mg/kg seed) and control (without any seed treatment) with 4 replications by using CRD. Nanoparticles concentrations were standardized after one month of seed treatment and also to study the nanoparticle concentration to increase the seed storability.

Seed Treatment

Seed treatment was done through artificial seed treater in seed processing unit at Nongwoo Seed India Private Limited, Yelahanka New Town, Bangalore. Stock solutions were prepared in 50 ml falcon tubes as per treatments scheduled and solution was poured in seed bin of seed treater for uniform distribution of solution to all seeds in the form of dry treatment. After the seed treatment, the seeds were incubated for a short period to achieve equilibration and treated seed were kept in cloth bag under ambient room temperature for further observation. After one month, all the seed quality parameters were evaluated as per ISTA (2013).

The following parameters were studied from the experiment to standardize the nanoparticle based seed treatment *viz.,* Seed germination (%), Speed of germination, Mean shoot length (cm), Mean root length (cm), Mean seedling length (cm), Mean seedling dry weight (mg/seedling), Seedling Vigour Index-I (SVI-I), Seedling Vigour Index-II (SVI-II), Field emergence (%), Electrical conductivity of seed leachate (µS/cm/g) and Total dehydrogenase activity (A_{480}nm).

Results and Discussion

Standardization of nano particle concentrations and their effect on seed quality parameters were studied.

Table 1

Initial seed quality parameters of Pigeonpea cv. BRG-5

Seed Quality Parameters	
Initial seed moisture content (%)	9.16
Seed Germination (%)	96.00
Seedling shoot length (cm)	19.38
Seedling root length (cm)	20.50
Mean seedling length (cm)	39.88
Seedling vigour index-I	3868
Seedling vigour index-II	4462
Electrical conductivity (µS/cm/g)	25.66
Total dehydrogenase activity (A_{480}nm)	2.013

Immediately after collection of seed material the seed quality parameters were analyzed without any seed treatment (Table 1). One month after seed treatment (Table 2), seed moisture content does not vary much (9.10-9.14%) among concentrations treated compared to initial moisture content.

Seed germination was recorded at 96.00 per cent immediately after harvest. During standardization of concentrations green zinc recorded better (96.25%) in maintaining the seed germination after one month of seed treatment compared to control (92.00%) which was significantly different (Table 2). Among chemical nanoparticles higher germination was recorded with seeds treated with zinc oxide @ 500 ppm (95.50%) followed by silicon dioxide @ 250 ppm (93.50%).

Field emergence significantly differed among concentrations used. Green zinc oxide @ 1250 ppm (94.50 %) is on par with green silicon dioxide @ 750 ppm (94.00 %), which is followed by green zinc oxide @ 1000 ppm (93.25 %) in recording field emergence. Among chemical NPs highest field emergence recorded in chemical zinc oxide @ 500 ppm (92.50 %) compared to the spinosad (91%) and control (90 %) (Table 2).

TABLE 2

Influence of seed treatment with nanoparticles on seed moisture content, germination, field emergence, speed of germination, seedling shoot length, seedling root length and mean seedling length in pigeonpea cv. BRG-5

	Seed Moisture Content (%)	Germination (%)	Field Emergence (%)	Speed of Germination	Seedling Shoot Length (cm)	Seedling Root Length (cm)	Mean Seedling Length (cm)
C_0	9.14	92.75	90.00	21.63	19.72	19.14	38.86
C_1	9.12	93.25	90.75	22.97	22.02	18.02	40.04
C_2	9.13	93.50	92.25	23.71	23.80	20.00	43.80
C_3	9.11	94.75	93.25	27.51	27.03	22.48	49.51
C_4	9.11	96.25	94.50	28.64	29.23	24.32	53.55
C_5	9.13	93.00	90.50	21.26	22.10	19.39	41.49
C_6	9.10	96.00	93.25	25.98	22.30	22.11	44.41
C_7	9.13	95.50	94.00	26.17	26.03	21.74	47.77
C_8	9.14	93.50	91.50	23.51	21.83	20.47	42.29
C_9	9.13	93.00	92.25	22.71	22.00	19.63	41.63
C_{10}	9.11	95.50	92.50	24.28	23.48	20.24	43.71
C_{11}	9.12	93.50	90.50	22.61	23.19	19.88	43.07
C_{12}	9.14	94.00	90.75	24.29	22.14	18.86	41.00
C_{13}	9.12	93.25	91.00	24.91	22.80	18.18	40.98
Mean	9.12	94.13	91.93	24.30	23.41	20.32	43.72
SEm (±)	0.03	0.78	0.79	0.56	0.58	0.50	0.83
CD@0.01	0.10	2.97	3.02	2.13	2.21	1.91	3.17
CV (%)	0.59	1.66	1.72	4.58	4.95	4.93	3.80

C_0- Control
C_1- Green ZnO 500 ppm
C_2 - Green ZnO 750 ppm
C_3 - Green ZnO 1000 ppm
C_4 - Green ZnO 1250 ppm
C_5 - Green SiO_2 250 ppm
C_6 - Green SiO_2 500 ppm
C_7 - Green SiO_2 750 ppm
C_8 - Green SiO_2 1000 ppm
C_9 - Chemical ZnO 250 ppm
C_{10}- Chemical ZnO 250 ppm
C_{11}- Chemical SiO_2 500 ppm
C_{12} - Chemical SiO_2 500 ppm
C_{13} - Spinosad 4.4 mg/kg seed

Higher precursor activity of nanoscale Zn in production of essential biomolecules that activates various enzymes which are responsible for driving many metabolic reactions in seeds and might mediate the ROS production leads to activation of pre-germinative metabolism in seed, Whereas, silica nanoparticles could increase cell extension by formatting complexes of Si polyphenol or lignin, which facilitate cell wall loosening to increase the seed germination, results are in conformity with Dragisic *et al.* (2007); Shyla & Natarajan (2014) and Korishettar *et al*. (2016).

Speed of germination significantly differed among concentrations, highest speed in germination of seedlings were recorded in green zinc oxide @ 1250 ppm (28.64) and it is on par with green zinc oxide @ 1000 ppm (27.51), which is immediately followed by green silicon dioxide @ 750 ppm (26.17), green silicon dioxide @ 500 ppm (25.98). Among chemical NPs, speed of germination in chemical zinc oxide @ 500 ppm (24.28), chemical silicon dioxide @ 250 ppm (22.61) compared to the spinosad (24.91) and control (21.63) were recorded (Table 2). The reason for rapid germination could be NPs may form new pores on seed coat during penetration facilitating the influx of water inside the seed thereby enhanced the speed of germination, similar findings were also reported by Sridhar (2012) and Mahakam *et al.* (2016).

Seedling shoot length significantly differed among concentrations used (Fig. 1). Highest shoot length was recorded in seeds treated with green zinc oxide @ 1250 ppm (29.23 cm) and it is on par with green zinc oxide @ 1000ppm (27.03 cm). Among chemical NPs, shoot length recorded in chemical zinc oxide @ 500 ppm (23.48 cm) followed by chemical silicon dioxide @ 250 ppm (23.19 cm) compared to the spinosad (22.80 cm) and control (19.72 cm). Seedling root length significantly differed among green and chemical NPS (Fig. 1). green zinc oxide @ 1250 ppm (24.32 cm) recorded highest root length and it is on par with green zinc oxide @ 1000ppm (22.48 cm). Among chemical NPs, chemical zinc oxide @ 500 ppm (20.24 cm), chemical silicon dioxide @ 250 ppm (19.88 cm) compared to the spinosad (18.18 cm) and control (19.14 cm) was recorded.

Green zinc oxide @ 1250 ppm (53.55 cm) better in showing seedling length and it is on par with green zinc oxide @ 1000 ppm (49.51 cm), which is immediately followed by green silicon dioxide @ 750 ppm (47.77 cm), chemical zinc oxide @ 500 ppm

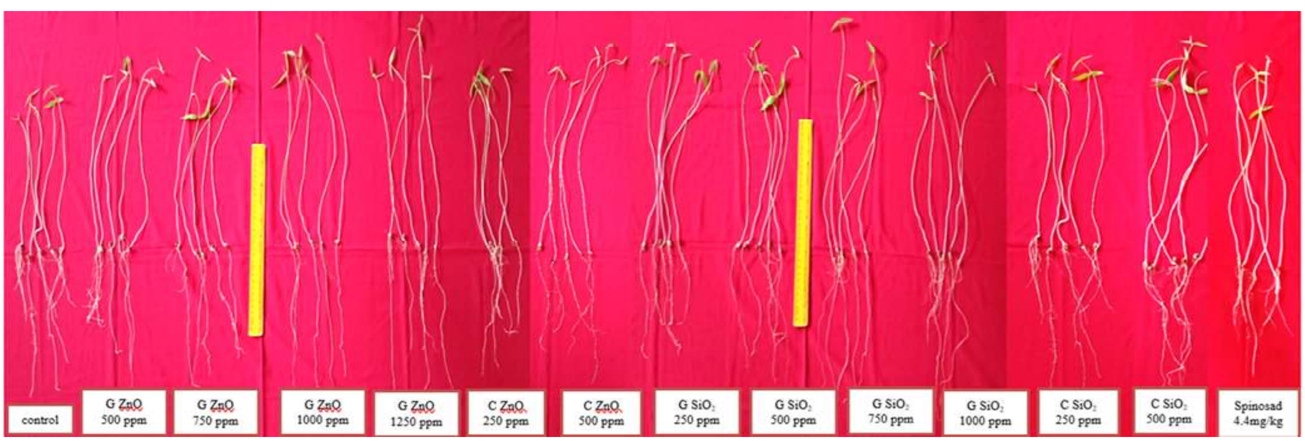

Fig. 1: Influence of seed treatment with nanoparticles on mean seedling length in pigeonpea cv. BRG-5

C_0- Control

C_1- Green ZnO 500 ppm
C_2 -Green ZnO 750 ppm
C_3 -Green ZnO 1000 ppm
C_4 -Green ZnO 1250 ppm
C_{13} -Spinosad 4.4 mg/kg seed

C_5 -Green SiO_2 250 ppm
C_6 -Green SiO_2 500 ppm
C_7 -Green SiO_2 750 ppm
C_8 -Green SiO2 1000 ppm

C_9 - Chemical ZnO 250 ppm
C_{10}-Chemical ZnO 250 ppm
C_{11}- Chemical SiO_2 500 ppm
C_{12} -Chemical SiO2 500 ppm

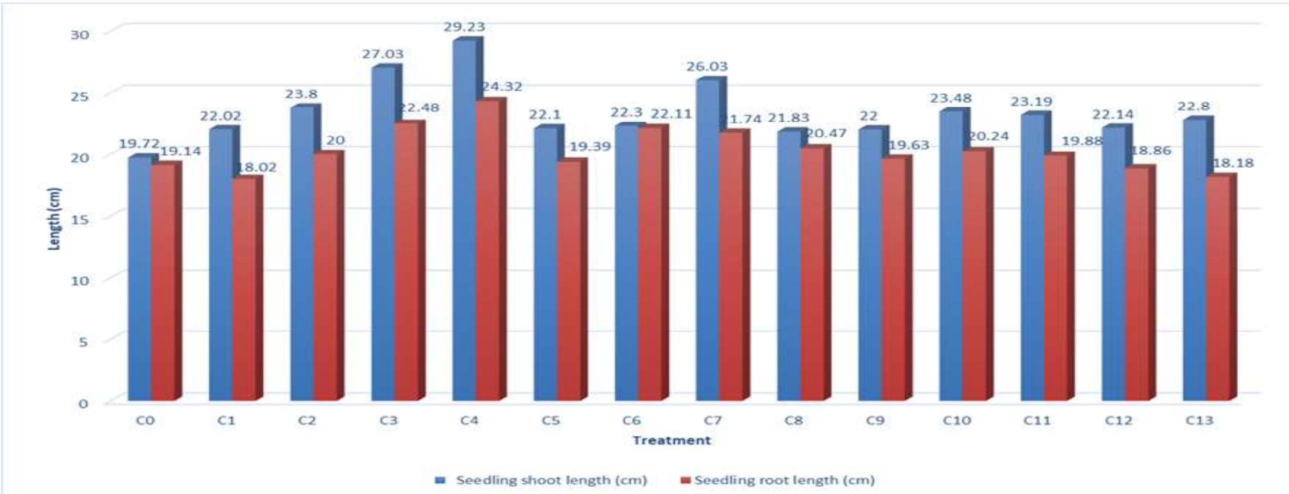

Fig. 2: Influence of seed treatment with nanoparticles on seedling shoot and root length (cm) in pigeonpea cv. BRG-5

C_0- Control
C_1- Green ZnO 500 ppm
C_2 -Green ZnO 750 ppm
C_3 -Green ZnO 1000 ppm
C_4 -Green ZnO 1250 ppm
C_5 -Green SiO_2 250 ppm
C_6 -Green SiO_2 500 ppm
C_7 -Green SiO_2 750 ppm
C_8 -Green SiO_2 1000 ppm
C_9 - Chemical ZnO 250 ppm
C_{10}-Chemical ZnO 250 ppm
C_{11}- Chemical SiO_2 500 ppm
C_{12} -Chemical SiO_2 500 ppm
C_{13} -Spinosad 4.4 mg/kg seed

(43.71 cm) compared to the spinosad (40.98 cm) and control (38.86 cm) in Fig. 2.

The probable reason could be due to the excess absorption at higher concentration resulted in penetration of NPs in to cell wall and plasma membrane of epidermal layers in shoot, root and accumulation in vascular tissues thereby enhancement in cell division and cell elongation in pigeonpea, similar findings were reported by Korishettar *et al.* (2016) in pigeonpea.

Mean seedling dry weight significantly differed among concentrations of Zn and Si NPs (Table 3). Green zinc oxide @ 1250 ppm (60.5 mg) recorded highest dry weight of seedling and it is on par with green zinc oxide @ 1000 ppm (60.5 mg), which is followed by green silicon dioxide @ 750 ppm (59.0 mg). Among chemical NPs, dry weight of seedlings were recorded in chemical zinc oxide @ 500 ppm (56.3 mg) followed by spinosad (53.0 mg) compared to the control (47.0 mg). During seed germination and early seedling growth, soluble sugars are mobilized from seeds resulted in higher biomass of the plants, similar findings were reported by Mahakam *et al.* (2016).

A significant variation for seedling vigour index-I and II was observed among concentrations used. Highest SVI-I recorded in green zinc oxide @ 1250 ppm (5153) and is on par with green zinc oxide @ 1000 ppm (4692). Among chemical NPs, chemical zinc oxide recorded @ 500 ppm (4174), chemical silicon dioxide @ 500 ppm (4026) compared with spinosad (3821) and control (3575) (Table 3). The highest SVI-II recorded in seeds treated with green zinc oxide @ 1250 ppm (5823) and it on par with green silicon dioxide @ 750 ppm (5635). Among chemical NPs, chemical zinc oxide @ 500 ppm (5371), followed by green silicon dioxide @ 1000 ppm (5071) compared to the spinosad (4942) and control (4324) (Table 3). Vigour enhancement by in corporation of nanoparticles (SNPs) increased cell division within the apical meristem of seedling, similar results were reported by Harish and Rame Gowda (2017).

Electrical conductivity significantly differed among concentrations used. Among NPs, green zinc oxide @ 1250 ppm (26.49 µS/cm/g) recorded lowest electrical conductivity and it is on par with green silicon dioxide @ 500 ppm (28.05 µS/cm/g). Among chemical NPs chemical zinc oxide @ 500 ppm

TABLE 3

Influence of seed treatment with nanoparticles on mean seedling dry weight, seedling vigour index- I, seedling vigour index- II, Electrical conductivity and total dehydrogenase activity in Pigeonpea cv. BRG-5

	Mean Seedling Dry Weight (mg)	Seedling Vigour Index- I	Seedling Vigour Index- II	Electrical Conductivity (µS/cm/g)	Total Dehydrogenase Activity (Absorbance at 480nm)
C_0	47.0	3575	4324	35.87	1.773
C_1	52.1	3734	4856	30.93	1.956
C_2	52.5	4095	4912	29.90	2.037
C_3	57.8	4692	5472	29.14	2.187
C_4	60.5	5153	5823	26.49	2.353
C_5	49.4	3858	4597	30.30	1.885
C_6	49.8	4262	4776	28.05	1.849
C_7	59.0	4563	5635	28.35	2.163
C_8	54.2	3955	5071	29.20	2.188
C_9	53.4	3872	4963	32.93	2.028
C_{10}	56.3	4174	5371	31.88	2.021
C_{11}	52.0	4026	4862	34.71	1.915
C_{12}	51.9	3853	4875	35.79	1.839
C_{13}	53.50	3821	4942	34.22	2.004
Mean	53.53	4116.64	5034.21	31.27	2.014
SEm (±)	8.36	84.21	85.46	0.54	0.05
CD@0.01	3.18	321.30	326.08	2.04	0.19
CV (%)	3.12	4.09	3.40	3.42	4.96

C_0- Control
C_1- Green ZnO 500 ppm
C_2 -Green ZnO 750 ppm
C_3 -Green ZnO 1000 ppm
C_4 -Green ZnO 1250 ppm
C_5 -Green SiO_2 250 ppm
C_6 -Green SiO_2 500 ppm
C_7 -Green SiO_2 750 ppm
C_8 -Green SiO_2 1000 ppm
C_9 - Chemical ZnO 250 ppm
C_{10}-Chemical ZnO 250 ppm
C_{11}- Chemical SiO_2 500 ppm
C_{12} -Chemical SiO_2 500 ppm
C_{13} -Spinosad 4.4 mg/kg seed

(31.88 µS/cm/g) followed by chemical silicon dioxide 250 ppm (34.71 µS/cm/g) compared to the spinosad (34.22 µS/cm/g) and control (35.87µS/cm/g) were recoded (Table 3). Electrical conductivity of seed leachate indicates the seed coat or membrane integrity, nanoparticles at lower concentration had no negative effect on the seed surface thereby reduced leakage of solute or electrolytes from the seeds by maintaining the seed coat integrity, these results are in conformity with study of Surabhi *et al*. (2021).

Total dehydrogenase activity (Table 3) of seeds treated with green zinc oxide @ 1250 ppm was highest (2.353 A_{480}nm) and it is on par with green silicon dioxide 1000 ppm (2.188 A_{480}nm) followed by chemical zinc oxide 500 ppm (2.021 A_{480}nm) compared to the spinosad (2.004 A_{480}nm) and control (1.773 A_{480}nm). The increased availability of these micronutrients at nanoscale with increased chemical reactivity resulted in the increase in synthesis and activity of the dehydrogenase enzymes, similar results were reported by Vijayalaxmi *et al.* (2013).

Among fourteen concentrations of zinc and silica nanoparticles, green synthesized nanoparticles found better in all seed quality parameters. Among green nanoparticles, zinc oxide @ 1250 ppm found better in seed germination (96.25%), mean seedling length (53.55cm), seedling vigour index I (5153) and field emergence (94.50%). Among chemical nanoparticles, zinc oxide @ 500 ppm found better in seed germination (95.50%), mean seedling length (43.71), seedling vigour index (4174) and field emergence (92.50%). It was clearly concluded that, seed treatment with nanoparticles is promising technology which improves the seed quality in pigeonpea.

References

Balogun, S. W., James, O. O., Sanusi, Y. K. and Olayinka, O. H., 2020, Green synthesis and characterization of zinc oxide nanoparticles using bashful (*Mimosa pudica*), leaf extract: a precursor for organic electronics applications. *Sn Applied Sci.*, **2** (3) : 1 - 8.

Dragisic M. J., Bogdanovic J., Maksimovic V. and Nikolic M., 2007, Silicon modulates the metabolism and utilization of phenolic compounds in cucumber (*Cucumis sativus* L.) grown at excess manganese. *J. Plant. Nutr. Soil Sci.,* **170** (6) : 739 - 744.

Harish, M. S. and Rame Gowda, 2017, Effect of nanoscale zinc oxide on plant growth seed yield and quality in groundnut, *Mysore J. Agric. Sci*., **51** (3) : 637 - 643.

Khan, J., Chandra, J., Xalxo, R., Korram, J., Satnami, M. L. and Keshavkant, S., 2020, Amelioration of ageing Associated Alterations and Oxidative Inequity in Seeds of *Cicer arietinum* by Silver Nanoparticles. *Plant Growth Regul*., **8** : 1 - 11.

Korishettar, P., Vasudevan, S. N., Shakuntala, N. M., Doddagoudar, S. R., Sharangouda, H. and Kisan, B., 2016, Seed polymer coating with Zn and Fe nanoparticles: An innovative seed quality enhancement technique in pigeonpea. *J. App. Nat. Sci.*, **8** (1) : 445 - 450.

Mahakham, W., Theerakulpisut, P., Maensiri, S., Phumying, S. and Sarmah, A. K., 2016, Environmentally benign synthesis of phytochemicals-capped gold nanoparticles as nanopriming agent for promoting maize seed germination. *Sci. Total Environ.,* **573** : 1089 - 1102.

Sarkar, J., Chakraborty, N., Chatterjee, A., Bhattacharjee, A., Dasgupta, D. and Acharya, K., 2020, Green synthesized copper oxide nanoparticles ameliorate defence and antioxidant enzymes in *Lens culinaris*. *J. Nanomate,*. **10** (2) : 312.

Shyla, K. K. and Natarajan, N., 2014, Customizing zinc oxide, silver and titanium dioxide nanoparticles for enhancing groundnut seed quality. *Indian J. Sci. Technol.,* **7** (9) : 1376 - 81.

Sridhar, C., 2012, Effect of nanoparticles for the maintenance of tomato seed vigour and viability. *M.Sc. (Agri.) Thesis,* TNAU, Coimbatore (India).

Surabhi, V. K., Rame Gowda and Nethra, N., 2021, Standardization of seed treatment protocol with nanoparticles for enhancing seed quality in pigeonpea, *Mysore J. Agric. Sci.,* **52** (3) : 588 - 596.

Vijayalaxmi, V., Ramamoorthy, K. and Natarajan, N., 2013, Effect of nanoparticle (TiO_2) on naturally aged seeds of maize (*Zea mays* L.). Paper presented in: 13th Nation. Seed Sem., Innovations in Seed Research and Development, UAS, Banglore, June, 8 - 10 ; 90.