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EFFECT OF FOLIAR APPLICATION OF ZNO NANOPARTICLES ON SEED QUALITY OF COWPEA (*Vigna unguiculata* L. Walp)

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ABSTRACT

The zinc oxide nanoparticles (ZnO NPs) were synthesized through biological method using leaf extract of *Azadirachta indica*. The efficacy of green synthesized ZnO NPs was attributed on seed quality of cowpea. The present investigation was carried out during *kharif* 2023 and 2024 using two varieties of cowpea V_1: Phule Sonali and V_2: Phule Rakhumai to determine effect of the foliar application of green synthesized zinc oxide nanoparticles (ZnO NPs) which was undertaken at 30, 45 and 60 days after sowing at different concentrations as a treatments viz. T_0: Untreated Control, T_1: Zinc oxide nanoparticle (ZnO NPs) @ 50 ppm, T_2: Zinc oxide nanoparticle (ZnO NPs) @ 75 ppm, T_3: Zinc oxide nanoparticle (ZnO NPs) @ 100 ppm, T_4: Zinc oxide nanoparticle (ZnO NPs) @ 125 ppm and T_5: Zinc oxide nanoparticle (ZnO NPs) @ 150 ppm. The foliar application of green synthesized ZnO NPs @ 125 ppm (T_4) had higher 100 seed weight, germination percentage, seedling length, seedling dry weight, vigour index I and vigour index II with lower electrical conductivity and seed mycoflora percentage followed by ZnO NPs @ 150 ppm (T_5) and which was superior over control (T_0).

***Keywords*:** Green synthesis, seed quality, zinc oxide nanoparticles, cowpea, seed quality.

Introduction

Cowpea (*Vigna unguiculata* L. Walp.) is a diploid species (2n=2x=22). It is an annual legume belonging to family Fabaceae (Maréchal *et al.*, 1978) or Leguminoceae and the subfamily Papilionaceae (Cobley and Steele, 1976). However, Sellsschop (1962) and Purseglove (1976) stated that all cultivars belong to the wild cowpea, *Vigna unguiculata* L. Walp, which is indigenous to tropical Africa (central Africa). In India zinc (Zn) is considered as fourth most important yield limiting nutrient in agricultural crops. Zinc deficiency in Indian soils is likely to increase from 49 to 63 per cent by 2025 (Arunachalam *et al.*, 2013). Zinc is required for chlorophyll production, pollen function, fertilization, germination and for biomass production (Pandey *et al.*, 2006). Sufficient amounts of micronutrients are required for optimal cowpea growth and development. These include zinc (Zn) because its deficiency negatively affects plant reproductive functions and pollen development, reduces leaf area, induces chlorosis and abnormal growth of plant structures (Pandey *et al.*, 2006). This deficiency can also result in higher sensitivity to biotic and abiotic stress (Obata *et al.*, 1999). Most importantly, Zn agronomic management includes foliar application and soil fertilization. Zinc involved in photosynthesis, DNA replication and transcription, hydrocarbon metabolism, regulation of auxin production and cell membrane integrity (Sturikova *et al.*, 2018). Zinc is a micro nutrient involved in many aspects of plant metabolic and structural process such as photosynthesis, respiration, the production of chlorophyll and chloroplast function (Zulfiqar *et al*., 2020).

Zinc oxide is regarded as one of the most important nano materials among nanoparticles because of its stability, non-toxicity, low cost of manufacture, and environmental friendliness. The physiochemical properties of ZnO nanoparticles allow them to penetrate the plant tissue and interfere with several metabolic processes. According to Miralles *et al.* (2012), ZnO can pierce the seed coat and induce embryonic differentiation by activating enzymes involved in seed dormancy breakdown. Zinc oxide nanoparticles not only enhance seed germination but they also improve seed longevity (Gayathri, 2019). Zinc oxide nanoparticles are also shown to have a beneficial effect on the response of phytohormones, particularly Indole Acetic Acid (IAA), enhancing phytostimulatory effects. Zinc-rich ZnO NPs may enhance the level of IAA in roots (sprouts), hence increasing seedling development rates (Pandey *et al*., 2010). Zinc nanoparticles (ZnO NPs) have received significant interest because of their low cost, large surface area, appearance, UV-filtering, antifungal, antibacterial and photochemical features and high catalytic activity. Recent studies have demonstrated ZnO efficacy as a fungicide due to their ability to prevent the growth of disease-causing fungi in plants. Applications of nanotechnology in improving seed germination, emergence and growth of seedlings (Zhang *et. al.,* 2006) and for early pathogen detection (Alocilja and Radke, 2003) are few of the multifarious beneficial interventions in the field of agriculture.

Materials and Methods

The field experiment was laid down during *kharif*, 2023 and 2024 in Factorial Randomized Block Design (FRBD) with three replications and the experiment was conducted at the field of Botany Farm, MPKV, Rahuri. The seed of cowpea cultivars V_1: Phule Sonali and V_2: Phule Rakhumai used for this experiment. The foliar application of green synthesized zinc oxide nanoparticles (ZnO NPs) was undertaken at 30, 45 and 60 days after sowing using treatments T_0: Untreated Control, T_1: Zinc oxide nanoparticle (ZnO NPs) @ 50 ppm, T_2: Zinc oxide nanoparticle (ZnO NPs) @ 75 ppm, T_3: Zinc oxide nanoparticle (ZnO NPs) @ 100 ppm, T_4: Zinc oxide nanoparticle (ZnO NPs) @ 125 ppm and T_5: Zinc oxide nanoparticle (ZnO NPs) @ 150 ppm.

Observations recorded

100 seed weight (g)

Hundred seeds in each treatment were counted manually and the weight was measured by using electronic weighing balance. The hundred seed weight was recorded in grams.

Electrical conductivity (dsm^{-1})

Five (5) g of seeds were soaked in 25 ml distilled water and kept in incubator maintained at 25 ±1° C for 24 hrs. After soaking and gently swirling the solution. The seeds were discarded and the leachate decanted and final volume made up to 25 ml by adding distilled water. This leachate was used for measuring the electrical conductivity with help of digital electrical conductivity meter and expressed in dSm^{-1} (Presley, 1958).

Germination (%)

Four replications of 100 seed from each treatment were kept for germination at 25±1°C for 10 days in between paper (BP) method. The germination percentage was expressed on the basis of normal seedlings as described in ISTA Rules (Anon., 2013).

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

Seedling length (cm)

Ten normal seedlings in each replication were randomly selected at final count of the germination test and the length was measured from the tip of the primary root to the point of attachment of cotyledons. The mean of ten seedling length was expressed in centimetres.

Seedling dry weight (g)

Ten normal seedlings were selected for seedling length measurement after removing cotyledons (remnant seed) were kept in an oven maintained at 80 ± 1°C for 24 hours. After drying, the weight of dry seedlings was recorded and means weight was calculated and expressed in grams.

Vigour index I

At the end of germination test ten normal seedlings from each replication were selected for calculation of vigour index by adopting the formula suggested by (Abdul- Baki and Anderson, 1973) and expressed in whole number as given below,

$$\text{Vigour index I} = \text{Germination (\%)} \times \text{Mean seedling length (cm)}$$

Vigour index II

Vigour index II was computed by multiplying standard germination percentage and mean seedling dry weight by adopting the formula suggested by Abdul Baki and Anderson (1973) and expressed in whole number as given below, Vigour index II = Germination (%) × Mean seedling dry weight (g)

Seed mycoflora (%)

Standard blotter paper method recommended by International Seed Testing Association (Anonymous, 1966 and Agarwal, 1976) were used to detect the seed mycoflora. Three discs of blotter paper was moistened with sterilized water; excess of water removed and placed it at the bottom of each petri-plate and labelled for each treatment. The 10 seeds in three replications of each treatment were placed at equidistance on blotter paper in each petri-plate under aseptic condition in the laminar air flow cabinet, after which the petri dishes were kept in an incubator at 20 ± 2^0C for 7 days with a cycle of 12 hours light and 12 hours darkness. On the 8^{th} day the seeds were examined for the seed mycoflora under stereoscopic binocular microscope and the observations of the fungi identified on the basis of sporulation and their fruiting structures were recorded. The percent incidence of fungi in seeds was expressed using the formula given below.

$$\text{Seed mycoflora (\%)} = \frac{\text{Number of seeds infected}}{\text{Total number of seeds incubated}} \times 100$$

Results and Discussion

100 seed weight (g)

From analysis confirmed the superiority of V_1 (Phule Sonali), which recorded a mean 100-seed weight of 10.61 g, as compared to 7.52 g in V_2 (Phule Rakhumai). Among the treatments, T_4 (ZnO NPs @ 125 ppm) recorded the highest 100-seed weight with a pooled mean of 9.85 g which was followed by T_5 (ZnO NPs @ 150 ppm), with a pooled mean of 9.55 g which was significantly superior over the control (T_0), The interaction between varieties and treatments on 100-seed weight was non-significant during both the years on pooled analysis, indicating a similar response of both varieties to the applied treatments. The highest 100-seed weight was observed under V_1T_4 (Phule Sonali + ZnO NPs @ 125 ppm) (Table 1).

Similar results were reported by Nalini *et al.* (2013) observed that foliar application of zinc at 0.1 per cent significantly improved seed size and seed weight in zinc-deficient black gram. Laware and Raskar (2014) reported in onion, Amin Farnia and Saeed Khodabandehloo (2015) found in maize. Likewise, Valadkhan *et al.* (2015) reported in chickpea. Hanumanthappa *et al.* (2019) observed higher test weight in groundnut with foliar application of nano-zinc. Dobariya and Patel (2021) reported in cowpea, Further, Tondey *et al.* (2022) in maize. Wankhade *et al.* (2025) also resulted that foliar application of ZnO NPs @ 150 ppm enhanced 1000 seed weight in onion.

Electrical Conductivity (dS m^{-1})

Electrical conductivity differed significantly between the varieties during both the years on pooled analysis. Variety V_2 (Phule Rakhumai) which recorded significantly lower values with a pooled mean of 0.244 dSm^{-1} compared to variety V_1 (Phule Sonali) which recorded higher values with a pooled mean of 0.278 dSm^{-1}. Among the treatments T_4 (ZnO NPs @ 125 ppm) recorded the lowest values with pooled mean of 0.248 dSm^{-1}, followed by T_5 (ZnO NPs @ 150 ppm) and T_3 (ZnO NPs @ 100 ppm) were found statistically at par with T_4 and significantly superior over control treatment (T_0). The interaction between varieties and treatments was non-significant during both the years on pooled analysis. Lower values were observed under the combination V_2T_4 (Phule Rakhumai + ZnO NPs @ 125 ppm). The results are in accordance with findings of Kumar *et al.* (2023) in chickpea. They reported that dry dressing with nano ZnO at 250 mg kg^{-1} significantly lower the electrical conductivity of seed leachates. The reduced electrical conductivity observed under nano ZnO treatment was attributed to improved membrane integrity which resulted in higher seed quality (Table 2).

Seed Germination (%)

Among the varieties V_2 (Phule Rakhumai) recorded significantly higher germination with a pooled mean of 95.56 % compared to V_1 (Phule Sonali) with pooled mean of 94.08 %. This indicates a better germination potential in Phule Rakhumai compared to Phule Sonali. The highest germination percentage was recorded under treatment T_4 - ZnO NPs @ 125 ppm with pooled mean of 97.50% which was significantly superior to control and followed by T_5 - ZnO NPs @ 150 ppm with pooled mean of 96.17%. The interaction effect between varieties and treatments was found non-significant during both years as well as in pooled data. The maximum germination was observed in V_2T_4 (Phule Rakhumai + ZnO NPs @ 125 ppm) (Table 3).

The findings are in close conformity with Pandey *et al.* (2010) reported that ZnO nanoparticles enhanced seed germination in *Cicer arietinum*. Similarly, Prasad *et al.* (2012) observed in peanut. Sedghi *et al.* (2013), Gokak and Taranath (2015), Maithreyee and Gowda (2015) and Snehalbhai (2016) who documented improved germination following nano zinc treatments across the different crops such as soybean, okra, maize and groundnut respectively. Rai and Jajoo (2021) observed increased amylase activity and water uptake in wheat seeds primed with ZnO nanoparticles. Further support is provided by Kumar *et al*. (2023) in chickpea.

Seedling Length (cm)

Among the varieties, V_2 (Phule Rakhumai) recorded significantly higher seedling length with a pooled mean of 26.24 cm compared to V_1 (Phule Sonali) with a pooled mean of 23.93 cm. The differences between the varieties were significant during both years on pooled analysis. The maximum seedling length was recorded under treatment T_4 (ZnO NPs @ 125 ppm) with a pooled mean of 27.40 cm which was significantly superior to control and followed by T_5 (ZnO NPs @ 150 ppm) with a pooled mean of 26.68 cm. The interaction between varieties and treatments was non-significant during both years on pooled analysis. The highest seedling length was observed in V_2T_4 (Phule Rakhumai + ZnO NPs @ 125 ppm (Table 4).

The results of present investigation, is in closely relate with the findings of Pandey *et al*. (2010) who reported in chickpea by increasing the activity of indole acetic acid (IAA). Dhoke *et al*. (2013) observed in mung bean. Shailesh *et al*. (2013) in mung bean indicating that nanoparticles facilitate improved nutrient uptake and metabolic activity during early growth stages. Patra *et al*. (2013) reported in cluster bean. Sedghi *et al*. (2013), reported in soybean. Gokak and Taranath (2015) observed in okra. Similarly, Maithreyee and Gowda (2015) in maize, Lakshmi *et al*. (2017) in mungbean, Rawat *et al*. (2018) in wheat, Tejaswini *et al*. (2019) reported in groundnut, Arshi *et al*. (2020) observed in tomato, Peiman *et al.,* (2022), in withania plants. Kumar *et al*. (2023) in chickpea reported that seed treatment with ZnO nanoparticles significantly enhanced seedling length.

Seedling Dry Weight (g)

Among the varieties, V_1 (Phule Sonali) recorded significantly higher seedling dry weight with a pooled mean of 0.064 g compared to V_2 (Phule Rakhumai) with a pooled mean of 0.049 g. Seedling dry weight was higher in seeds treated with ZnO nanoparticles compared to the control (T_0). The highest seedling dry weight was recorded in T_4 (ZnO NPs @ 125 ppm) with a pooled mean of 0.062 g followed by T_5 (ZnO NPs @ 150 ppm) with a pooled mean of 0.060 g. The interaction between varieties and treatments was non-significant during both years on pooled analysis. The highest seedling dry weight was observed in V_1T_4 (Phule Sonali + ZnO NPs @ 125 ppm) (Table 5).

The present findings are in close conformity with the results of Sedghi *et al*. (2013), who reported in soybean. Tarafdar *et al*. (2013) reported that foliar application of nanoscale ZnO in cluster bean significantly enhanced plant biomass. Adhikari *et al*. (2015), who observed in maize treated with nano-ZnO compared to conventional zinc fertilizers. Mendez *et al*. (2016) who reported in *Capsicum annuum*. Rawat *et al*. (2018) reported in wheat, Arshi *et al*. (2020) and Velasco *et al*. (2020), who reported in tomato and Peiman *et al.,* (2022) in withania plants.

Vigour Index I

Among the varieties V_2 (Phule Rakhumai) recorded significantly higher vigour index I compared to V_1 (Phule Sonali) during both years on pooled data. V_2 registered higher values with a pooled mean of 2493 than V_1 recorded with a pooled mean of 2254. The highest vigour index I was recorded under T_4 (ZnO NPs @ 125 ppm) with a pooled mean of 2672, which was significantly superior over control and followed by T_5 (ZnO NPs @ 150 ppm) with a pooled mean of 2564. The interaction between varieties and treatments was non-significant indicating a similar response pattern of both varieties to ZnO nanoparticle treatments. The highest vigour index I was recorded in V_2T_4 (Phule Rakhumai +ZnO NPs @ 125 ppm) (Table 6).

Vigour Index II

Among the varieties V_1 (Phule Sonali) recorded significantly higher vigour index II compared to V_2 (Phule Rakhumai) during both experimental years on pooled data. V_1 (Phule Sonali) recorded higher values with a pooled mean of 6.05 compared to V_2 (Phule Rakhumai) recorded values with a pooled mean of 4.67. Among the treatments the higher values was recorded under T_4 (ZnO NPs @ 125 ppm) with a pooled mean of 6.08 followed by T_5 (ZnO NPs @ 150 ppm) with a pooled mean of 5.75 The interaction between varieties and treatments was non-significant during both years on pooled analysis. The highest pooled vigour index II was recorded in V_1T_4 (Phule Sonali + ZnO NPs @ 125 ppm) (Table 7).

Similar findings were reported by Prasad *et al*. (2012), who observed in groundnut seeds. Maithreyee and Gowda (2015), who reported in maize. Snehalbhai (2016) in groundnut. Rawat *et al*. (2018), who reported in wheat seed. Rai-Kalal and Jajoo (2021) in wheat. Kumar *et al*. (2023) in chickpea.

Seed Mycoflora (%)

Among the varieties V_1 (Phule Sonali) recorded a seed mycoflora with a pooled mean of 0.56%, whereas V_2 (Phule Rakhumai) recorded lower pooled mean of 0.28%. All ZnO NPs treatments completely suppressed seed mycoflora, recording 0% infection during both years on pooled analysis compared to the control (T_0) (pooled mean of 2.50%). In contrast, the interaction between varieties and treatments was non-significant. Similar results also reported by Kumar *et al.* (2023) highlighting the protective role of nano zinc in improving seed health and subsequent crop performance (Table 8).

Table 1: Effect of varieties, zinc oxide nanoparticle (ZnO NPs) and their interactions on 100 seed weight (g) of cowpea.

Year	2023			2024			Pooled		
Treat/Var.	**V_1**	**V_2**	**Mean**	**V_1**	**V_2**	**Mean**	**V_1**	**V_2**	**Mean**
T_0	8.74	6.49	**7.62**	10.20	6.49	**9.11**	9.47	7.25	**8.36**
T_1	9.14	6.59	**7.86**	10.22	6.59	**9.18**	9.68	7.36	**8.52**
T_2	9.59	6.77	**8.18**	11.25	6.77	**9.73**	10.42	7.48	**8.95**
T_3	9.77	6.87	**8.32**	11.79	6.87	**10.02**	10.78	7.56	**9.17**
T_4	11.01	7.25	**9.13**	12.75	7.25	**10.57**	11.88	7.82	**9.85**
T_5	10.39	7.07	**8.73**	12.49	7.07	**10.38**	11.44	7.67	**9.55**
Mean	**9.77**	**6.84**		**11.45**	**8.21**		**10.61**	**7.52**	
	V	**T**	**VxT**	**V**	**T**	**VxT**	**V**	**T**	**VxT**
SEm (±)	0.16	0.27	0.39	0.25	0.43	0.60	0.14	0.24	0.34
CD @ 1 %	0.63	1.10	NS	0.98	1.70	NS	0.53	0.92	NS

NS- Non Significant

Table 2: Effect of varieties, zinc oxide nanoparticle (ZnO NPs) and their interactions on Electrical Conductivity (dSm^{-1}) of cowpea.

Year	2023			2024			Pooled		
Treat/Var.	**V_1**	**V_2**	**Mean**	**V_1**	**V_2**	**Mean**	**V_1**	**V_2**	**Mean**
T_0	0.329	0.278	**0.304**	0.272	0.278	**0.252**	0.301	0.255	**0.278**
T_1	0.315	0.273	**0.294**	0.253	0.273	**0.239**	0.284	0.249	**0.267**
T_2	0.309	0.275	**0.292**	0.249	0.275	**0.236**	0.279	0.249	**0.264**
T_3	0.305	0.268	**0.287**	0.248	0.268	**0.233**	0.277	0.243	**0.260**
T_4	0.291	0.259	**0.275**	0.235	0.259	**0.220**	0.263	0.232	**0.248**
T_5	0.296	0.261	**0.279**	0.239	0.261	**0.223**	0.268	0.234	**0.251**
Mean	**0.308**	**0.269**		**0.249**	**0.218**		**0.278**	**0.244**	
	V	**T**	**VxT**	**V**	**T**	**VxT**	**V**	**T**	**VxT**
SEm (±)	0.003	0.004	0.006	0.003	0.004	0.006	0.002	0.003	0.004
CD @ 1 %	0.011	0.017	NS	0.011	0.017	NS	0.008	0.011	NS

NS- Non Significant

Table 3: Effect of varieties, zinc oxide nanoparticle (ZnO NPs) and their interactions on Germination (%) of cowpea.

Year	2023			2024			Pooled		
Treat/Var	**V_1**	**V_2**	**Mean**	**V_1**	**V_2**	**Mean**	**V_1**	**V_2**	**Mean**
T_0	92.00 (73.65)	94.00 (75.85)	**93.00 (74.75)**	90.00 (71.62)	92.00 (73.65)	**91.00 (72.64)**	91.00 (72.64)	93.00 (74.75)	**92.00 (73.69)**
T_1	93.00 (74.68)	95.67 (78.10)	**94.33 (76.39)**	92.00 (73.65)	94.00 (75.95)	**93.00 (74.80)**	92.50 (74.17)	94.83 (77.03)	**93.67 (75.60)**
T_2	93.67 (75.49)	95.33 (77.58)	**94.50 (76.54)**	94.00 (75.95)	94.00 (75.95)	**94.00 (75.95)**	93.83 (75.72)	94.67 (76.77)	**94.25 (76.24)**
T_3	94.33 (76.47)	97.00 (80.12)	**95.67 (78.30)**	94.00 (75.95)	96.00 (78.72)	**95.00 (77.33)**	94.17 (76.21)	96.50 (79.42)	**95.33 (77.82)**
T_4	97.67 (81.26)	98.33 (83.97)	**98.00 (82.61)**	96.00 (78.72)	98.00 (83.44)	**97.00 (81.08)**	96.83 (79.99)	98.17 (83.70)	**97.50 (81.85)**
T_5	96.33 (79.14)	96.33 (79.14)	**96.33 (79.14)**	96.00 (78.72)	96.00 (78.72)	**96.00 (78.72)**	96.17 (78.93)	96.17 (78.93)	**96.17 (78.93)**
Mean	**94.50 (76.78)**	**96.11 (79.13)**		**93.67 (75.77)**	**95.00 (77.74)**		**94.08 (76.28)**	**95.56 (78.43)**	
	V	**T**	**VxT**	**V**	**T**	**VxT**	**V**	**T**	**VxT**
SEm (±)	0.57	0.99	1.40	0.73	1.27	1.80	0.44	0.76	1.07
CD @1%	2.28	3.96	NS	NS	5.07	NS	1.65	2.85	NS

NS- Non Significant Figures in parenthesis are arcsine transformed values

Table 4: Effect of varieties, zinc oxide nanoparticle (ZnO NPs) and their interactions on Seedling length (cm) of cowpea.

Year	2023			2024			Pooled		
Treat/Var.	**V_1**	**V_2**	**Mean**	**V_1**	**V_2**	**Mean**	**V_1**	**V_2**	**Mean**
T_0	21.80	22.20	**22.00**	23.30	24.20	**23.75**	22.55	23.20	**22.88**
T_1	22.40	23.50	**22.95**	24.00	25.30	**24.65**	23.20	24.40	**23.80**
T_2	22.10	25.30	**23.70**	24.40	25.60	**25.00**	23.25	25.45	**24.35**
T_3	23.30	25.60	**24.45**	24.70	26.30	**25.50**	24.00	25.95	**24.98**
T_4	25.60	28.60	**27.10**	25.90	29.50	**27.70**	25.75	29.05	**27.40**
T_5	24.80	28.10	**26.45**	25.10	28.70	**26.90**	24.95	28.40	**26.68**
Mean	**23.33**	**25.88**		**24.53**	**26.60**		**23.93**	**26.24**	
	V	**T**	**VxT**	**V**	**T**	**VxT**	**V**	**T**	**VxT**
SEm (±)	0.25	0.43	0.60	0.27	0.48	0.67	0.18	0.31	0.43
CD @ 1 %	0.98	1.70	NS	1.09	1.90	NS	0.67	1.16	NS

NS- Non Significant

Table 5: Effect of varieties, zinc oxide nanoparticle (ZnO NPs) and their interactions on Seedling dry weight (g) of cowpea.

Year	2023			2024			Pooled		
Treat/Var.	**V_1**	**V_2**	**Mean**	**V_1**	**V_2**	**Mean**	**V_1**	**V_2**	**Mean**
T_0	0.053	0.040	0.047	0.065	0.047	0.056	0.059	0.043	0.051
T_1	0.055	0.041	0.048	0.066	0.049	0.057	0.061	0.045	0.053
T_2	0.060	0.045	0.053	0.066	0.051	0.058	0.063	0.048	0.055
T_3	0.062	0.048	0.055	0.067	0.052	0.059	0.064	0.050	0.057
T_4	0.069	0.053	0.061	0.071	0.056	0.064	0.070	0.055	0.062
T_5	0.066	0.051	0.059	0.069	0.053	0.061	0.067	0.052	0.060
Mean	**0.061**	**0.046**		**0.067**	**0.051**		**0.064**	**0.049**	
	V	**T**	**VxT**	**V**	**T**	**VxT**	**V**	**T**	**VxT**
SEm (±)	0.003	0.004	0.006	0.003	0.004	0.006	0.002	0.003	0.004
CD @ 1 %	0.011	NS	NS	0.011	NS	NS	0.007	NS	NS

NS- Non Significant

Table 6: Effect of varieties, zinc oxide nanoparticle (ZnO NPs) and their interactions on Vigour Index I of cowpea.

Year	2023			2024			Pooled		
Treat/Var.	**V_1**	**V_2**	**Mean**	**V_1**	**V_2**	**Mean**	**V_1**	**V_2**	**Mean**
T_0	2006	2086	**2046**	2096	2225	**2160**	2051	2156	**2103**
T_1	2083	2249	**2166**	2207	2377	**2292**	2145	2313	**2229**
T_2	2069	2412	**2240**	2292	2405	**2349**	2181	2409	**2295**
T_3	2196	2483	**2340**	2320	2523	**2422**	2258	2503	**2381**
T_4	2500	2811	**2656**	2485	2890	**2687**	2493	2851	**2672**
T_5	2388	2706	**2547**	2407	2754	**2580**	2398	2730	**2564**
Mean	**2207**	**2458**		**2301**	**2529**		**2254**	**2493**	
	V	**T**	**VxT**	**V**	**T**	**VxT**	**V**	**T**	**VxT**
SEm (±)	20.09	34.79	49.20	14.10	24.42	34.54	12.78	22.14	31.31
CD @ 1 %	80.07	138.68	NS	56.21	97.36	137.69	48.23	83.54	118.15

NS- Non Significant

Table 7: Effect of varieties, zinc oxide nanoparticle (ZnO NPs) and their interactions on Vigour Index II of cowpea.

Year	2023			2024			Pooled		
Treat/Var.	V_1	V_2	Mean	V_1	V_2	Mean	V_1	V_2	Mean
T_0	4.87	3.76	**4.32**	5.89	4.31	**5.10**	5.38	4.04	**4.71**
T_1	5.12	3.92	**4.52**	6.10	4.59	**5.35**	5.61	4.26	**4.93**
T_2	5.63	4.29	**4.96**	6.20	4.82	**5.51**	5.92	4.55	**5.24**
T_3	5.87	4.66	**5.26**	6.26	5.01	**5.64**	6.06	4.84	**5.45**
T_4	6.74	5.22	**5.98**	6.86	5.50	**6.18**	6.80	5.36	**6.08**
T_5	6.37	4.92	**5.65**	6.63	5.09	**5.86**	6.50	5.01	**5.75**
Mean	**5.77**	**4.46**		**6.32**	**4.89**		**6.05**	**4.67**	
	V	**T**	**VxT**	**V**	**T**	**VxT**	**V**	**T**	**VxT**
SEm (±)	0.25	0.43	0.60	0.26	0.45	0.64	0.16	0.28	0.40
CD @ 1 %	0.98	NS	NS	1.04	NS	NS	0.62	NS	NS

NS- Non Significant

Table 8: Effect of varieties, zinc oxide nanoparticle (ZnO NPs) and their interactions on Seed Mycoflora (%) of cowpea.

Year	2023			2024			Pooled		
Treat/Var.	V_1	V_2	Mean	V_1	V_2	Mean	V_1	V_2	Mean
T_0	3.33 (6.14)	3.33 (6.14)	**3.33 (6.14)**	3.33 (6.14)	0.00 (0.00)	**1.67 (3.07)**	3.33 (6.14)	1.67 (3.07)	**2.50 (4.61)**
T_1	0.00 (0.00)	0.00 (0.00)	**0.00 (0.00)**	0.00 (0.00)	0.00 (0.00)	**0.00 (0.00)**	0.00 (0.00)	0.00 (0.00)	**0.00 (0.00)**
T_2	0.00 (0.00)	0.00 (0.00)	**0.00 (0.00)**	0.00 (0.00)	0.00 (0.00)	**0.00 (0.00)**	0.00 (0.00)	0.00 (0.00)	**0.00 (0.00)**
T_3	0.00 (0.00)	0.00 (0.00)	**0.00 (0.00)**	0.00 (0.00)	0.00 (0.00)	**0.00 (0.00)**	0.00 (0.00)	0.00 (0.00)	**0.00 (0.00)**
T_4	0.00 (0.00)	0.00 (0.00)	**0.00 (0.00)**	0.00 (0.00)	0.00 (0.00)	**0.00 (0.00)**	0.00 (0.00)	0.00 (0.00)	**0.00 (0.00)**
T_5	0.00 (0.00)	0.00 (0.00)	**0.00 (0.00)**	0.00 (0.00)	0.00 (0.00)	**0.00 (0.00)**	0.00 (0.00)	0.00 (0.00)	**0.00 (0.00)**
Mean	**0.56**	**0.56**		**0.56**	**0.00**		**0.56**	**0.28**	
	V	**T**	**VxT**	**V**	**T**	**VxT**	**V**	**T**	**VxT**
SEm (±)	0.98	1.69	2.39	0.72	1.25	1.77	0.61	1.05	1.49
CD @1%	NS	NS	NS	NS	NS	NS	NS	NS	NS

NS- Non Significant Figures in parenthesis are arcsine transformed values

Conclusion

Variety Phule Sonali (V_1) had higher 100 seed weight, seedling dry weight, vigour index II while variety Phule Rakhumai (V_2) had higher germination percentage, shoot length, root length, seedling length, seedling vigour index I with lower electrical conductivity and seed mycoflora percentage. The foliar application of green synthesized ZnO NPs @ 125 ppm (T_4) had higher 100 seed weight, germination percentage, shoot length, root length, seedling length, seedling dry weight, vigour index I and vigour index II with the lower electrical conductivity and seed mycoflora percentage followed by ZnO NPs @ 150 ppm (T_5) which was superior over control. The interaction V_1T_4 (Phule Sonali + ZnO NPs @ 125 ppm) had higher 100 seed weight, seedling dry weight, vigour index II while the interaction V_2T_4 (Phule Rakhumai + ZnO NPs @ 125 ppm) had higher germination percentage, shoot length, root length, seedling length, seedling vigour index I with lower electrical conductivity and seed mycoflora percentage during both the years on pooled basis.

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