

Influence of green synthesized silver and zinc nanoparticle on seed quality attributes in Red sanders (*Pterocarpus santalinus* Linn.)

Comment [Z1]: Please change attributes to attributes.

Background and Justification:

Comment [Z2]: Well justified

Abstract: Red Sanders (*Pterocarpus santalinus*), or Rakta Chandana, is an endangered tree native to the Southern Indian peninsula, valued for its medicinal and dye properties. However, its seed germination is hindered by a hard seed coat, phenolic compounds and hormonal imbalances. The laboratory experiment was conducted at the Green Nano-Technology Laboratory and Department of seed science and technology, University of Agricultural Sciences, Dharwad, during 2023, the experiment used a Completely Randomized Design (CRD) with three replications and evaluated seven pre-sowing treatments: T₁ (AgNPs at 1000 ppm for 24 hours), T₂ (ZnNPs at 2000 ppm for 24 hours), T₃ (Conc. H₂SO₄ for 12 minutes), T₄ (NaDC 500 ppm), T₅ (Dewinging), T₆ (Soaking in hot water at 100°C for 5 minutes) and T₇ (Control). Results indicated that seeds treated with ZnNPs at 2000 ppm (T₂) exhibited highest germination rate (56%), germination speed (1.04) along with root length (17.05 cm), shoot length (16.33 cm) and highest dry weight (6.22 g) and highest Seedling Vigour Index-I (1869). In comparison, H₂SO₄ treatment (T₃) achieved 51.67 per cent germination and a speed of 0.95, with root length (15.28 cm), shoot length (16.22 cm), dry weight (5.24 g) and Seedling Vigour Index (1628) slightly lower but still significant. The control (T₇) had the lowest performance with 31 per cent germination, a speed of 0.36, root length (15.28 cm), shoot length (16.22 cm), lower dry weight (1.63 g) and the lowest Seedling Vigour Index (649). Overall, ZnNPs at 2000 ppm (T₂) were the most effective in enhancing germination, seedling growth and overall vigour in Red Sanders.

Comment [Z3]: What type of randomization method have you applied?

Keywords: *Pterocarpus santalinus* Linn, Seed priming, Zn nanoparticle

I. Introduction

Red sanders (*Pterocarpus santalinus* Linn.) is an endangered, endemic tree of the southern Indian peninsula, belonging to the Fabaceae family. It thrives in dry, hilly, rocky areas. The wood of red sanders is prized for its rich red colour and fine grain, making it highly sought after for furniture, musical instruments, and carvings. Its high market value drives both legal and illegal trade. But it has poor and staggered germinability (33%) (Anuradha et al., 2019) due to the presence of phenolic compounds, hormonal imbalance and dormancy in pod and seed

respectively. Seed germination and early seedling establishment depend on several factors, with seed dormancy being a major constraint in forest species. Dormancy, where viable seeds fail to germinate under suitable conditions, can be due to environmental factors or the seeds own inhibitory mechanisms, which can be external, internal, or both. The process of dormancy breaking, germination improvement is a complex phenomenon through silver and zinc nanoparticles and comprises of a series of physiological and biochemical changes. Hence the present investigation is to evaluate the effect of green synthesised silver (Ag) and zinc (Zn) nanoparticles and different treatments on seed quality of Red sanders.

II. Materials and Methods

The laboratory experiment was conducted to investigate the Influence of green synthesized Ag and Zn nanoparticles on seed quality parameters in Red sander (*Pterocarpussantalinus*Linn.) during 2023 at the Department of Seed Science and Technology and green synthesis of nanoparticles carried out in Green Nanotechnology Laboratory, University of Agricultural Sciences, Dharwad. The experiment was laid out **incompletely** randomized design with 3 replications and 17 treatments viz., Red sander seeds were primed with Silver and Zinc nanoparticles (ZnNPs) of concentration 500, 1000, 1500 and 2000 ppm respectively for 12 h and 24 h and Seed primed with 1mM AgNO₃ and 1 mM ZnSO₄, respectively along with control (Untreated). The seeds to solutions ratio for nanopriming treatment was 1:2. Later seeds were shade dried to bring down to original moisture content under ambient conditions. Observations on seed quality parameters were recorded.

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Synthesis, characterization and standardization of nanoparticles silver (Ag) and zinc (Zn) were confirmed through UV-visible spectroscopy, PSA, SEM and XRD. The effective concentration of Ag and ZnNPs for priming duration was standardized through seed priming. The observations were recorded for germination, root length, shoot length and best priming duration and concentration of AgNPs and ZnNPs were standardized and primed the seeds with 17 different treatments. Red sander seeds were primed as per best silver and zinc nanoparticles treatment T₁ and T₂ was carried out with other final treatments. The treated seeds were evaluated for seed quality parameters *i.e.* seed germination (%), speed of germination, final mean germination, shoot length, root length, seedling dry weight and seedling vigour index. Treatment details are given below,

T₁: Seed priming with AgNPs at 1000 ppm for 24 h, T₂: Seed priming with ZnNPs at 2000 ppm for 24 h T₃: Seed treatment with Concentrated H₂SO₄ for 12 min, T₄: Seed treatment with NaDC 500 ppm for 15 min, T₅: Dewinging by secature, T₆: Soaking seeds in hot water at 100° for 5 minutes, T₇: Control (Untreated).

III. Observations to be recorded

Germination (%)

The standard germination test was conducted (ISTA, 1976) by sowing the seeds insand media in four replications. On final count of germination test, number of seeds germinated were counted and expressed as germination percentage.

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

Comment [Z6]: Represent the formula as --- equation 1

Speed of germination

Number of normal seedlings was counted everyday till the end of test period. The speed of emergence was calculated using the formula suggested by Maguire (1962).

$$\text{Speed of emergence} = \frac{n_1/d_1 + n_2/d_2 + n_3/d_3 + \dots}{\dots}$$

Comment [Z7]: Represent the formula as --- equation 2

Where, n = number of emerged seedlings, d= number of days.

Final mean germination

Final mean germination was calculated by dividing the cumulative germination percentage/total number of days.

Seedling dry weight (g)

From the germination test the same ten normal seedlings used for measuring the root and shoot length was kept in a butter paper packet and dried in hot air oven maintained at $80 \pm 2^\circ\text{C}$ for 24 hours. Then the seedlings were cooled in desiccators for 30 minutes and the weight of the dry seedlings were recorded using electronic balance and was expressed in milligrams.

Ten normal seedlings after observing root and shoot length they are put in butter paper (Hot air oven).

Root length (cm)

From the germination test, the same ten normal seedlings which are used for measuring the shoot length was used for measuring the root length. The root length was measured from tip of root to the hypocotyl point and the mean length was calculated and expressed in centimeter.

Shoot length (cm)

From the germination test, ten normal seedlings were randomly selected from each treatment on twelfth day and the shoot length was measured from the tip of shoot to the hypocotyl point and the mean length was calculated and expressed in centimeter.

Seedling Vigour Index

The Seedling Vigour Index was calculated by using the formula suggested by Abdul-Baki and Anderson (1973).

Seedling vigour index = Germination (%) x [root length (cm) + shoot length (cm)]

Comment [Z8]: Represent the formula as --- equation 3

SVI-I = Germination (%) × Total seedling length (cm)

Comment [Z9]: Represent the formula as --- equation 4

SVI-II = Germination (%) × Seedling dry weight (mg)

Comment [Z10]: Represent the formula as --- equation 5

IV. Results and Discussion

Among the treatments significantly higher seed germination (56.00 %), speed of germination (1.04), final mean germination (1.20) was recorded in T₂ (ZnNPs 2000 ppm) primed seeds compared to control T₇ seeds (31.00 %), (0.36), (0.45) respectively (Table. 1). Thenanoparticles entering into seed coat pores, causes increased penetration of water molecules and inducing ROS-generating/starch-degrading enzyme activity for physiological enhancement of seed germination (Mahakhamet *al.*, 2017; nanoprimering technology for enhanced seed germination and starch metabolism of aged rice seeds using photosynthesized silver nanoparticles, Khodakovskaya *et al.*, 2011). Zinc is essential for the preservation and defence of cell membrane structural stability reported by Cakmak (2000), Ajouriet *al.* (2004) found that Zn seed priming significantly improved barley seed germination and seedling development; these findings may indicate that high Zn concentration in seeds plays critical physiological roles during seed germination and early seedling growth (Prasad *et al.*, 2012). Zinc oxidenanoparticles additionally boosted peanut germination, growth and yield. Nanoparticles contribute positively in enhancing seed quality by implying in process that involves oxidation and reduction during germination. This is achieved by generating superoxide ion radicals, which effectively neutralize free radicals present during seed germination. Enhanced oxygen produced in this process leads to higher respiration and this further encourage germination. These results are in compliance with the findings (Shyla and Natarajan, 2014).

Significantly higher mean shoot (cm) length and root length was recorded in T₂ (ZnNPs 2000 ppm) primed seeds (16.33 and 17.05 cm) respectively as compared to control i.e.,

T₇(Untreated) (11.02 and 9.92 cm) respectively in Table.2. This is mainly due to the enhancement of the growth hormones in seed by the treatment of different concentrations of zinc oxide nanoparticles improved other growth parameters in green gram (Lakshmi *et al.*, 2017). Zinc nanoparticles enhance plant growth and improve nutrient uptake due to their small size and high surface area, making zinc more available for absorption by plant roots. Micronutrient is vital for physiological processes such as enzyme activation, protein synthesis and hormone regulation Raliya *et al.* (2019).

Significantly higher mean seedling dry weight was recorded in T₂ (ZnNPs 2000 ppm for 24 h) primed seeds (6.22 g) as compared to control i.e., T₇ (Untreated) (1.63 g). Suriyaprabha *et al.* (2012) reported that the content of organic substances in the primed seeds, including protein, chlorophyll, and phenols, was increased by the application of nanoparticles to the seed. Due to increase in seedling length (cm) through nanopriming, primed seeds recorded higher seedling dry weight.

Significantly higher mean Seedling Vigour Index-I and Seedling Vigour Index – II was recorded in T₂ (ZnNPs 2000 ppm for 24 h) primed seeds (1869 and 125) respectively as compared to control i.e., T₇ (Untreated) (649 and 51) respectively in Table 3. This is due to the result of increased synthesis of tryptophan and auxin, a hormone that stimulates longitudinal growth, as well as the effects of nanoparticles on boosting cell division and growth, particularly in meristematic points (Kaya and Higgs, 2002). This could lead to an increase in the length and height of internodes. This conclusion is consistent with findings from studies on encouraging and acceleration of longitudinal growth in *Tanacetum parthenium* (Mahajan *et al.*, 2011; Shahhoseini *et al.*, 2020).

The mechanism of NP uptake was generally considered as active transport that includes cellular processes such as signaling and regulation of the plasma membrane (Etxeberria *et al.*, 2006). NPs can move through tissues via: apoplastic and symplastic routes. Apoplastic transport occurs outside the plasma membrane and through the extracellular spaces, cell walls of adjacent cells, and xylem vessels (Sattelmacher, 2001); in contrast, symplastic transport involves movement of water and substances between the cytoplasm of adjacent cells through specialized structures called plasmodesmata (Roberts and Oparka, 2003) and sieve plates. The apoplastic pathway is important for radial movement within plant tissues; it allows nanomaterials to reach the root central cylinder and vascular tissue.

Compared to the untreated control, the seed treatment with nanoparticles considerably changed the seed quality parameters and consistently preserved the viability and vigour of the seeds. In terms of seed germination (%), speed of germination, final mean germination, seedling

shoot length (cm), seedling root length(cm), dry weight and Seedling Vigour Indices seeds treated with nanoparticles significantly increased as compared to control. The seed treatment with ZnNPs outperforms other treatments examined, including the control.

Significance of the study:

Comment [Z11]: Please highlight the significance, direction, recommendation and limitation of the study.

V. Conclusion

Green synthesis of AgNPs and ZnNPs was successfully carried out through leaf extracts of guava (*Psidiumguajava*) as reducing and capping agents. As compared to control significantly higher seed quality parameters were recorded in seeds primed with ZnNPs (2000 ppm for 24 h) recorded in Red sander seeds. The outcome of this experiment unequivocally demonstrates that ZnNPs can be used as an effective priming agent for enhancing seed quality.

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Table 1: Effect of green synthesized silver and zinc nanoparticles along with other treatments on seed germination, speed of germination and final mean germination of Red sander

Treatments	Germination (%)	Speed of germination	Final mean germination
T ₁ - Seed priming with AgNPs at 1000 ppm for 24 h	42.00 (40.38)*	0.77	0.76
T ₂ - Seed priming with ZnNPs at 2000 ppm for 24 h	56.00 (48.43)*	1.04	1.20
T ₃ - Seed treatment with Conc H ₂ SO ₄ for 12 min	51.67 (45.94)*	0.95	1.16
T ₄ - Seed treatment with NaDC 500 ppm for 15 min	35.33 (36.45)*	0.50	0.60
T ₅ - Dewinging by secature	48.33 (44.03)*	0.89	0.93
T ₆ - Soaking seeds in hot water at 100 °C for 5 min	37.67 (37.85)*	0.62	0.64
T ₇ - Control (Untreated)	31.00 (33.82)*	0.36	0.45
S.Em (±)	1.09	0.02	0.02
CD at 1%	3.31	0.05	0.07

Note : * values in the parenthesis are arc sine transformed

AgNPs: Silver nanoparticles,

ZnNps : Zinc nanoparticles, Conc

H₂SO₄ : Concentrated sulphuric acid,

NaDC : Sodium de-oxycholate

Comment [Z12]: What about log transformation?

Table 2: Effect of green synthesized silver and zinc nanoparticles along with other treatments on root length, root length and seedling dry weight of Red sander

Treatments	Shoot length (cm)	Root length (cm)	Seedling dry weight (g)/10 seedlings
T ₁ - Seed priming with AgNPs at 1000 ppm for 24 h	13.75	12.17	2.97
T ₂ - Seed priming with ZnNPs at 2000 ppm for 24 h	16.33	17.05	6.22
T ₃ - Seed treatment with Conc H ₂ SO ₄ for 12 min	16.22	15.28	5.24
T ₄ - Seed treatment with NaDC 500 ppm for 15 min	11.67	11.79	1.96
T ₅ - Dewinging by secature	14.39	13.05	5.09
T ₆ - Soaking seeds in hot water at 100 °C for 5 min	12.55	12.08	2.48
T ₇ - Control (Untreated)	11.02	9.92	1.63
S.Em (±)	0.33	0.31	0.08
CD at 1%	1.00	0.95	0.24

Table 3: Effect of green synthesized silver and zinc nanoparticles along with other treatments on seedling vigour indices of Red sander

Treatments	Seedling Vigour Index-I	Seedling Vigour Index-II
T ₁ - Seed priming with AgNPs at 1000 ppm for 24 h	1089	125
T ₂ - Seed priming with ZnNPs at 2000 ppm for 24 h	1869	348
T ₃ - Seed treatment with Conc H ₂ SO ₄ for 12 min	1628	271
T ₄ - Seed treatment with NaDC 500 ppm for 15 min	829	69
T ₅ - Dewinging by secature	1326	246
T ₆ - Soaking seeds in hot water at 100°C for 5 min	928	93
T ₇ - Control (Untreated)	649	51
S.Em (±)	28.47	3.76
CD at 1%	86.36	11.40

REVIEW

fig .1 Effect of green synthesized silver and zinc nanoparticles along with other treatments on germination (%), speed of germination (%) and final mean germination of Red sander (*Pterocarpus santalinus* Linn.)

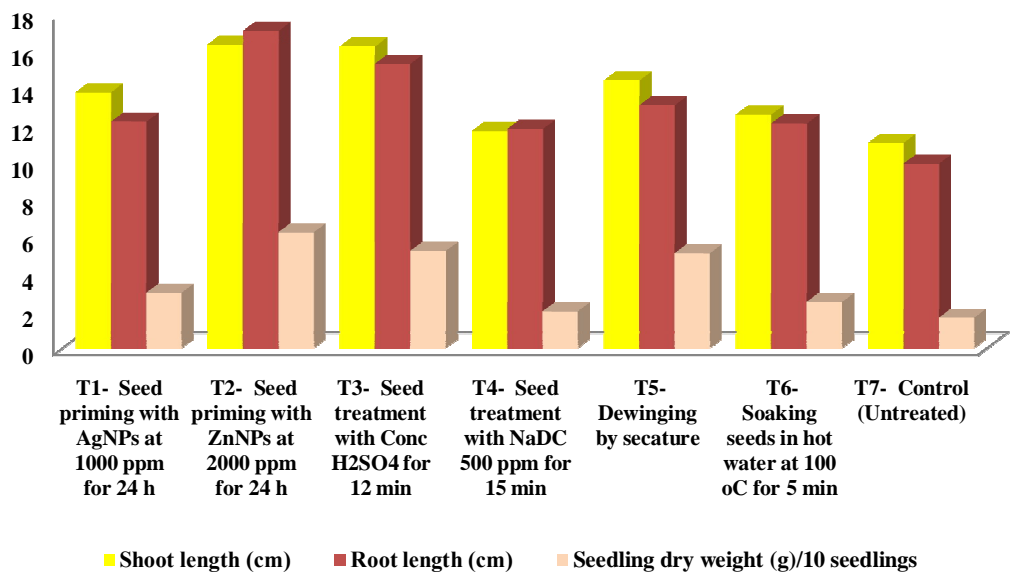
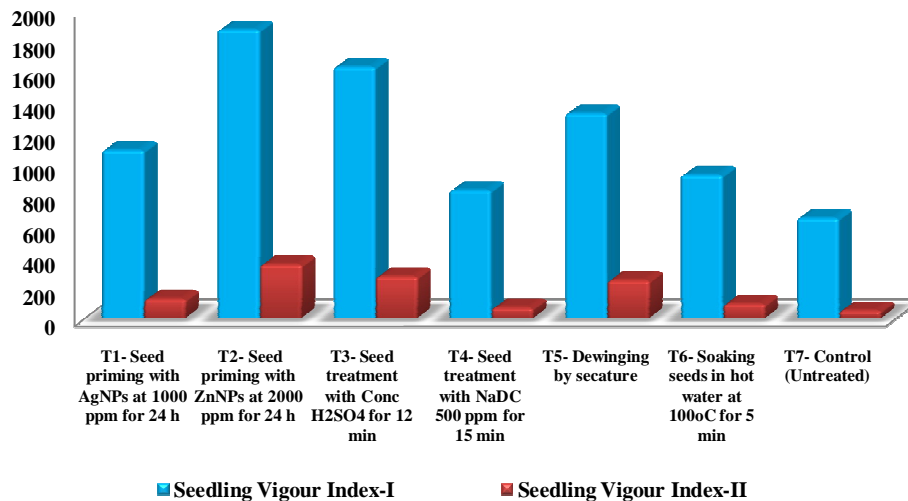


fig . 2 Effect of green synthesized silver and zinc nanoparticles along with other treatments on seedling vigour indices of Red sander (*Pterocarpus santalinus* Linn.)



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