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Biogenic nano seed treatment studies in pigeon pea under pot culture

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Abstract
An experiment was conducted to know the effect of biogenic nanoparticle seed treatment in pigeon pea under pot culture during 2021-22 at NSP, GKVK, Bangalore. Seeds were sown in pots after imposing eight treatments in various concentrations including control *viz*., green zinc oxide (1000 ppm & 1250 ppm), green silicon dioxide (500 ppm & 750 ppm) also chemical zinc oxide, chemical silicon dioxide and Spinosad (2 ppm). Among the different nanoparticles studied, green zinc oxide @ 1250 ppm recorded maximum plant height (153.48 cm), number of nodules per plant (99.75), pod length (7.92 cm), number of seeds per pod (5.15), number of pods per plant (115.09), seed yield per plant (53.73 g), and resultant seed obtained from green zinc oxide @ 1250 ppm shown highest 100 seed weight (14.71 g), seed germination (94.00%), mean seedling length (43.02 cm), seedling dry weight (51.02 mg/seedling), seedling vigor index-I (4043), seedling vigor index-II (4795), total dehydrogenase activity (2.013 A_{480} nm) and lowest electrical conductivity (20.44 µS/cm/g). These findings suggest that seed treatment with green zinc oxide @ 1250 ppm is better for obtaining good seed yield and quality parameters in pigeon pea.

Keywords: Nano seed treatment, pigeon pea, pot culture

Introduction

Pulses truly are nutritious seeds for a sustainable future and can make an important contribution to the achievement of many of the Sustainable Development Goals of 2030. *Cajanus cajan* is a perennial member of the family Fabaceae, commonly known as 'Pigeon pea' or red gram. Pigeon pea is an important pulse crop in India, which is the major source of dietary protein for most of the vegetarian population and it is the backbone of the nutritional security of our country.

The biological route of NP synthesis is most preferred nowadays, as it is eco-friendly, non-toxic, biocompatible and economical too. 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 environmentally friendly in nature, which are considered green nanoparticles. Nanotechnology is one of the most effective and novel areas of research in agriculture. The potential uses and benefits of nanotechnology are enormous in the field of agriculture. Green Nanotechnology is defined as the use of biological routes such as those involving microorganisms and plants for the synthesis of nanoparticles (Balogun *et al*., 2020) [2].

In agriculture, rapid and uniform seed germination and seedling emergence are important determinants of successful stand establishment. In recent years, several green NPs (Ag, Au, CuO, MgO, Fe, TiO_2, ZnO) have been applied as seed pre-treatment agents can internalize seed coat, and support water uptake inside seeds, could possibly interact with α-amylase enzyme or act as Nano catalyst; 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) [6].

The effect of nanoparticles has also been documented on several biochemical parameters related to plant growth and development. NPs are capable of influencing plant growth and development by altering some of the physiological processes in plants. Most studies indicate that NPs can cause toxic effects above a certain concentration, the current phytotoxicity profile of nanoparticles is in preliminary stage and ill effects of the unique characteristics of are not well understood, hence more studies on toxicity are required for commercial application (Duhan *et al*., 2017) [3]. Hence, the current research was conducted to know the effect of nano seed treatment in pigeon pea under pot culture.

Materials and Methods

Method of seed treatment

Seeds of pigeon pea cv. BRG-5 dried to safer and uniform moisture level (8-9%) were treated with different nanoparticles form as dry treatment at different concentrations *viz*., Control, green zinc oxide (500, 750, 1000 and 1250 mg/Kg of seed, green silicon dioxide (250, 500, 750 and 1000 mg/Kg of seed) and chemical Zinc oxide (250, 500 mg), chemical silicon dioxide (250, 500 mg). Both nanoparticles and seeds were thoroughly mixed as per treatment combinations at Nang woo seeds, Yelahanka using seed coater for the uniform absorption of nanoparticles.

Two sets of treated pigeon pea seeds (three replicates) were sown in pots filled with equal quantities of soil and watered to field capacity. Proper care was taken to use similar soil in all the pots to minimize soil heterogeneity effects. After germination, one plant per pot was maintained throughout. Proper agronomic and plant protection management was done to all the treated plants for their maximum growth expression.
Treatment details (per kg of seed)
From the experiment, we studied the following parameters under pot culture as follows, plant height (cm), number of nodules per plant, number of pods per plant, pod length (cm), number of seeds per pod, seed yield per plant (g), hundred seed weight (g), seed germination (%), mean seedling length (cm), seedling vigor index-I (SVI-I), seedling vigor index-II (SVI-II), electrical conductivity of seed leachate (μS/cm/g), total dehydrogenase activity (A @ 480 nm).

Fig 1: Nanoparticles used for seed treatment in pigeon pea

Results and Discussion

Plant height (cm) at 30 DAS differed significantly among eight seed treatments. The treatment T_2 (green ZnO @ 1000 ppm) recorded highest plant height (36.77 cm) followed by T_3 (green ZnO @ 1250 ppm), T_5 (green SiO_2 @ 750 ppm) and T_4 (green SiO_2 @ 500 ppm) which are on par with each other (36.65 cm, 36.46 cm and 35.76 cm, respectively) and lowest was recorded in control (29.79 cm). Plant height (cm) at 60 DAS differed significantly among eight seed treatments. The treatment T_3 (green ZnO @ 1250 ppm) recorded highest plant height (119.61 cm) followed by T_2 (green ZnO @ 1000 ppm), T_5 (green SiO_2 @ 750 ppm) and T_4 (green SiO_2 @ 500 ppm) which are on par with each other (118.15 cm, 117.35 cm and 114.61 cm, respectively) and lowest was recorded in control (105.68 cm) (Table.1). Plant height (cm) at 90 DAS differed significantly among eight seed treatments. The treatment T_3 (green ZnO @ 1250 ppm) recorded highest plant height (153.48 cm) followed by T_5 (green SiO_2 @ 750 ppm), T_2 (green zinc oxide @ 1000 ppm), and T_7 (chemical SiO_2 @ 250 ppm) which are on par with each other (149.55 cm, 147.87 cm and 147.26 cm, respectively) and lowest was recorded in control (145.5 cm).

Zn is an important cofactor for many enzymes which are involved in many physiological activities of plants during their growth. So higher precursor activity of nano scale zinc helpful at tissue development, cell division and enhanced the plant growth through development of vigorous root system and it enable the plant to derive available soil nutrients lead to increasing the plant height. Nano silicon in maize increased plant height might be attributed to an increased level of gibberellic acid (GAs) as GA is mainly responsible for shoot elongation (Kukreti, *et al.*, 2020) [7]. Similar results were reported by Harish., 2017 on nano Zinc Oxide (ZnO), FeO NPs, $CaCO_3$ NPs, TiO_2 NPs and Bulk ZnO, Bulk FeO, Bulk $CaCO_3$, Bulk TiO_2 in groundnut; Ali *et al.*, 2019 on combined use of biochar and zinc oxide nanoparticle as a foliar spray in rice under cadmium stress.

The number of nodules per plant differed significantly among eight seed treatments (Fig. 2 and 6). The treatment T_3 (green ZnO @ 1250 ppm) recorded the highest number of nodules per plant (99.75) followed by T_5 (green SiO_2 @ 750 ppm) (95.58) and lowest was recorded in control (65.75 cm). There is synergetic relationship between zinc and nitrogen fixation, zinc might be Zn nutrition might be attributed to an increase in leghemoglobin, and increased plant growth, resulting in enhanced activity of N fixing organisms (Shukla and Yadav 1982) [10]. Similar results were reported by Harish., 2017 on nano zinc Oxide (ZnO), FeO NPs, $CaCO_3$ NPs, TiO_2 NPs and Bulk ZnO, Bulk FeO, Bulk $CaCO_3$, Bulk TiO_2 in groundnut.

The number of pods per plant differs significantly among different eight seed treatments. The treatment T_3 (green ZnO @ 1250 ppm) recorded highest number of pods (115.09) followed by T_5 (green SiO_2 @ 750 ppm) and T_2 (green zinc oxide @ 1000 ppm) which are on par with each other (110.03 and 109.41 respectively) while lowest number of pods per plant was recorded in control (99.08). The increase in growth parameters may be due to the release of more Zn^{2+} ions for plant development. The application of ZnO NPs significantly increased the grain zinc content. Higher zinc content resulted in higher photosynthetic potential and more photosynthetic substances, thereby development of higher number of panicles in rice (Zhang *et al.*, 2021) [11]. Similar results were obtained by Prasad *et al.*, (2012) [8] on nanoscale zinc oxide in ground nut and Harish., 2017 on nano Zinc Oxide (ZnO), FeO NPs,

$CaCO_3$ NPs, TiO_2 NPs and Bulk ZnO, Bulk FeO, Bulk $CaCO_3$, Bulk TiO_2 in groundnut.

Pod length (cm) significantly not differed among different seed treatments (Fig. 6). But, among the treatments, T_3 (green ZnO @ 1250 ppm) recorded highest pod length (7.92 cm) followed by T_2 (green ZnO @ 1000 ppm) and T_5 (green SiO_2 @ 750 ppm) which are on par with each other (7.69 and 7.46 cm respectively) while lowest pod length was recorded in control (6.93 cm). Zinc is an essential component of hundreds of plant enzymes that participate in chlorophyll and auxin synthesis and the synthesis and transformation of carbohydrates. Enhanced growth in early stages may help in faster and enhanced growth of pod.

Number of seeds per pod significantly not differed among treatments (Fig. 6). Among the treatments, T_3 (green ZnO @ 1250 ppm) recorded highest number of seeds per pod (5.15) followed by T_7 (chemical SiO_2 @ 250 ppm) (4.95). While the lowest number of seeds per pod was recorded in control (4.60) (Table.1). Mobility of the nanoparticles is known to be very high which ensures the phloem transport and also the nutrient movement to all parts of the plant. It may help in higher filling of pod (Prasad *et al.,* 2012) [8]. Resultant action of zinc nanoparticles in early stages may result in a higher number of seeds per pod. Similar results were obtained with study of Hanumanthappa *et al.,* 2019 [4] on seed priming with zinc sulphate @ 0.25% fb spraying of nano zinc @ 300 ppm at 30 DAS in groundnut and Harish., 2017 on nano Zinc Oxide (ZnO), FeO NPs, $CaCO_3$ NPs, TiO_2 NPs and Bulk ZnO, Bulk FeO, Bulk $CaCO_3$, Bulk TiO_2 in groundnut.

Pod weight per plant significantly differed among treatments. Among the treatments, T_3 (green ZnO @ 1250 ppm) recorded highest Pod weight per plant (104.99g) followed by T_5 (green SiO_2 @ 750 ppm) (94.45g) (Table.1 and Fig. 3). While lowest Pod weight per plant was recorded in control (77.81g). Nanoparticle seed treatment in early stages may enhance the photosynthesis process and translocation of photosynthetic products, this may result in higher pod weight. Similar results were obtained with study of Hanumanthappa *et al.,* 2019 [4] on seed priming with zinc sulphate @ 0.25% fb spraying of nano zinc @ 300 ppm at 30 DAS in groundnut.

Seed yield per plant significantly differed among treatments (Fig. 3). Among the treatments, T_3 (green ZnO @ 1250 ppm) recorded highest seed yield per plant (53.73g) followed by T_2 (green ZnO @ 1000 ppm) (50.39 g), while lowest seed yield per plant was recorded in control (32.72 g). The main objective of seed production is achieving maximum seed yield with good quality which is related to partitioning ability of the photosynthates in a particular plant. Nanoparticles might enhance the activities during early stages, which increased the pod number and pod growth, ultimately the yield. Reynolds (2002) suggested that micronutrients in the form of NPs can be used in crop production to increase yield. The inherent small size and the associated large surface area of nano scale ZnO fertilizer may increase the uptake. All these factors may be responsible to give higher yields for nano scale ZnO compared to chelated or bulk ZnO. Similar results were obtained by Harish., 2017 on nano Zinc Oxide (ZnO), FeO NPs, $CaCO_3$ NPs, TiO_2 NPs and Bulk ZnO, Bulk FeO, Bulk $CaCO_3$, Bulk TiO_2 in groundnut.

Hundred seed weight significantly differed among treatments. Among the treatments, T_3 (green ZnO @ 1250 ppm) recorded highest hundred seed weight (14.71g) followed by T_2 (green ZnO @ 1000 ppm) (14.27g). While the lowest hundred seed weight was recorded in T_8 (12.37g). Sound filling of seed may result in higher test weight, Similar results were obtained with study of Hanumanth appa *et al.,* 2019 [] on seed priming with zinc sulphate @ 0.25% fb spraying of nano zinc @ 300 ppm at 30 DAS recorded the higher test weight.

Seed germination significantly differed among treatments (Fig. 4). Among the treatments, T_3 (green ZnO @ 1250 ppm) recorded highest seed germination (94.00%) followed by T_2 (green ZnO @ 1000 ppm) (92.25%). While the lowest seed germination was recorded in T_1 (90.25%). Resultant seed which obtained from the nano treated one is having higher seed quality, this may result higher seed germination. Seedling length significantly differed among treatments. Among the treatments, T_3 (green ZnO @ 1250 ppm) recorded highest seedling length (43.02 cm) followed by T_2 (green ZnO @ 1000 ppm) (41.22 cm). While the lowest seedling length was recorded in T_1 (38.04 cm) (Table.2 & Figure 2).

Resultant seed has shown increase in length of shoot and root due to seeds with more test weight shall have higher potential and seedling growth, thus in turn increase the metabolic activity through micronutrients and its translocation leading to early germination, cell division and elongation of cells leading to improving seedling length.

Seedling dry weight significantly differed among treatments. Among the treatments, T_3 (green ZnO @ 1250 ppm) recorded highest seedling dry weight (51.02 mg) followed by T_6 (chemical ZnO @ 750 ppm) (47.79 mg). While the lowest seedling dry weight was recorded in T_1 (43.68 mg). The resultant seed has shown increased the seedling length and fresh weight automatically leads to an increase in seedling dry weight.

Seedling vigor index-I & II significantly differed among treatments. Among the treatments, T_3 (green ZnO @ 1250 ppm) recorded highest seedling vigor index-I and II (4043 and 4795 respectively) followed by T_2 (green ZnO @ 1000 ppm) and T_5 (green SiO_2 @ 750 ppm) (4507 and 3802 respectively). While lowest was recorded in T_1 (3432 and 3941 respectively) (Figure 5).

Electrical conductivity significantly differed among treatments. Among the treatments, T_3 (green ZnO @ 1250 ppm) recorded lowest electrical conductivity (20.444 µS/cm/g) followed by T_4 (green SiO_2 @ 500 ppm) (20.453 µS/cm/g). While highest electrical conductivity was recorded in T_1 (26.048 µS/cm/g). Total dehydrogenase significantly differed among treatments. Among the treatments, T_3 (green ZnO @ 1250 ppm) recorded the highest total dehydrogenase (2.013 $A_{480@nm}$) followed by T_2 (green ZnO @ 1000 ppm) (1.968 $A_{480@nm}$). While the lowest total dehydrogenase was recorded in T_1 (1.757 $A_{480@nm}$). High vigorous seeds have greater membrane stability which releases less seed leachate. This might be the reason resultant seed obtained from nano seed treatment has shown lower electrical conductivity and also higher dehydrogenase activity.

Table 1: Effect of nano zinc oxide and silicon dioxide nanoparticles on field parameters in pigeon pea cv. BRG-5

Treatments	Plant Height @ 30 DAS (cm)	Plant Height @ 60 DAS (cm)	Plant Height @ 90 DAS (cm)	Number of nodules per plant @ 65 DAS	Pod length (cm)	Number of seeds per pod	Number of pods per plant	Pod weight (g/plant)	Seed yield (g/ plant)
T_1- Control	29.79	105.68	145.45	65.75	6.93	4.60	99.08	77.81	32.72
T_2 -GZnO 1000 ppm	36.77	118.15	147.87	94.00	7.69	4.90	109.41	90.35	50.39
T_3-GZnO 1250ppm	36.65	119.61	153.48	99.75	7.92	5.15	115.09	104.99	53.73
T_4-GSiO_2 500 ppm	35.76	114.61	144.16	94.00	7.27	4.70	104.08	87.01	36.41
T_5-GSiO_2 750 ppm	36.46	117.35	149.55	95.58	7.46	4.85	110.03	94.45	43.15
T_6-CZnO 500 ppm	33.07	113.22	140.60	93.50	7.02	4.65	106.00	84.37	39.66
T_7-CSiO_2 250 ppm	33.62	110.64	147.26	88.50	7.53	4.95	107.08	93.05	40.21
T_8-Spinosad 2 ppm	34.25	116.46	145.93	86.50	7.37	4.90	107.75	92.44	38.73
Mean	34.54	114.46	146.79	89.70	7.40	4.84	107.31	90.56	41.87
S.Em (±)	0.600	1.572	2.576	2.89	0.206	0.100	2.485	3.671	1.14
CD@ (0.01P)	2.374	6.218	10.190	11.440	0.815	0.396	9.830	14.521	4.50
CV (%)	3.475	2.747	3.510	6.449	5.571	4.134	4.632	8.108	5.43

Table 2: Effect of nano zinc oxide and silicon dioxide nanoparticles on seed quality parameters in pigeon pea cv. BRG-5

Treatments	100 seed weight (g)	Germination (%)	Speed of germination	Mean shoot length (cm)	Mean root length(cm)	Mean seedling dry weight (mg)	EC (µS/cm/g)	TDH activity (Absorbance at 480 nm)
T_1- Control	13.81	90.25	19.60	20.43	17.61	43.680	26.048	1.757
T_2 -GZnO 1000 ppm	14.27	92.25	20.90	21.89	19.33	47.270	23.423	1.968
T_3-GZnO 1250 ppm	14.71	94.00	21.78	23.12	19.90	51.020	20.444	2.013
T_4-GSiO_2 500 ppm	13.89	91.00	20.90	20.62	18.67	46.415	21.453	1.811
T_5-GSiO_2 750 ppm	13.69	92.25	21.00	21.53	18.80	48.868	20.544	1.952
T_6-CZnO 500 ppm	13.15	90.50	20.52	20.80	18.17	47.790	20.843	1.909
T_7-CSiO_2 250 ppm	12.96	90.75	20.96	20.47	18.39	46.138	21.150	1.889
T_8-Spinosad 2 ppm	12.37	90.75	20.79	20.76	18.35	47.278	20.823	1.856
Mean	13.60	91.47	20.80	21.20	18.65	47.307	21.841	1.894
S.Em (±)	0.364	0.418	0.300	0.334	0.295	1.003	0.517	0.021
CD@ (0.01P)	1.439	1.652	1.188	1.319	1.165	3.969	2.044	0.083
CV (%)	5.350	0.913	2.887	3.146	3.159	4.242	4.732	2.212

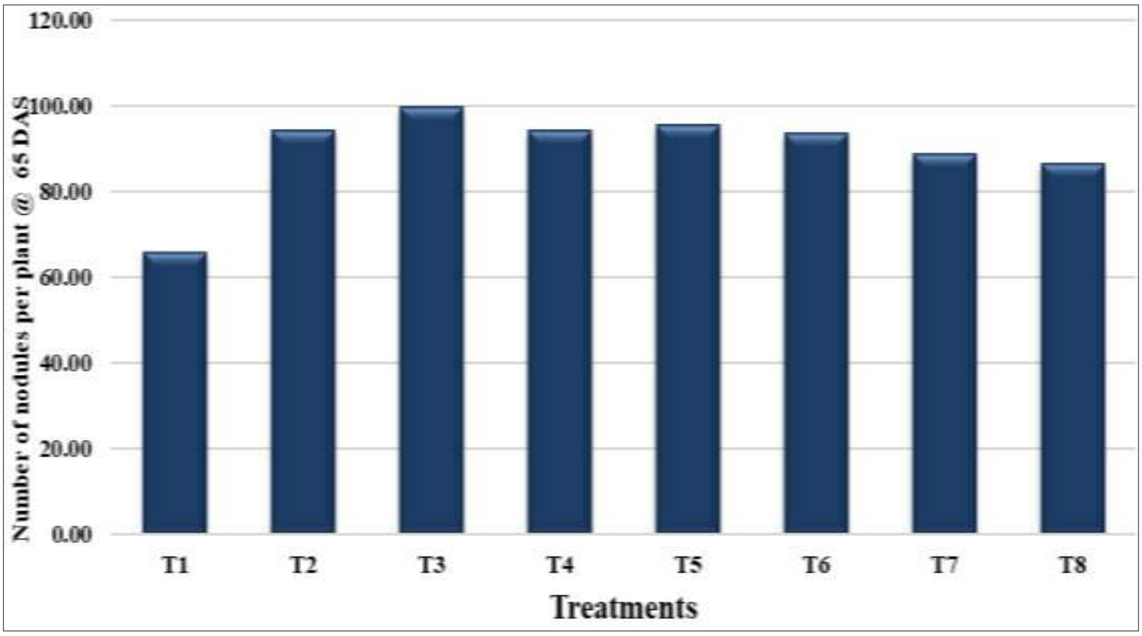

Fig 2: Effect of nano seed treatment on number of nodules per plant of pigeon pea cv. BRG-5 grown under greenhouse conditions in pot culture

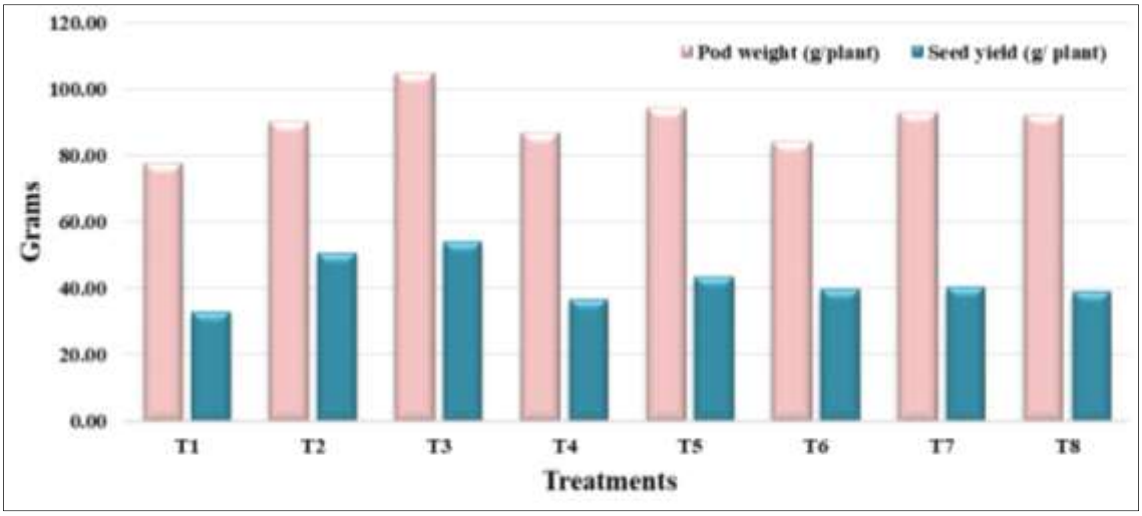

Fig 3: Effect of nano seed treatment on pod weight (g/plant), seed yield (g/ plant) of pigeon pea cv. BRG-5 grown under greenhouse conditions in pot culture

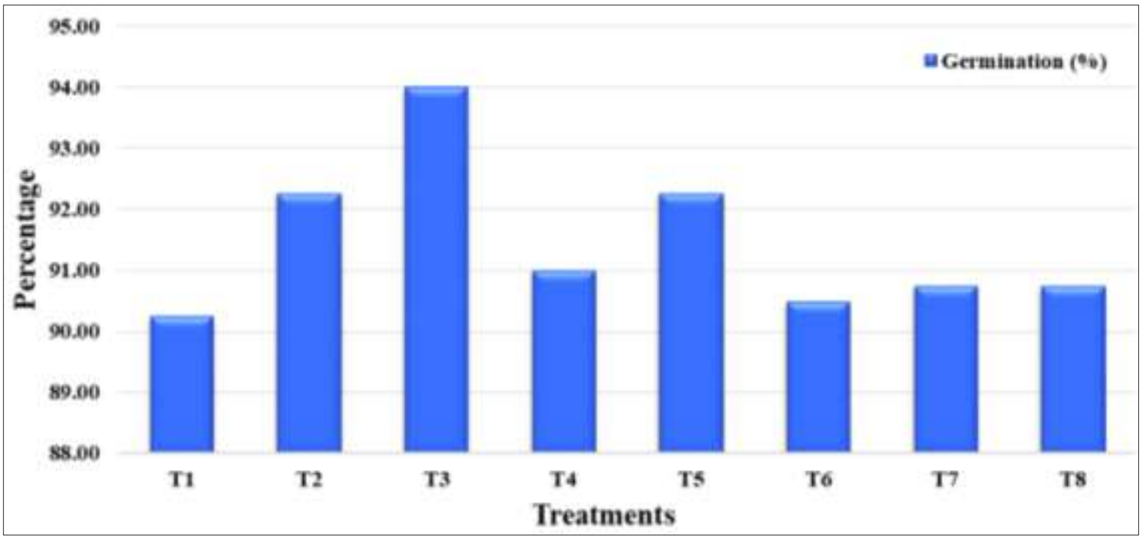

Fig 4: Effect of nano zinc oxide and silicon dioxide on germination (%) of pigeon pea cv. BRG- 5

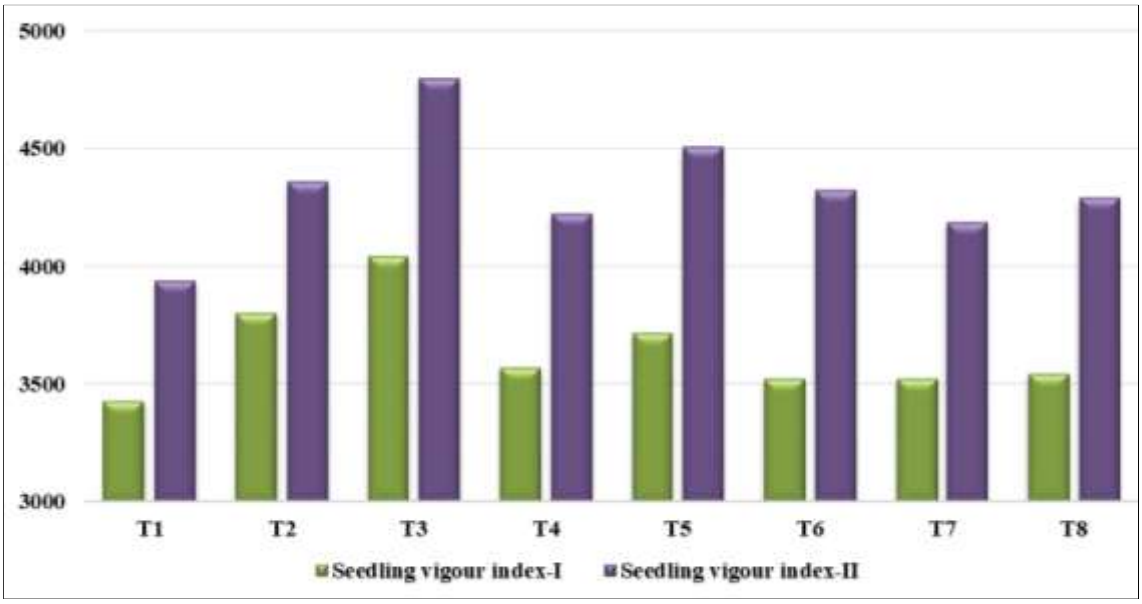

Fig 5: Effect of nano zinc oxide and silicon dioxide nanoparticles on seedling vigor index in pigeon pea cv. BRG-5

Fig 6: Effect of nanoparticle seed treatment on growth, seed yield of pigeon pea under pot culture

Conclusion

In the current study seed treatment with green ZnO @ 1250 ppm resulted a maximum number of root nodules per plant (99.75) and seed yield per plant (53.73 g) over control (65.75 and 32.72 g respectively) and the resultant seed of green ZnO @ 1250 ppm has shown highest seed germination (77.25%), seedling vigor index-I & II (4043 & 4795), TDH (2.013 $A_{480@nm}$). Application of NPs as seed treatment could be advocated for better field performance and seed quality parameters, so as to get higher seed yield with good quality seeds.

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