

Original Research Article

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

ABSTRACT

Aims: To evaluate the seed viability percent and enhancing germination enhancement percentage through plant growth hormones in *Anogeissus latifolia*.

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) TZ-solution. About 50g seeds were pre-treated with Gibberellic acid (GA₃) and Indole Butyric acid (IBA) GA₃ and IBA 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 ($P \leq 0.001$) impact of PGRs and their interaction with the indices under investigation. The best treatment was demonstrated by GA₃ 750 ppm concentrations overall, followed by GA₃ 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

Anogeissus latifolia, 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

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Comment [TY2]: P/se check! Indole-3-Butyric Acid (IBA)

Comment [TY3]: Is this true (GA 750 is better than GA 1000 in Table 4a) for average root length, average shoot length & allometric coefficient?? P/se check & correct it.

Comment [TY4]: P/se write the full scientific name for the 1st mention. *Anogeissus latifolia* (Roxb. ex DC.) Wall. Ex Guill. & Perr.

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, *Anogeissus-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 way of life by fostering economies that uphold ecological equilibrium.

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.

Overall, The concern with *A. nogoissus 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.

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e.g. et. al., or et al.
Yadav et. al. 2019

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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 Seed Sampling: A set of 5 replications is taken to perform the test. Each replication has 150 seeds placed in a Petri plate of diameter 15 cm.

2.2.2 Pre-Treatment: The fruits are small and it is difficult to remove each seed from the fruit so they are first soaked in distilled water to soften the seed coat for about 12-16 hrs, which facilitates the penetration of the staining solution later.

2.2.3 Staining: 1 gram of Tetrazolium chloride solution (TZ) is dissolved in one hundred milliliters of distilled water to create a 1% TZ solution. Immerse the seeds in a 1% TZ (according to ISTA rules), typically at 28-30°C, for about 18 h.

2.2.4 Interpretation: After staining, the seeds are examined under a Dewinter microscope (10X). The staining pattern is analyzed to determine the viability of the seeds. Fully stained embryos are considered viable, while partially stained or unstained embryos are non-viable.

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

2.3 Hormonal pre-treatment experiment

PGRs (plant growth hormones) used- Gibberellic acid (GA₃) and Indole Butyric acid (IBA)

50 g of dried seeds were pre-treated with PGR- GA₃ (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 treatment was repeated three times. The germination percentage was calculated on the 15th day after sowing. 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 15th day from the day of sowing. Calculations were made and their significance was examined.

2.4 Studied indices and calculations

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1. cite a reference.
2. mention the color of viable & non-viable seeds.
3. also include other findings.

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Indole-3-Butyric Acid (IBA)

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6 treatments, 150 seeds/replication, 5 replications

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What about Replication?? What is the difference b/n them?

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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)	$MGT = \frac{\sum ni * di}{N}$	day	El-Nour and Attia, 2022
Root length	ruler	cm	Khalaki et al 2019
Shoot length	ruler	cm	Khalaki et al 2019
Allometric coefficient (AC)	$Ac = SI/RI$	-	Khalaki et al 2019
Vigour index (Vi)	$Vi = (RI+SI) * GP$	-	Khalaki et al 2019

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

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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 was analyzed independently and **Welch's test tested the test for** homogeneity of variance to assess the unequal variance means of each studied indices **was performed by Welch's test**.

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3. RESULTS AND DISCUSSION

3.1 Seed Viability study test

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 . The viability percent was determined using a 1% TZ solution. The viability percentage indicates the seed vigor and establishes the capacity of the seed to germinate. Therefore, this is a crucial factor that must be evaluated before seed germination or seed sowing.

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Then, p/se discuss main findings with previous results by comparing and contrasting & citing relevant & important references to your findings.

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

	Minimum	Maximum	Mean	Mean Std. Error	Std. Deviation
Stained	19.00	73.00	50.600	9.841	22.006
Total	150	150	150	-	-

Viability percent	12.67	48.67	33.736	6.561	14.669
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3.2 Germination standard and studied indices using varied concentrations of PGRs

An ANOVA test was performed to assess whether pre-treatments affected seed germination and studied indices—germination speed, mean germination time, root length, shoot length, allometric coefficient, and vigor index. The analysis of ANOVA test found a highly 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.

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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 germination time	Between groups	6	0.045	0.008	31.27	<.001
	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			
Average-allometric coefficient	Between groups	6	0.044	0.007	25.115	<.001
	Within groups	14	0.004	0		
	Total	20	0.048			
Avg-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 (Table 3) was carried out which further indicated that they have highly significant differences between their means.

Table 3: Welch's test statistics result for studied indices and its significance in comparing means of independent groups with mean-variance differences

Studied indices	Statistic ^a	df1	df2	P-value
germination percent	67.696	6	6.15	<.001
speed of germination	40.675	6	6.134	<.001
mean germination time	66.951	6	6.165	<.001
Avg. root length (cm)	17.813	6	6.198	<.001
Avg. shoot length (cm)	49.498	6	6.194	<.001
Avg. allometric coefficient	118.339	6	6.015	<.001
Avg. vigor index	172.612	6	5.837	<.001

a. Asymptotically F distributed

Table 4a: 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

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IBA 750 ppm	2.417±0.116	1.326±0.085	0.137±0.018	4.133±0.033	3.333±0.033	0.806±0.02	18.057±1.01
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

Table 4b: Influence of hormonal treatment and interaction with each other on the studied indices

I	J	Mean difference (I-J)						
		Germ ination percent (%)	Germ ination speed (N/D)	mean germination time (day)	Avg. root length (cm)	Avg. shoot length (cm)	Avg. allometric coefficient	Vigor index
IBA 500 ppm	control	0.4±0.178	0.2233±0.0917	0.013±0.0126	0.0667±0.0591	0.2±0.0617	0.0373±0.0139	3.23±1.272
	IBA 750 ppm	0.616±0.178**	0.051±0.0917	0.035±0.0126	0.133±0.0591	0.2±0.0617	0.0233±0.0139	5.213±1.272*
	IBA 1000 ppm	1.47±0.178*	0.407±0.0917*	0.0626±0.0126*	0.333±0.0591	0.1±0.0617	0.01867±0.0139	10.533±1.272*
	GA3 500 ppm	0.533±0.178*	0.1553±0.0917	0.0306±0.0126*	0.233±0.0591**	0.433±0.0617*	0.059±0.0139**	5.345±1.272*
	GA3 750 ppm	1.483±0.178*	0.550±0.0917*	0.0883±0.0126*	0.3667±0.0591*	0.633±0.0617*	0.0793±0.0139*	13.853±1.272*
	GA3 1000	1±0.1	0.391	0.0606	0.5±0.0	0.7667	0.0836	10.663

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	ppm	78*	±0.09 17*	±0.012 6*	591*	±0.061 7*	±0.013 9*	±1.272 *
IBA 750 ppm	control	1.016 ±0.17 8*	0.274 ±0.09 17	0.0483 ±0.012 6**	0.2±0.0 591	0.4±0. 0617*	0.0606 ±0.013 9**	8.443± 1.272*
	IBA 500 ppm	0.616 7±0.1 78*	0.051 ±0.09 17	0.035± 0.0126	0.1333 ±0.059 1	0.2±0. 0617	0.0233 ±0.013 9	5.213± 1.272* *
	IBA 1000 ppm	2.086 7±0.1 78*	- 0.458 ±0.09 17*	0.0976 ±0.012 6*	0.1667 ±0.059 1	0.3±0. 0617*	0.042± 0.0139	15.746 ±1.272 *
	GA3 500 ppm	0.083 ±0.17 8	- 0.104 3±0.0 917	0.0043 ±0.012 6	- 0.1±0.0 591	- 0.233± 0.0617 **	- 0.0356 ±0.013 9	- 1.333± 1.272
	GA3 750 ppm	- 0.866 6±0.1 78*	- 0.499 ±0.09 17*	- 0.0533 ±0.012 6**	- 0.2333 ±0.059 1**	- 0.433± 0.0617 *	- 0.056± 0.0139 **	- 8.64±1 .272*
	GA3 1000 ppm	- 0.383 ±0.17 8	- 0.34± 0.091 7*	- 0.0256 ±0.012 6	- 0.3667 ±0.059 1*	- 0.5667 ±0.061 7*	- 0.060± 0.0139 **	- 5.45±1 .272**
IBA 1000 ppm	control	- 1.07± 0.178 *	- 0.183 6±0.0 917	- 0.0493 ±0.012 6**	0.033± 0.0591	0.1±0. 0617	0.0186 7±0.01 39	- 7.3026 ±1.272 *
	IBA 500 ppm	- 1.470 ±0.17 8*	- 0.407 ±0.09 17*	- 0.0626 ±0.012 6*	- 0.033± 0.0591	- 0.1±0. 0617	- 0.0186 7±0.01 39	- 10.532 6±1.27 2*
	IBA 750 ppm	- 2.086 7±0.1 78*	- 0.458 ±0.09 17*	- 0.0976 ±0.012 6*	- 0.1667 ±0.059 1	- 0.3±0. 0617*	- 0.042± 0.0139	- 15.746 ±1.272 *
	GA3 500 ppm	- 2.003 ±0.17 8*	- 0.562 3±0.0 917*	- 0.093± 0.0126 *	- .2667± 0.0591 *	- 0.533± 0.0617 *	- 0.0776 ±0.013 9*	- 15.879 ±1.272 *
	GA3 750 ppm	- 2.953 ±0.17 8*	- 0.957 ±0.09 17*	- 0.151± 0.0126 *	- 0.4±0.0 591*	- 0.733± 0.0617 *	- 0.0980 ±0.013 9*	- 24.386 ±1.272 *

	GA3 1000 ppm	- 2.470 ±0.17 8*	- 0.798 ±0.09 17*	- 0.123± 0.0126 *	- 0.533± 0.0591 *	- 0.8667 ±0.061 7*	- 0.1023 ±0.013 9*	- 21.196 ±1.272 *
GA3 500 ppm	control	0.933 3±0.1 78*	0.378 ±0.09 17*	0.044± 0.0126 **	0.3±0.0 591*	0.633± 0.0617 *	0.0963 ±0.013 9*	8.576± 1.272*
	IBA 500 ppm	0.533 3±0.1 78	0.155 ±0.09 17	0.0306 7±0.01 26	0.233± 0.0591 **	0.433± 0.0617 *	0.059± 0.0139 **	5.346± 1.272* *
	IBA 750 ppm	- 0.083 3±0.1 78	0.104 ±0.09 17	- 0.0043 ±0.012 6	0.1±0.0 591	0.233± 0.0617 **	0.0356 ±0.013 9	0.1333 ±1.272
	IBA 1000 ppm	2.003 ±0.17 8*	0.562 ±0.09 17*	0.0933 ±0.012 6*	0.2667 ±0.059 1*	0.533± 0.0617 *	- 0.0776 ±0.013 9*	15.879 ±1.272 *
	GA3 750 ppm	- 0.95± 0.178 *	- 0.395 ±0.09 17*	- 0.0576 ±0.012 6*	- 0.133± 0.0591	- 0.2±0. 0617	- 0.0203 3±0.01 39	- 8.5067 ±1.272 *
	GA3 1000 ppm	- 0.466 7±0.1 78	- 0.235 ±0.09 17*	- 0.03±0 .0126	0.2667 ±0.059 1*	0.333± 0.0617 *	- 0.0246 ±0.013 9	- 5.3167 ±1.272 *
GA3 750 ppm	control	1.883 3±0.1 78*	0.773 ±0.09 17*	0.1016 ±0.012 6*	0.433± 0.0591 *	0.833± 0.0617 *	0.1167 ±0.013 9*	17.083 ±1.272 *
	IBA 500 ppm	1.483 3±0.1 78*	0.55± 0.091 7*	0.0883 ±0.012 6*	0.3667 ±0.059 1*	0.633± 0.0617 *	0.0793 ±0.013 9*	13.853 ±1.272 *
	IBA 750 ppm	0.866 7±0.1 78*	0.499 ±0.09 17*	0.0533 ±0.012 6**	0.2333 ±0.059 1**	0.433± 0.0617 *	0.056± 0.0139 *	8.64±1 .272*
	IBA 1000 ppm	2.953 ±0.17 8*	0.957 ±0.09 17*	0.151± 0.0126 *	0.4±0.0 591*	0.733± 0.0617 *	0.098± 0.0139 *	24.386 ±1.272 *
	GA3 750 ppm	0.95± 0.178 *	0.394 ±0.09 17*	0.0576 ±0.012 6*	1.333± 0.0591 *	0.2±0. 0617	0.0203 ±0.013 9	8.5067 ±1.272 *
	GA3 1000	0.483 3±0.1	0.159 ±0.09	0.0276 ±0.012	- 0.1333	- 0.133±	- 0.0043	3.19±1 .272

	ppm	78	17	6*	±0.059 1*	0.0617	±0.013 9	
GA ₃ 1000 ppm	control	1.4±0 .1788	0.614 3±0.0 917*	0.074± 0.0126 *	0.5667 ±0.059 1*	0.9667 ±0.061 7*	0.121± 0.0139 *	13.893 ±1.272 *
	IBA 500 ppm	1±0.1 78*	0.391 ±0.09 17*	0.060± 0.0126 *	0.5±0.0 591*	0.7667 ±0.061 7*	0.0836 7±0.01 39*	10.663 ±1.272 *
	IBA 750 ppm	0.383 3±0.1 78	0.34± 0.091 7*	0.025± 0.0126	0.3667 ±0.059 1*	0.5667 ±0.061 7*	0.0603 ±0.013 9*	5.45±1 .272**
	IBA 1000 ppm	2.47± 0.178 *	0.798 ±0.09 17*	0.123± 0.0126 *	0.533± 0.0591 *	0.8667 ±0.061 7*	0.1023 ±0.013 9*	21.196 ±1.272 *
	GA ₃ 500 ppm	0.466 7±0.1 78	0.235 6±0.0 917	0.030± 0.0126	0.2667 ±0.059 1*	0.333± 0.0617 *	0.0246 7±0.01 39	5.3167 ±1.272 **
	GA ₃ 750 ppm	- 0.483 3±0.1 78	- 0.159 ±0.09 17	- 0.0276 ±0.012 6	0.1333 ±0.059 1	0.133± 0.0617	0.0043 ±0.013 9	- 3.19±1 .272

*p<.001

**p<.01

3.3. Germination percent standard: The germination percent means of some of the groups differ significantly according to posthoc comparison using Tukey's HSD test ($\alpha=0.05$; $P<.001$) (Table 4b). The pre-treatments given to the *A. latifolia* seeds of IBA (500 ppm, 750 ppm, 1000 pm) and GA₃ (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 (Table 4b) except IBA 500 ppm which showed a lower significance difference ($P=.332$). Pre-treatment with IBA 5000 ppm, IBA 750 ppm, GA₃ 500 ppm, GA₃ 500 ppm and GA₃ 1000 pm showed an increased mean germination percent i.e. $1.8\pm0.057\%$, $2.417\pm0.116\%$, $2.333\pm0.120\%$, $3.283\pm0.072\%$, and $2.8\pm0.057\%$ respectively. When compared to other treatments and their interaction to control higher significant differences in their germination percent means were observed (Table 4b) ($P<.001$). GA₃ 750 ppm and GA₃ 1000 pm are more effective pre-treatments than others as they show higher differences in germination percent means (Table 4b) compared to the 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. 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 ($0.330\pm0.260\%$) when compared to others and control. Overall, these three pre-treatments- IBA 750 ppm, GA₃ 750 ppm, and GA₃ 1000 ppm had a greater positive effect on the mean germination percent of *A. latifolia* seeds.

Comment [TY29]:

Comment [TY30]:

Comment [TY31]: P/se reconsider these points:

1. put each finding of germination percent, speed, mean germination time, average root length, average shoot length, allometric coefficient, and vigor index in separate paragraph.
2. Check & revise your finding thoroughly including figures.
3. Focus on main findings in accordance with your objectives.

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Comment [TY32]: 5000 or 1000??

Comment [TY33]: 500 or 750??
p/se check all the figures.

In terms of germination speed (Table 4a), when compared to the control (1.051 ± 0.041 number per day) GA₃ 750 ppm showed the highest speed of germination means (1.825 ± 0.107 number per day) followed by GA₃ 1000 ppm (1.666 ± 0.032 number per day), and is highly significant ($P < .001$). This shows the early germination of seeds using these gibberellic acid concentrations. 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 ($\alpha = .05$). Likewise in terms of mean germination time (Table 4a) when compared to the control (0.089 ± 0.0004 day), GA₃ 750 ppm showed the highest speed of germination means (0.191 ± 0.004 day; $P < .001$) followed by GA₃ 1000 ppm (0.163 ± 0.006 day; $P < .001$) but no difference when their means are grouped (Table 4b) which indicates they have a somewhat similar effect on mean germination time. IBA 1000 ppm showed the lowest means of germination speed (0.040 ± 0.126 day; $P < .01$).

The average root length (Table 4a) was found maximum in GA₃ 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 GA₃ 1000 ppm (3.900 ± 0.057 cm) and minimum in IBA 1000 ppm treatment (3.033 ± 0.033 cm). 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, IBA 750 ppm, GA₃ 750 ppm, and GA₃ 1000 ppm treated average shoot length differences in their means (Table 4b) 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 ($RI = 4.00 \pm 0.057$; $SI = 3.133 \pm 0.033$) and 750 ppm ($RI = 4.133 \pm 0.033$; $SI = 3.333 \pm 0.033$) length means combined were significantly different (Table 4a, $P < .01$) from the length means of GA₃ 750 ppm ($RI = 4.367 \pm 0.033$; $SI = 3.767 \pm 0.033$) and 1000 ppm ($RI = 44.500 \pm 0.057$; $SI = 3.900 \pm 0.057$) but less differences in their means when they are grouped (Table 4b). 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 differences in average allometric coefficient means of IBA pre-treatments and GA₃ hormone pre-treatments are significantly different from each other. Each concentration of GA₃ i.e. GA₃ 500 ppm (0.0963 ± 0.0139 ; $P < .001$), GA₃ 750 ppm (0.1167 ± 0.0139 ; $P < .001$), and GA₃ 1000 ppm (0.121 ± 0.0139 ; $P < .001$) when compared to the control shows highly significant allometric coefficient mean differences (Table 4b) 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 average vigor index is seen to decrease in the highest concentrations (1000 ppm) of both the hormones, i.e. Gibberellic acid and Indole-butyric acid IBA when compared to their own other two concentrations (500 ppm and 1000 ppm). The maximum seed vigor index is reported in GA₃ 750 ppm (26.697 ± 0.435) as compared to the rest of the pre-treatments (Table 4a). The lowest indicated in IBA 1000 ppm (2.310 ± 1.820) even

compared to the control (9.613 ± 0.401) which indicates repressing activity or a negative effect on seed germination.

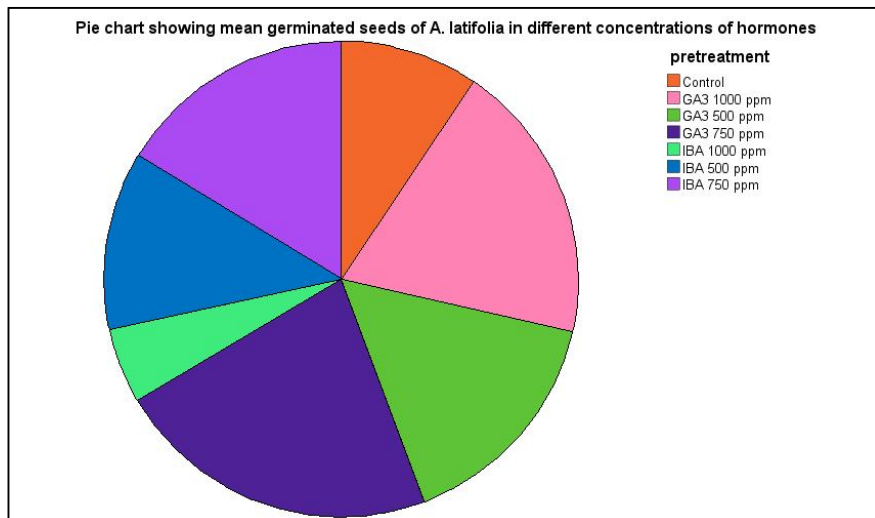


Figure 1: Mean germinated seeds of *A. latifolia* in different concentrations of hormones using a pie chart for comparison

Comment [TY34]: I think better to indicate the figures on the Chart to easily understand.

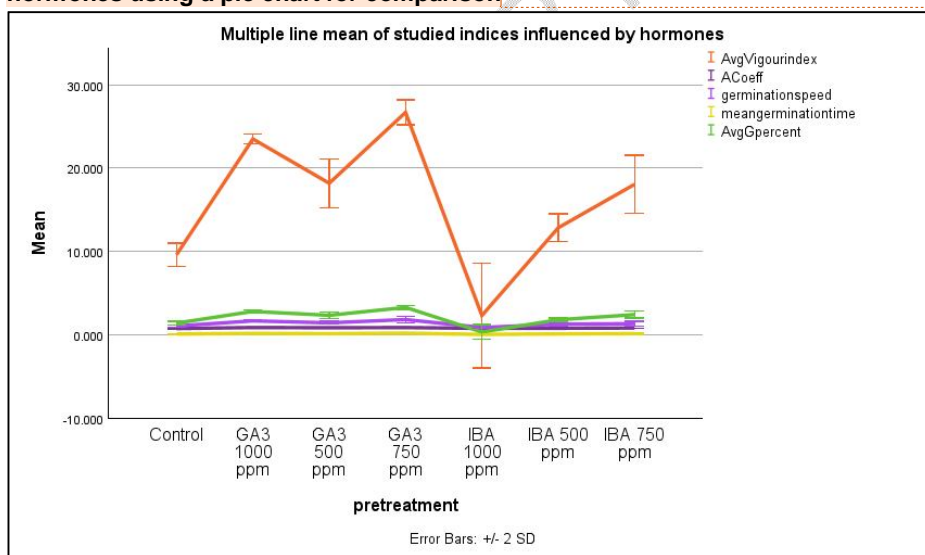


Figure 2: Multiple line graph comparing the means of studied indices due to the influence of varied concentrations of hormones

Comment [TY35]: Sorry, I don't think so Fig. 2 is important to indicate this result. So, p/se check it again.

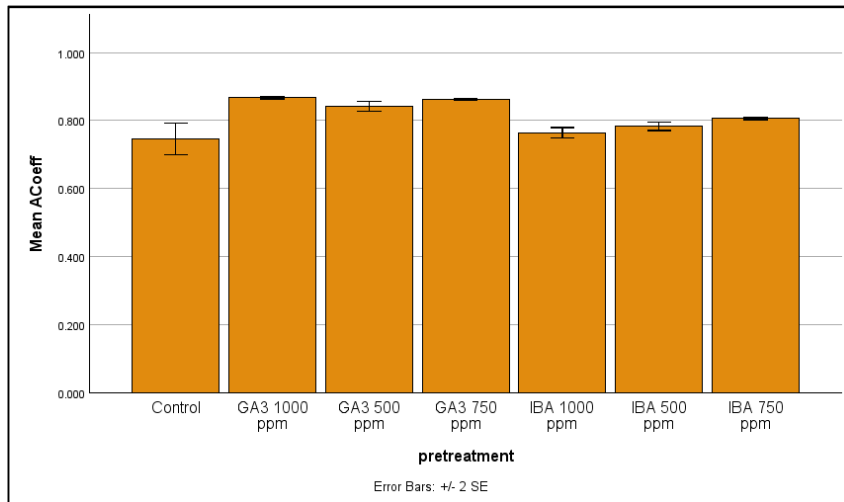


Figure 3: Positive impact on average allometric coefficient means of *A. latifolia* seeds using different concentrations of hormones when compared to control

4. CONCLUSION & Recommendation

The study's results highlight the significance of employing plant growth hormones to improve *Anogeissus latifolia* seed germination. Gibberellic acid helped in increasing the number of germinated seeds, thereby increasing the overall germination percentage (Figure 1). It 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 GA₃ 750 ppm and GA₃ 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.

Comment [TY36]: P/se focus germination percent, speed, mean germination time, average root length, average shoot length, allometric coefficient, and vigor index to conclude your results. Again, highlight in short & clear sentences.

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 *Anogeissus Latifolia* with Brine Shrimp Lethality Assay, *World Journal of Pharmaceutical Research*. 2018; 7(8):1182-1189.
- Byraiah Dinesh. Pollen biology and morphology of *Anogeissus latifolia*, (Roxb. ex DC) Wall. ex Bedd. (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. ex Benth. *Phyton*. 2023: 92(7)
- Khalaki, Masoomeh & Ghorbani, Ardavan & Dadjou, Farid. Influence of Nano-Priming on *Festuca ovina* 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 Bhagat Jaiswal. Analgesic, anti-inflammatory and antipyretic activities of ethanolic extract of stem bark of *Anogeissus latifolia* 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: *Anogeissus latifolia*, *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 *Anogeissus Latifolia*, *FLORA AND FAUNA*. 2019; 25:03-06.