Original Research Article

Evaluating seed viability and germination enhancement through plant growth hormones in *Anogeissuslatifolia* (Roxb. ex DC.) Wall. Ex Guill. &Perr.

ABSTRACT

Aims: To evaluate the seed viability and enhancing germination percentage through plant growth hormones in *Anogeissuslatifolia*.

Study design: A randomized complete block design was used to conduct the investigation.

Place & Duration of Study: Plant nursery, ICFRE- AFRI, Jodhpur, Rajasthan, India; between Feb 2023 and June 2023

Methodology: The viability test was analyzed using a 1% Tetrazolium chloride solution (TZ). About 50g seeds were pre-treated with Gibberellic acid (GA_3) and Indole-3-Butyric acid (IBA) and hormones of concentrations 500, 750, and 1000 ppm each. 1000 pre-treated seeds of each treatment were sowed in the mother bed of the nursery and germination was recorded for up to 15 days. Further germination indices such as germination percentage, germination speed, mean germination time, root length, shoot length, allometric coefficient, and vigor index were calculated.

Results: *A. latifolia* contained around 33.74±6.561% viable seeds, according to a viability analysis using a 1% TZ solution. An analysis of variance in the data revealed a substantial (P≤.001) impact of PGRs and their interaction with the indices under investigation. The best treatment overall was demonstrated by GA_3 750 ppm concentrationfollowed by GA_3 1000 ppm and IBA 750 ppm concentrations. IBA 1000 ppm, however, showed a decreased effect on germination studies.

Conclusion: Gibberellic acid overall had a beneficial effect on germination and all the studied indices. These hormonal treatments can be used to propagate the plant species for plantation, afforestation, or conservation purposes.

Keywords: germination, PGRs, viability, vigor index

1. INTRODUCTION

Anogeissuslatifolia (Roxb. ex DC.) Wall. Ex Guill. &Perr., commonly known as Axle wood and locally known as ‘Safed Dhok’, is a moderate-sized Indigenous economic tree of India and is distributed throughout East Asia and native to countries of Nepal, Myanmar, and Sri Lanka (Yadav et. al. 2019; Wealth of India). In India, it is distributed in deciduous forests of the Himalayas and South Indian Hills (Sharma et al, 2020) except East Bengal and Assam (Patil& Gaikwad, 2011). The tree is about 15-20 meters tall and has an erect trunk with drooping branchlets and smooth white-grey bark that exfoliates in irregular thin scales (Shetty and Singh, 1987). Leaves are oblong, glabrous, opposite or sub-opposite,and coriaceous. Flowers are yellow to pinkish yellow in color, sessile, and in dense heads. Fruits are small, compressed, winged with beak, and seed ovoid. Pollen grains are yellow, prolate–spherical, manods, radially symmetrical, tricolporate with three subsidiary colpi and exine surface micro regulates-

echinate (Dinesh, 2018). The flowering and fruiting of the plant is from September to March (Bagayatkar and Garge, 2018).

At regional and global levels, *A. latifolia*forests have been crucial in reducing carbon emissions and preventing climate change (Chauhan et.al. 2020). Bulk density and other physical characteristics of the soil including soil pH considerably dropped when reforestation was done on degraded land (Mutanal et. al. 2016). Compared to non-forest locations, forest sites produced more nutrients, while *A. latifolia* had the highest capacity for producing nutrients in comparison with the other forest-type species (Sheikh et. al. 2010). The plant helps in erosion control as it is a good survivor on eroded land therefore used in river bank stabilization (Nag et. al. 2007). It improves soil as it contributes to soil nutrient cycling, exhibiting high leaf-litter decomposition rates. The potential worth of its timber is enormous, and the leaves are regarded as superior feed for livestock (Kumar et. al. 2012). The wood is used for erecting fences on field bunds and thus acts as a boundary barrier or support. The Indian gum known as "Ghatti gum," extracted from the plant is used in the paper industry and calico painting, (Singh et. al. 2022). In addition to its significant medicinal and commercial value, *A. latifolia* supports the local population's livelihoods by fostering economies that uphold ecological equilibrium.

The sole way to reproduce naturally in a plant species is through seeds. Despite this fact, factors influencing seed germination include the genetic makeup of the seed, the conditions surrounding its harvest, and the pre-treatment methods used to break dormancy. Both internal and external factors affect the germination and quality of seeds (Lamichhane et al, 2018). Seed dormancy, seed viability, and genetic makeup are internal elements that are passed down from parent plants that affect the seed germination potential and subsequent seedling growth. Variations in the seed supply between species can have a major impact on the growth and quality of the seed. The growth environment, which includes temperature, moisture content, light, and soil characteristics (Han and Yang, 2015), is influenced by external elements that also affect seedling germination and establishment. Furthermore, external treatments including chemical treatments, scarification, and seed priming can alter the characteristics of the seed coat, end dormancy, or promote the growth of seedlings (Huang et al, 2023).

The physiological traits of seeds that govern their capacity to both survive a variety of primarily unfavorable environmental conditions and to sprout quickly in the soil are referred to as their "vigor of viability" (Milošević et al, 2010). The three main environmental elements that impact seed deterioration and loss of viability are moisture, temperature, and oxygen percentage (De Vitis et al, 2020). Before cultivation, sale, and planting, it is imperative to ascertain the germination state and viability of seeds. This is because seed viability plays a major role in seed quality features that indicate potential for seed germination.

Several studies have been conductedin the field of plant physiology and it has been found that gibberellic acid followed by indole-butyric acid is the most effective hormone in breaking seed dormancy. A state of metabolic pause known as seed dormancy keeps seeds from sprouting in ideal circumstances. Many plant species depend on this phenomena to survive and spread because it keeps seeds viable until they are exposed to environments that support their growth. Plant hormones called gibberellic acid (GA) and indole-3-butyric acid (IBA) are essential for thawing seed dormancy (Bewley and Black, 1994). IBA is known to encourage root growth and development,

whereas GA is a strong activator of cell growth and division. The concern with *A. latifolia* is that they are slow-growing and have low seed viability and germination rates, which makes the surviving established seedlings difficult to thrive in the wild. As a result, demographic instability is present in more than 50% of reported species. In several species, the seedling-to-sapling and sapling-to-adult tree growth rates have been comparatively low (Singh and Singh, 2011). Therefore, this study aims to give an idea of the species' seed viability and further contribute knowledge on seed germination using plant growth hormones for maximum germination and establishment of the understudied species.

2. MATERIALS AND METHODS

2.1 Seed collection, handling, processing and storage

Freshly harvested seeds were collected from dry deciduous forests of Shahbad, Baran, Rajasthan, India (N: 25°12.996', E: 77°08.360') in January 2023. Seeds were simply cleaned from the unwanted particles and dust and stored in sealed plastic containers at room temperature (28±4°C). The moisture percent of mature seeds determined was approximately 0.5-1%.

2.2 Seed viability assessment

2.2.1 Pre-Treatment:*Anogeissuslatifolia*fruits being small and view its difficulty to remove each seed from them, they were first soaked in distilled water to soften their seed coat for about 12-16 hr. This operation facilitated the water solution penetration to the staining later.

2.2.2 Seed Sampling and staining: A solution of 1% Tetrazolium chloride (TZ) were obtained by dissolving 1 gram of TZ in one hundred milliliters of distilled water. Experiment consisted of immersing 150 seeds in this 1% TZ solution contained in a Petri plate of diameter 15 cm (ISTA rules, 2017). A set of 5 replications (Petri plates) was taken to perform the test typically at 28-30°C, for about 18 h.

2.2.3 Interpretation: After staining, the seeds were examined under a Dewinter microscope (10 X). The staining pattern of the seeds were analyzed to determine the seed viability. Fully stained embryos were considered viable, while partially stained or unstained embryos were non-viable (ISTA 2017, rules).

Viability percent= (No. of seeds stained /total number of seeds) × 100%

2.3 Hormonal pre-treatment experiment

PGRs (plant growth hormones) used- Gibberellic acid (GA_3) and Indole-3-Butyric Acid (IBA)

50 g of dried seeds were pre-treated with PGR- GA_3 (500 ppm, 750 ppm, 1000 ppm) and IBA (500 ppm, 750 ppm, 1000 ppm) for 18 hrs. The seeds were then sown in the mother bed of the nursery having soil: sand (1:1) ratio. The seeds were watered regularly to maintain the moisture.

2.3 Seed Germination standard and growth

2000 pre-treated seeds along with control were sowed in the mother bed of the nursery of dimension (100cm × 480 cm × 60 cm) at ICFRE-Arid Forest Research Institute, Jodhpur. Each of the six treatments [2 hormones (GA3 and IBA) × 3 concentrations (500, 750 and 1000 ppm)] was repeated three times. The germination percentage was calculated on the 15^{th} day after sowing (DAS). The seed was considered to have germinated when the radicle showed through the seed coat and was longer than 2-3 mm. When the quantity of germinated seeds did not rise for five days in a row, the germination process was declared over. The germinated seedlings in each treatment were recorded and replicated three times. Counting of the number of seeds that germinated continued until the fifteenth day from the day of sowing. Calculations were made and their significance was examined.

2.4 Studied indices and calculations

List 1 :Studied indices and calculations

Studied indices	Calculation formula	Unit of measurement	Used References
Germination percentage (GP)	GP= Ni/N * 100	%	Huang et al 2023
Speed of germination (S)	S= ni/di	number/day	Khalaki et al 2019
Mean germination time (MGT)	MGT = ∑ni∗di/N	day	El-Nour and Attia, 2022
Root length (Rl)	ruler	cm	Khalaki et al 2019
Shoot length (Sl)	ruler	cm	
Allometric coefficient (AC)	Ac= Sl/ Rl	-	
Vigour index (Vi)	Vi= (Rl+Sl) * GP	-	

N: Total number of seeds, Ni: germinated seeds at the end of counting days, ni: germinated seeds per day, and di: counting day,

2.5 Statistical Analysis

SPSS 29.0.2.0 software was used for statistical analysis and the data means were analyzed using Tukey's HSD test for statistical significance (P<.01). Each test of studied indices (List 1) was analyzed independently using one way ANOVA and Welch's test tested the test for homogeneity of variance to assess the unequal variance means of each studied indices.

3. RESULTS

3.1 Viability study

In Table 1, the mean number of stained seeds found was 50.6 ± 9.842 and the mean viability percent recorded was 33.74 ± 6.561. Seed viability percentage indicates its vigor and establishes their capacity to germinate. Therefore, this is a crucial factor that must be evaluated before seed germination or seed sowing. The viability percent determines that approximately 34% of the seeds are viable and may or may not germinate depending on the external factors as discussed or due to the major cause i.e. dormant seeds.

Table 1: Mean descriptive statistics of *A. latifolia* seed viability

	Mean	Mean Std. Error	Std. Deviation
Stained	50.600	9.841	22.006
Total	150	-	-
Viability percent	33.736	6.561	14.669

3.2 Effect of pre-treatments on seed viability and seedling vigor

ANOVA results showed that pre-treatments significantly (*P*<.001) affected seed germination and studied indices- germination speed, mean germination time, root length, shoot length, allometric coefficient, and vigor index. The analysis found a high statistically significant difference in the means of germination percent, speed, mean germination time, average root length, average shoot length, allometric coefficient, and vigor index. For germination percent, the F-value (Table 2) is 60.366, (P <.001); the germination speed F-value is 26.135, (P <.001); mean germination time F-value is 31.270, (P<.001); average root length F-value is 26.606, (P<.001); average shoot length F-value is 73.378, (P<.001); allometric coefficient F-value is 23.758, (P<.001); and Vigour index F-value is 86.138, (P<.001). There were six degrees of freedom between groups and fourteen within the groups.

Table 2: Analysis of variance results showing the effect of pre-treated seeds on studied indices of *A. latifolia*

Source of Variance		df	sum of squares	Mean square	F-value	P-value
germination percent	Between groups	6	17.225	2.871	60.366	<0.001
	Within groups	14	0.66	0.048		
	Total	20	17.891			
speed of germination	Between groups	6	1.978	0.33	97.782	<.001
	Within groups	14	0.177	0.013		
	Total	20	2.155			
mean	Between	6	0.045	0.008	31.27	<.001

germination time	groups					
	Within groups	14	0.003	0		
	Total	20	0.048			
Average root length (cm)	Between groups	6	0.836	0.139	26.606	<.001
	Within groups	14	0.073	0.005		
	Total	20	0.91			
Average shoot length (cm)	Between groups	6	2.552	0.435	81.212	<.001
	Within groups	14	0.073	0.005		
	Total	20	2.626			
Averageallometric coefficient	Between groups	6	0.044	0.007	25.115	<.001
	Within groups	14	0.004	0		
	Total	20	0.048			
Average vigor index	Between groups	6	1254.841	209.14	83.28	<.001
	Within groups	14	35.158	2.511		
	Total	20	1289.999			

From Table 2, Analysis of variance signifies the studied indices means were highly significant (P<.001). Since the variance means of each indices were unequal, to assess the homogeneity of variance in all the indices, Welch's test was carried out which further indicated that they have highly significant differences between their means.

Table 3: Influence of hormonal treatment on the means of the studied indices (P<.001)

pre-treatment	Germination percent (%)	speed of germination (number per day)	mean germination time (day)	Avg. root length (cm)	Avg. shoot length (cm)	Avg. allometric coefficient	Vigor index
Control	1.400±0.057	1.051±0.041	0.089±0.004	3.933±0.033	2.933±0.067	0.746±0.023	9.613±0.401
IBA 500 ppm	1.800±0.057	1.275±0.066	0.103±0.004	4.000±0.057	3.133±0.033	0.783±0.006	12.843±0.482
IBA 750	2.417±0.	1.326±0.08	0.137±0.0	4.133±0.0	3.333±0.0	0.806±0.0	18.057±1.0

ppm	116	5	18	33	33	02	1
IBA 1000 ppm	0.330±0.260	0.868±0.031	0.040±0.005	3.967±0.033	3.033±0.033	0.765±0.008	2.311±1.82
GA3 500 ppm	2.333±0.120	1.430±0.049	0.133±0.009	4.233±0.033	3.567±0.033	0.843±0.007	18.190±0.852
GA3 750 ppm	3.283±0.072	1.825±0.107	0.191±0.004	4.367±0.033	3.767±0.033	0.863±0.001	26.697±0.435
GA3 1000 ppm	2.800±0.057	1.666±0.032	0.163±0.006	4.500±0.057	3.900±0.057	0.867±0.002	23.507±0.162

Germination percent standard: The germination percent means of some of the groups differ significantly according to posthoc comparison using Tukey's HSD test (α=0.05; P<.001). The pre-treatments given to the *A. latifolia* seeds of IBA (500 ppm, 750 ppm, 1000 pm) and GA_3 (500 ppm, 750 ppm, 1000 pm) concentration, all had a significant effect (P<.01) as compared to the control in terms of germination percent means except IBA 500 ppm which showed a low significance (P=.332). Pre-treatment with IBA 500 ppm, IBA 750 ppm, GA_3 500 ppm, GA_3 500 ppm and GA_3 1000 pm showed an increased mean germination percent i.e. 1.8±0.057 %, 2.417±0.116 %, 2.333±0.120 %, 3.283±0.072 %, and 2.8±0.057 % respectively. When compared to other treatments and their interaction to control, higher significant differences in their germination percent means were observed (P<.001). IBA 750 ppm pre-treatment (P<.001) has a highly significant effect on the germination percent means and has overall decreased the mean germination percent (0.330±0.260 %) when compared to others and control.

In terms of germination speed (Table 3), when compared to the control (1.051±0.041 number per day) GA_3 750 ppm showed the highest speed of germination means (1.825±0.107 number per day) followed by GA_3 1000 ppm (1.666±0.032 number per day), and is highly significant (P<.001). IBA 1000 ppm however showed the lowest means of germination speed (0.868±0.031 number per day) and no difference in their means when grouped with control according to Tukey's HSD test (α=.05). Likewise in terms of mean germination time (Table 3) when compared to the control (0.089±0.0004 day), GA_3 750 ppm showed the highest speed of germination means (0.191±0.004 day; P<.001) followed by GA_3 1000 ppm (0.163±0.006 day; P<.001) but no difference when their means are grouped. IBA 1000 ppm showed the lowest means of germination speed (0.040±0.126 day ; P<.01).

The average root length (Table 3) was found maximum in GA_3 1000 ppm (4.500±0.057 cm) and minimum in IBA 1000 ppm treatment (0.040±0.005 cm). Similarly, the average shoot length was found maximum in GA_3 1000 ppm (3.900±0.057 cm) and minimum in IBA 1000 ppm treatment (3.033±0.033 cm). IBA 500 ppm, IBA 750 ppm, GA_3 750 ppm, and GA_3 1000 ppm treated average shoot length differences in their means were found to be significant (P<.01) whereas, in IBA 500 ppm and IBA 1000 ppm, the average shoot length differences in their means were not significant (P=.068 and P=.673) when compared to control. IBA 500 ppm (Rl=4.00±0.057; Sl=3.133±0.033) and 750 ppm (Rl=4.133±0.033; Sl=3.333±0.033) length means combined were significantly different

(Table 3, P<.01) from the length means of GA_3 750 ppm (Rl=4.367±0.033; Sl=3.767±0.033) and 1000 ppm (Rl=44.500±0.057; Sl=3.900±0.057).

The differences in average allometric coefficient means of IBA pre-treatments and GA_3 hormone pre-treatments are significantly different from each other. Each concentration of GA_3 i.e. GA_3 500 ppm (0.0963±0.0139; P<.001), GA_3 750 ppm (0.1167±0.0139; P<.001), and GA_3 1000 ppm 0.121±0.0139; P<.001) when compared to the control shows highly significant allometric coefficient mean differences whereas IBA 500 ppm (0.0373±0.0139) and IBA 1000 ppm (0.01867±0.0139) allometric coefficient mean differences when compared to control were found to be non-significant (P=.777 and P=.825 respectively). IBA 750 ppm (0.0606±0.0139; P=.009) showed significant allometric coefficient means when compared to control means.

The maximum seed vigor index is reported in GA_3 750 ppm (26.697±0.435) as compared to the rest of the pre-treatments (Table 3) and minimum in IBA 1000 ppm (2.310±1.820) when compared to the control (9.613±0.401).

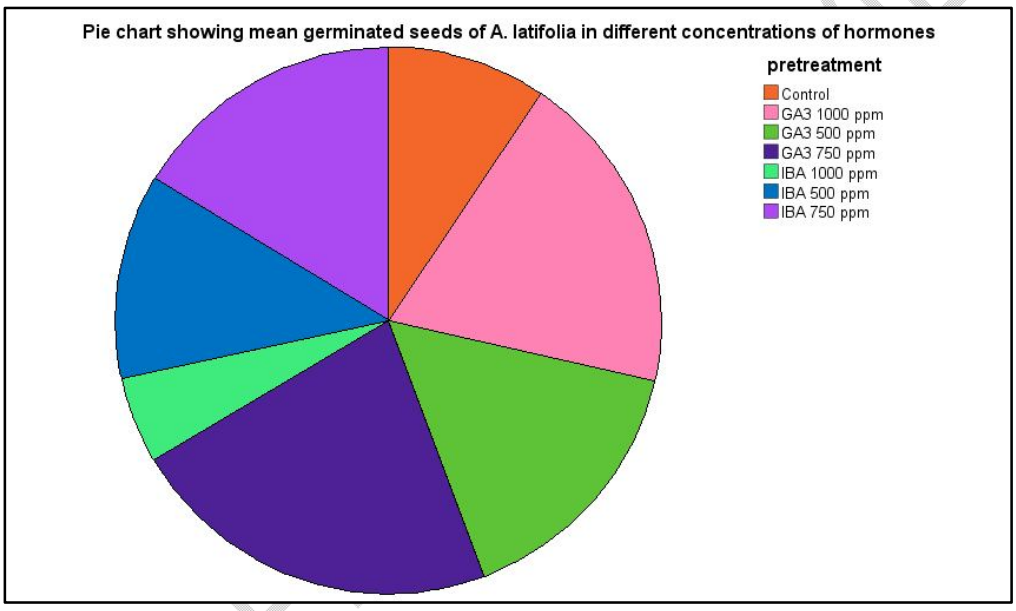

Figure 1: Mean germinated seeds of *A. latifolia*in different concentrations of hormones using a pie chart for comparison

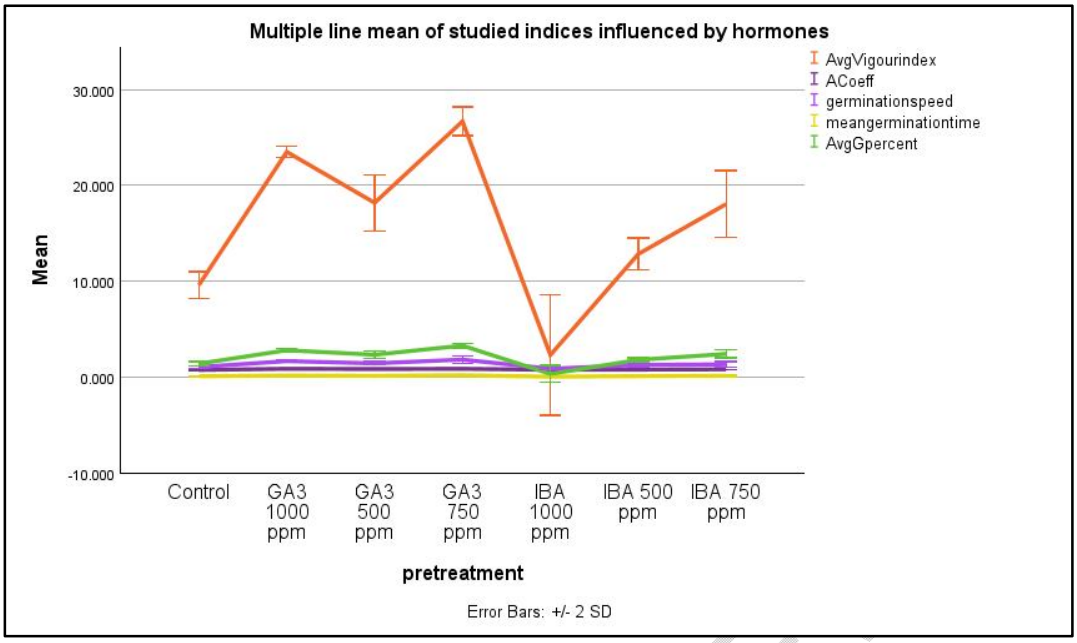

Figure 2: Multiple line graph comparing the means of studied indices due to the influence of varied concentrations of hormones

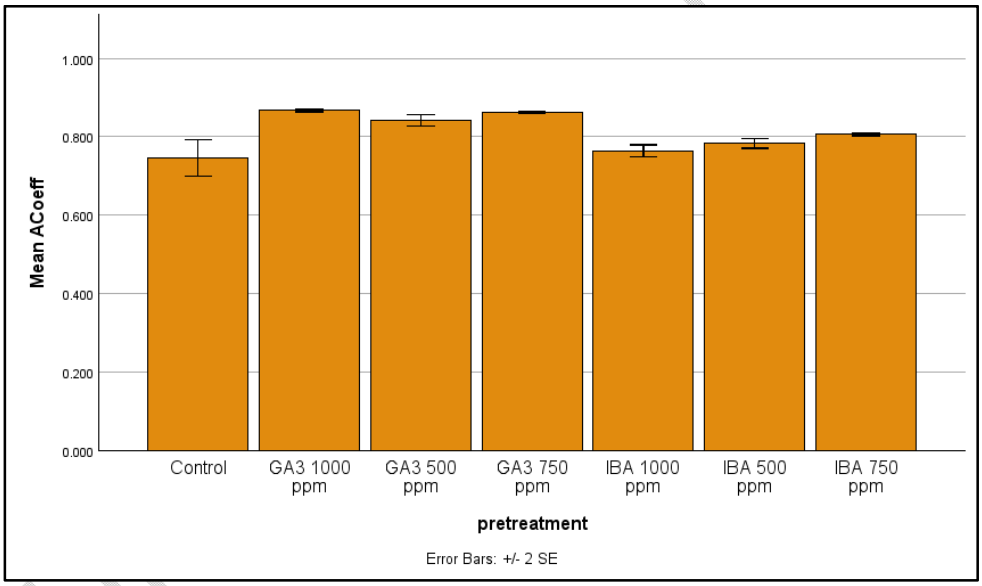

Figure 3: Positive impact on average allometric coefficient means of *A. latifolia* seeds using different concentrations of hormones when compared to control

4. DISCUSSION

Both $GA_3$and IBA hormones (except IBA 1000 ppm) showed an increase in their mean number of germinated seeds and hence mean germination percent. GA_3 750 ppm and GA_3 1000 pm are more effective pre-treatments than others as they show higher differences in germination percent means compared to rest but show less difference when they are grouped indicating similar germination percent means which indicates both the treatment can be used for maximum germination study. Overall these three pre-treatments- IBA 750 ppm, GA_3 750 ppm, and GA_3 1000 ppm had a greater positive

effect on the mean germination percent of *A. latifolia* seeds (Figure 2). Results show the early germination of seeds using the gibberellic acid concentrations (750 and 1000 ppm). GA_3 750 and GA_3 1000 ppm show no difference when their means are grouped according to the Tukey's test which indicates they have a somewhat similar effect on mean germination time and mean germination speed of *A. latifolia* seeds. Average root length and average shoot length means were found to increase more in seeds pre-treated with varied gibberellic acid concentrations. IBA 500 ppm and 750 ppm length means combined were significantly different (Table 3, $P<.01$) from the length means of GA_3 750 ppm and 1000 ppm but less differences in their means when they are grouped. This relates that both the gibberellic acid and indole-butyric acid concentrations had a positive impact separately but not much difference between the two concentrations of selected hormones was observed in terms of seedling length when grouped. Gibberellic acid had an overall high effect on seedling length. As shown in Figure 3, the pre-treatment by PGRs has improved the allometric coefficient when compared to the control and seen a positive effect. The average vigor index is seen to decrease in the highest concentrations (1000 ppm) of both the hormones i.e. Gibberellic acid and Indole-3 butyric acid when compared to their own other two concentrations (500 ppm and 1000 ppm). The lowest vigor index is observed in IBA 1000 ppm when compared to the control which indicates repressing activity or a negative effect on seed germination.

5. CONCLUSION

The study's results highlight the significance of employing plant growth hormones to improve *Anogeissuslatifolia* seed germination. Gibberellic acid helped in increasing the number of germinated seeds (Figure 1), thereby increasing the overall germination percentage. It also increased their average shoot lengths in addition to having a generally beneficial effect on germination (Figure 2). IBA 1000 ppm decreased the amount of seeds that germinated and damaged seed germination. The reason could be the balance of plant hormones required for germination, such as auxins, gibberellins, and cytokinins, may be upset by high hormone levels. The allometric coefficient was improved overall indicating the increase in shoot: root ratio in the pre-treated seedlings. The most successful treatment for a general improvement in the examined indices was demonstrated by GA_3 750 ppm and GA_3 1000 ppm as well as IBA 750 ppm. These hormonal treatments can be used to propagate the plant species for plantation, afforestation, or conservation purposes.

Disclaimer (Artificial intelligence)

Option 1:

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

Option 2:

Author(s) hereby declare that generative AI technologies such as Large Language Models, etchave been used during writing or editing of manuscripts. This explanation will include the name, version, model, and source of the generative AI technology and as well as all input prompts provided to the generative AI technology

Details of the AI usage are given below:

1.
2.
3.

REFERENCES

Abou El-Nour, Hala Hassan, and Reda Mohamed Attia. "Evaluate the effects of rare earth elements on sweet pepper seeds germination process, seedlings growth, and plants productivity." *GSC Advanced Research and Reviews.*2022; 13(1): 023-038.

Bagayatkar M.A. and Vaibhavi N. Garge. Evaluation of Cytotoxic Activity of Hydro Alcoholic Extract of*AnogeissusLatifolia*with Brine Shrimp Lethality Assay, *World Journal of Pharmaceutical Research*. 2018; 7(8):1182-1189.

Bewley, J. D., & Black, M. Seeds: Physiology of development and germination .1994(2nd ed.). Plenum Press.

Byraiah Dinesh. Pollen biology and morphology of*Anogeissuslatifolia,* (Roxb. ex DC) Wall. exBedd. (Combretaceae), *International Journal of Botany Studies*. 2018; 3(2):121-123.

De Vitis, Marcello, Fiona R. Hay, John B. Dickie, Clare Trivedi, Jaeyong Choi, and Rob Fiegener. "Seed storage: maintaining seed viability and vigor for restoration use" *Restoration Ecology.* 2020; 28: S249-S255.

Han, C., & Yang, P.. Studies on the molecular mechanisms of seed germination. *Proteomics*. 2015;*15*(10):1671-1679.

Huang, Y., Wei, F., Ma, Q., Lin, Y., Huang, J., Zhu, Y., & Tang, D. Substrate, Hormone, Winnowing, and Stratification Influence the Seed Germination of Ilex asprella (Hook. et Arn.) Champ. exBenth. *Phyton*. 2023: *92*(7)

Khalaki, Masoomeh&Ghorbani, Ardavan&Dadjou, Farid. Influence of Nano-Priming on Festucaovina Seed Germination and Early Seedling Traits under Drought Stress, in Laboratory Condition. 2019; 7:133-139.

Lamichhane, J. R., Debaeke, P., Steinberg, C., You, M. P., Barbetti, M. J., &Aubertot, J. N. Abiotic and biotic factors affecting crop seed germination and seedling emergence: a conceptual framework. *Plant and soil*. 2018; *432*:1-28.

Milošević, M., Vujaković, M., &Karagić, Đ. Vigour tests as indicators of seed viability. *Genetika*. 2010; *42*(1):103-118.

Prudente, D. O., &Paiva, R. Seed dormancy and germination: Physiological considerations. *Journal of Cell and Developmental Biology* 2018. *2*(1):2.

Sharma V. C., Kaushik Atul, Dey Y.N., Bhavana Srivastava, Wanjari M. and BhagatJaiswal. Analgesic, anti-inflammatory and antipyretic activities of ethanolic extract of stem bark of*Anogeissuslatifolia*Roxb, *Clinical Phytoscience*. 2020; 6(22):1-9.

Shetty B. V. and V. Singh. *Flora of Rajasthan*, Botanical Survey of India, 1:313-314.

Singh, J. S., & Singh, K. D. Silviculture of dry deciduous forests, India. *Silviculture in the tropics* 2011; 1:273-283.

U. H. Patil and D. K. Gaikwad. Ethno-pharmacological Review of a Herbal Drug:*Anogeissuslatifolia, International Journal of Pharma Sciences and Research*. 2011; 2(1):41-43.

Yadav V.K., Sharma Sonam and P. K. Khare. Application of Incubation-Drying-Separation Method for Viable and Dead Seeds Fractions in*AnogeissusLatifolia, FLORA AND FAUNA*. 2019; 25:03-06.