Influence of green synthesized silver and zinc nanoparticle on seed qualityattributesinRedsanders (*Pterocarpussantalinus* Linn.)

Abstract: Red Sanders (*Pterocarpussantalinus*), or Raktachandana, is an endangered tree native to the Southern Indian Peninsula, valued for its medicinal and dye properties.However, its germination is hindered by a hard seed coat, phenolic compounds and hormonal imbalances.Nanotechnology in forestry enhances product innovation, paper processes and sustainability through plant-based nanoparticle biosynthesis.The experiment used a completely randomized design(CRD) with three replications and evaluated seven presowing treatments: T_1(AgNPs at 1000 ppm for 24 hours), T_2 (ZnNPs at 2000 ppm for 24 hours), T_3(Conc. H_2SO_4 for 12 minutes), T_4 (NaDC 500 ppm), T_5 (Dewinging), T_6(soaking in hot water at 100°C for 5 minutes) and T_7 (control). Theresults indicated that seeds treated with ZnNPs at 2000 ppm (T_2) exhibited highest germination rate (56%), germination speed (1.04), root length (17.05cm), shoot length (16.33cm) and highest dry weight (6.22 g) and highest seedling vigour Index-I (1869). In comparisonH_2SO_4 treatment (T_3) delivered 51.67 per cent germination and a speed of germination(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 (T_7) 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 (T_2) were the most effective in enhancing germination, seedling growth and overall vigour in Red sanders.

Keywords: *Pterocarpussantalinus* Linn, Seedpriming, Znnanoparticle

I. Introduction

Red sanders (*Pterocarpussantalinus*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 desirable for furniture, musical instruments, and carvings. Its high market value drives both legal and illegal trade. Howeverit has poor and staggered germinability (33%) (Anuradha*et al.*, 2019)due to the presence of phenolic compounds, hormonal imbalance and dormancy in pods and seed respectively. Seed germination and early seedling establishment depend on moisture, temperature, photoperiod, oxygen and nutrient availabilitywith 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. In the process of dormancy breaking, germination improvement is a complex phenomenon through silver and zinc nanoparticles and comprises a series of physiological and biochemical changes. Hence the present investigation aimedto evaluate the effects of green synthesized silver (Ag) and zinc (Zn) nanoparticles and different treatments on theseed 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 2023at the Department of Seed Science and Technology and green synthesis of nanoparticles carried out in Green Nanotechnology Laboratory, University of Agricultural Sciences, Dharwad. The experimentused ancompletely 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 seeds primed with 1mM $AgNO_3$ and 1 mM$ZnSO_4$, respectively along with control (untreated). The seed-to-solution ratio for thenanopriming treatment was 1:2. Later the seeds were shade dried to bring down to original moisture content under ambient conditions. Observationsof theseed quality parameters were recorded.

Thesynthesis, characterization and standardization ofsilver (Ag) and zinc (Zn)nanoparticles were confirmed through UV-visible spectroscopy, PSA, SEM and XRD. The effective concentrationsof Ag and ZnNPs for priming duration were standardized through seed priming with guava leaf extract.Aqueous guava leaf extract was prepared using 20 g of air-dried guava (*Psidium guajava*) leaves were cut into fine pieces, mixed with 200 ml of double-distilled water and boiled for 30 minutes at 45-55°C temperature. The boiled mixture was cooled at room temperature, filtered with Whatman's No.1 filter paper and the filtered extract was stored at 4°C for future use. The observations of germination, root length, shoot length, priming duration and concentration of AgNPsand ZnNPs were standardized and the seeds wereprimedwith 17 different treatments.Red sander seeds were primed according tobest silver and zinc nanoparticle treatment,and T_1 and T_2 were subjected to 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 as follows:

T_1: Seed priming with AgNPs at 1000 ppm for 24 h, T_2: Seed priming with ZnNPs at 2000 ppm for 24 h, T_3: Seed treatment with Concentrated H_2SO_4 for 12 min, T_4: Seed treatment with NaDC 500 ppm for 15 min, T_5: Dewinging by secature, T_6:Soaking seeds in hot water at 100□ for 5 minutes, T_7: 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.For thefinal count of thegermination test, number of seeds germinated wascounted and expressed as germination percentage.

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

Speed of germination

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

Speed of emergence = n1/d1+n2/d2+n3/d3+ ----------

where, n = thenumber of emerged seedlings,

d= number of days.

Final mean germination

The final mean germination ratewas calculated by dividing the cumulative germination percentage/total number of days.

Seedling dry weight (g)

Forthe germination test, ten normal seedlings used for measuring the root and shoot lengthwerekept in a butter paper packet and dried in hot air oven maintained at 80 ± 2°C for 24 hours. The seedlings were subsequentlycooled in desiccators for 30 minutes and the weights of the dry seedlings were recorded using anelectronic balance and expressed in milligrams.

After observing the length of the roots and shoots, ten normal seedlings were placed on butter paper (hot air oven).

Root length (cm)

Forthe germination test, the ten normal seedlings which are used for measuring the shoot lengthwereused for measuring the root length. The root length was measured from thetip of theroot to the hypocotyl point and the mean length was calculated and expressed in centimetres.

Shoot length (cm)

The germination test, ten normal seedlings were randomly selected from each treatment on thetwelfth day and the shoot length was measured from the tip ofthe shoot to the hypocotyl point and the mean length was calculated and expressed in centimeters.

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)]

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

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

IV. Results and Discussion

Among the treatments, T_1 (Seed priming with AgNPs at 1000 ppm for 24 h), T_2 (Seed priming with ZnNPs at 2000 ppm for 24 h), T_3 (Seed treatment with Concentrated H_2SO_4 for 12 min), T_4 (Seed treatment with NaDC 500 ppm for 15 min), T_5 (Dewinging by secature), T_6 (Soaking seeds in hot water at 100□ for 5 minutes), T_7 (Control),T_2 (ZnNPs 2000 ppm) primed seeds recordedsignificantlyhigher seed germination (56.00 %),speed of germination (1.04), final mean germination (1.20)whencompared to control T_7seeds (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 (Mahakham*et al.*, 2017; nanopriming technology for enhancing 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, asreported by Cakmak (2000), and Ajouri*et al.* (2004) found that Zn seed priming significantly improved barley seed germination and seedling development; these findings may indicate that high Zn concentrations in seeds play critical physiological roles during seed germination and early seedling growth (Prasad *et al*., 2012). Zinc oxidenanoparticlesalso increasedpeanut germination, growth and yield.Nanoparticles positivelycontribute to increasing seed qualityvia processes that involveoxidation and reduction during germination. This is achieved by generating superoxide ion radicals, which effectively neutralize free radicals present during seed germination.The increased amount ofoxygen produced in this process leads toincreasedrespiration, which further encourages germination. These results are in accordancewith the findings ofShyla and Natarajan, 2014 and Savithramma*et al*., 2023.

Significantly higher mean shoot (cm) length and root lengthwererecorded in T_2 (ZnNPs 2000 ppm) primed seeds (16.33 and 17.05 cm) respectively as compared to control *i.e.,* T_7 (Untreated) (11.02 and 9.92 cm) respectively in Table.2. This is mainly due to theincrease inthe growth hormones in seeds caused by treatmentwithdifferent concentrations of zinc oxide nanoparticles, which improved other growth parameters in green gram (Lakshmi *et al*., 2017). Zinc nanoparticles enhance plant growth and improve nutrient uptake becauseoftheir small size and high surface area,increasingtheavailabilityofzincfor absorption by plant roots. Micronutrientsare 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 T_2 (ZnNPs 2000 ppm for 24 h) primed seeds (6.22 g) as compared to control *i.e*., T_7 (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 seeds. Owing totheincrease in seedling length (cm) through nanopriming, primed seeds recorded higher seedling dry weight.

Significantly higher mean Seedling Vigour Index-I and SeedlingVigourIndex – II was recorded in T_2 (ZnNPs 2000 ppm for 24 h) primed seeds (1869 and 125) respectively as compared to control *i.e.,* T_7 (untreated) (649 and 51) respectively in Table 3. This is due to the increased synthesis of tryptophan and auxin, a hormone that stimulates longitudinal growth, as well as the abilityof nanoparticlestoincreasecell 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 accelerating of longitudinal growth in *Tanacetumparthenium* (Mahajan*et al*., 2011; Shahhoseini*et al*., 2020; Guo *et al*., 2022).

The mechanism of NP uptake is generally considered active transport, which includes cellular processes such as signalling and regulation of the plasma membrane (Etxeberria*et al*., 2006). NPs canpasses 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, symplastictransportinvolves 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 withthe untreated control, 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 (%),thespeed of germination, final mean germination, seedling shoot length (cm), seedling root length(cm), dry weight and Seedling Vigour indices significantly increased in the seeds treated with nanoparticles compared with the control. The seed treatment with ZnNPs outperforms other treatments examined, including the control.

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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1.

2.

3.

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

Treatments	Germination	Speed of	Final mean

	(%)	germination	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_2SO_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 ^oC 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 transfomed

AgNPs : Silver nanoparticles,

ZnNps : Zinc nanoparticles, Conc

H_2SO_4 : Concentrated sulphuric acid,

NaDC : Sodium de-oxycholate

Table 2: Effects of green synthesized silver and zinc nanoparticles incombinationwith other treatments on the 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_2SO_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 ^oC 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: Effects of green synthesized silver and zinc nanoparticlesincombination with other treatments on the vigour indices of Red sander seedlings

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_2SO_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^oC for 5 min	928	93
T_7- Control (Untreated)	649	51
S.Em (±)	**28.47**	**3.76**
CD at 1%	**86.36**	**11.40**

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

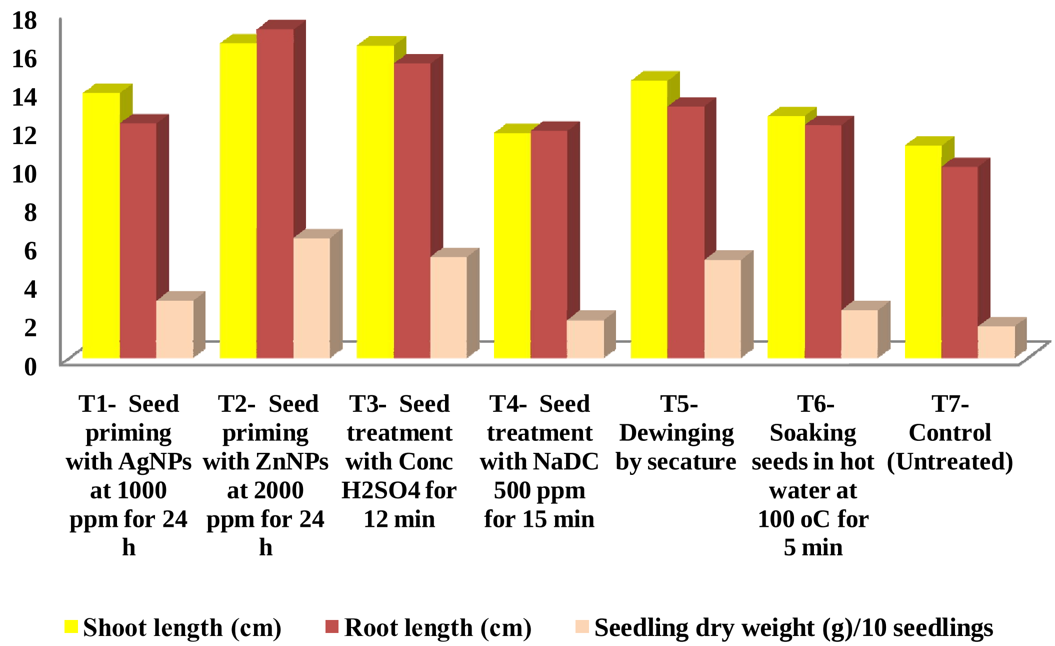
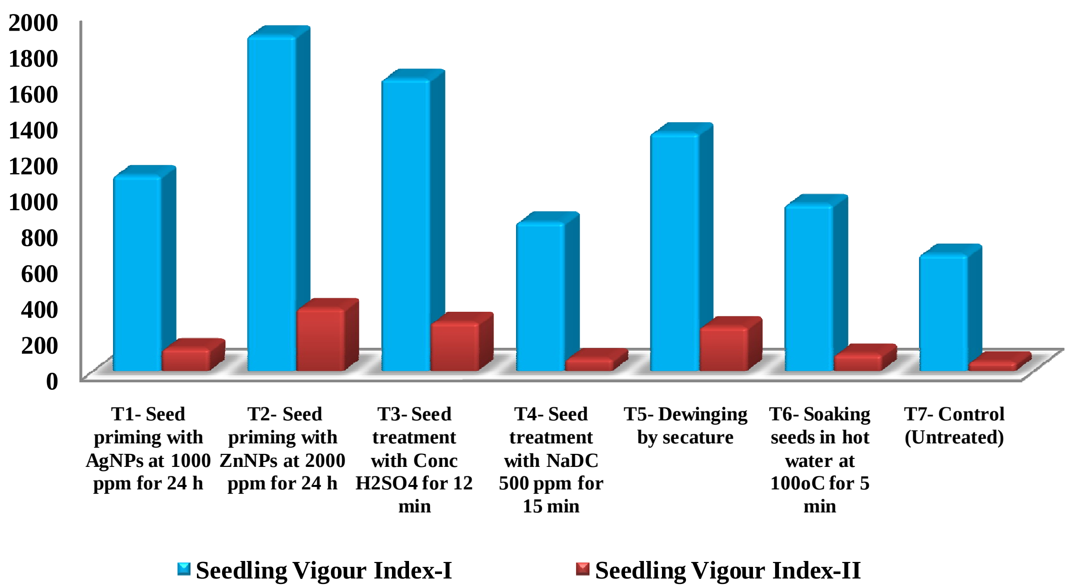


fig . 2 Effect of green synthesized silver and zinc nanoparticles along with other treatments on seedling vigour indices of Red sander (*Pterocarpus santalinus* Linn.)



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