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Plant growth regulator-mediated changes in phenology, canopy dynamics and physiological growth of Ashwagandha (*Withania somnifera* L. Dunal)

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Abstract

Ashwagandha (*Withania somnifera* L. Dunal) is an important medicinal crop whose growth and development can be influenced by plant growth regulators. A field experiment was conducted during 2017–18 at the Horticulture Research Farm, College of Horticulture, Mandsaur, Madhya Pradesh, India, to evaluate the effect of indole-3-butyric acid (IBA), 2,3,5-triiodobenzoic acid (TIBA) and chlormequat chloride (CCC) on phenological development and physiological growth characteristics of Ashwagandha. The experiment consisted of ten treatments comprising IBA at 100, 150 and 200 ppm; TIBA at 40, 50 and 60 ppm; CCC at 250, 500 and 750 ppm; and water spray as control, arranged in a randomized block design with three replications. Foliar applications were made at 30, 45 and 75 days after sowing. TIBA at 50 ppm accelerated phenological development and recorded the minimum number of days required for 50% germination, 50% flowering, physiological maturity and physical maturity. IBA at 100 ppm recorded the highest leaf area at 45 and 75 DAS and showed favourable canopy development and chlorophyll responses. CCC at 250 ppm recorded the highest leaf area at 105 DAS and the highest leaf area index and leaf area duration during the 75–105 DAS interval. Specific leaf area, crop growth rate and relative growth rate did not differ significantly among treatments. The results demonstrate that plant growth regulator responses in Ashwagandha were regulator-specific and growth-stage dependent.

Keywords: Ashwagandha, IBA, TIBA, CCC, phenology, leaf area, growth analysis, chlorophyll

1. Introduction

Ashwagandha (*Withania somnifera* L. Dunal) is an important medicinal crop cultivated for its roots and bioactive constituents. Plant growth regulators can influence developmental progression, canopy development and physiological activity. Growth-analysis parameters such as leaf area, leaf area index (LAI), leaf area duration (LAD), specific leaf area (SLA), crop growth rate (CGR), relative growth rate (RGR) and chlorophyll content provide a useful framework for evaluating treatment responses over successive crop-growth stages. A previous publication from the same experiment reported growth and yield attributes. Therefore, the present manuscript is restricted to the phenological and physiological growth-analysis dataset and does not reproduce the previously reported growth and yield results. The objective was to evaluate the influence of different concentrations of IBA, TIBA and CCC on phenological development and physiological growth characteristics of Ashwagandha.

1.1 Agronomic and physiological importance of Ashwagandha

Ashwagandha (*Withania somnifera* L. Dunal) is an economically important medicinal crop cultivated primarily for its roots, which are valued as a source of withanolides and other bioactive constituents. Its commercial importance has stimulated research on crop establishment, genotype selection, plant density, tillage, nutrient management and physiological regulation. Agronomic studies have shown that changes in crop management can substantially influence plant morphology, biomass production, root yield and quality, demonstrating the close relationship between crop growth and final medicinal raw-material production [1, 2, 16, 17]. The crop is commonly grown under relatively dry and subtropical to semi-arid conditions, and its performance is influenced by soil properties, season and genotype. Field studies have demonstrated substantial variation among Ashwagandha

genotypes for morphological, chemical and yield-related traits, indicating that crop productivity is governed by both genetic constitution and the production environment [18, 19]. Consequently, physiological measurements are useful because they help explain how a treatment modifies crop development before differences become visible at final harvest.

1.2 Plant growth regulators and developmental regulation

Plant growth regulators provide an experimental means of modifying plant development through changes in endogenous hormonal balance, organ growth and assimilate distribution. Auxin-related compounds, including indole-3-butyric acid (IBA), are widely associated with root development and growth regulation, while TIBA can interfere with polar auxin transport and thereby alter developmental responses. CCC is commonly used as a growth-regulating compound capable of modifying vegetative growth and plant architecture [3, 5, 20]. The magnitude and direction of these responses, however, depend on the compound, concentration, application timing and crop growth stage. Studies conducted specifically on Ashwagandha support the relevance of growth-regulator research. Kukreti *et al.* reported that growth regulators influenced growth, biomass and reproductive development of *Withania somnifera*, while the field experiment previously published from the present treatment series showed differential effects of IBA, TIBA and CCC on growth and yield attributes [21, 22]. Additional work combining biofertilizers with PGRs also demonstrated that regulator treatments can modify growth, root yield and alkaloid-related traits [23]. These studies establish that Ashwagandha responds to growth regulation, but they also show that responses cannot be generalized from one concentration or production environment to another.

1.3 Need for physiological growth analysis

Measurements such as leaf area, leaf area index (LAI), leaf area duration (LAD), specific leaf area (SLA), crop growth rate (CGR) and relative growth rate (RGR) provide complementary information about crop performance. Classical growth analysis emphasizes that final biomass is the integrated outcome of canopy development and the rate at which assimilates are accumulated over time [6, 8, 10, 11]. A treatment may therefore influence one component of growth, such as leaf expansion or canopy persistence, without producing a statistically significant response in every composite growth index. This distinction is particularly relevant for medicinal crops, where the production objective is not simply maximum vegetative biomass. The timing of canopy development, duration of physiological activity and progression toward maturity can influence the period during which assimilates are produced and subsequently partitioned to economically important organs. A field study of Ashwagandha that evaluated physiological parameters at successive stages demonstrated measurable differences in total leaf area, LAI, CGR, LAD, RGR and photosynthetic pigments under contrasting management practices [24]. These observations provide a useful framework for interpreting the present physiological dataset.

1.4 Rationale and objective of the present study

The present manuscript was developed to provide a focused analysis of the phenological and physiological observations generated from the field experiment. A separate publication from the same treatment series has already reported major growth and yield attributes; therefore, those previously published variables are not reproduced as primary results here [22]. Instead, the present study examines how IBA, TIBA and CCC at different concentrations influenced developmental timing, canopy establishment, canopy persistence and physiological growth indices. This approach provides an independent interpretation of the dataset and contributes to a more complete understanding of plant growth regulator responses in Ashwagandha. The specific objective was to evaluate the effects of different concentrations of IBA, TIBA and CCC on days to major phenological stages, leaf area, LAI, LAD, SLA, CGR, RGR and chlorophyll status of Ashwagandha cv. RVA-100 under field conditions.

2. Materials and Methods

2.1 Experimental site

The field experiment was conducted during the late Kharif season of 2017–18 at the Research Field of the Department of Plantation, Spices, Medicinal and Aromatic Crops, College of Horticulture, Mandsaur, Rajmata Vijayaraje Scindia Krishi Vishwavidyalaya (RVSKVV), Gwalior, Madhya Pradesh, India. The experimental period extended from October 2017 to March 2018. Mandsaur is located on the Malwa Plateau in the western part of Madhya Pradesh, between 23.45° and 24.13° N latitude and 74.44° and 75.18° E longitude, at an altitude of 435.02 m above mean sea level. The experimental soil was light black and loamy in texture, with low available nitrogen (192 kg ha⁻¹), low available phosphorus (7.6 kg ha⁻¹), medium available potassium (145.0 kg ha⁻¹), pH 8.36 and electrical conductivity of 0.18 dS m⁻¹.

2.2 Experimental material, design and crop management

Ashwagandha (*Withania somnifera* L. Dunal) cv. RVA-100 was used as the experimental material. The crop was sown at a spacing of 30 × 20 cm using a seed rate of 5–7 kg ha⁻¹. The experiment comprised ten treatments and three replications and was laid out in a Randomized Block Design (RBD). The treatments consisted of foliar application of indole-3-butyric acid (IBA), 2,3,5-triiodobenzoic acid (TIBA), and cycocel/chlormequat chloride (CCC) at different concentrations. Foliar sprays were applied at 30, 45 and 75 days after sowing (DAS). The untreated control received water spray.

2.3 Treatment details

T₁: IBA 100 ppm; T₂: IBA 150 ppm; T₃: IBA 200 ppm; T₄: TIBA 40 ppm; T₅: TIBA 50 ppm; T₆: TIBA 60 ppm; T₇: CCC 250 ppm; T₈: CCC 500 ppm; T₉: CCC 750 ppm; T₁₀: water spray (control).

2.4 Observations and physiological growth analysis

Phenological observations included days to 50% germination, 50% flowering, physiological maturity and physical maturity. Physiological growth observations included leaf area, leaf area index (LAI), leaf area duration (LAD), specific leaf area (SLA), crop growth rate (CGR),

relative growth rate (RGR) and chlorophyll content. Observations were recorded at successive crop-growth stages, as applicable. The growth-analysis indices were calculated using standard procedures described in classical crop growth analysis [6, 8]. The following formulae were used:

Leaf area index (LAI)

$LAI = \text{Total leaf area} / \text{Land area}$

For successive observations: $LAI = (LA_2 + LA_1) / 2P$

where LA_1 and LA_2 are leaf areas at two consecutive observations and P is the land area occupied by the plant [6].

Leaf area duration (LAD)

$LAD = [(LA_2 + LA_1) / 2] \times (t_2 - t_1) \text{ (cm}^2 \text{ day}^{-1}\text{)}$

where LA_1 and LA_2 represent leaf area at two successive time intervals (t_1 and t_2) [7].

Specific leaf area (SLA)

$SLA = \frac{1}{2} [(LA_2 / LW_2) + (LA_1 / LW_1)] \text{ (cm}^2 \text{ g}^{-1}\text{)}$

where LA_1 and LA_2 are leaf areas and LW_1 and LW_2 are the corresponding leaf dry weights at the two observations.

Crop growth rate (CGR)

$CGR = [(W_2 - W_1) / (t_2 - t_1)] \times (1 / P) \text{ (g cm}^{-2} \text{ of ground area day}^{-1}\text{)}$

where W_1 and W_2 are the dry weights of plants at times t_1 and t_2 , respectively, and P is the land area [6–8].

Relative growth rate (RGR)

$RGR = (\log W_2 - \log W_1) / (t_2 - t_1) \text{ (g g}^{-1} \text{ day}^{-1}\text{)}$

where W_1 and W_2 are the plant dry weights at times t_1 and t_2 , respectively.

2.5 Statistical analysis

The data were analysed according to the analysis of variance (ANOVA) procedure appropriate for a Randomized Block Design. Treatment means were compared using the critical difference (CD) at the 5% level of significance. Statistical analysis was carried out following the procedures described by Panse and Sukhatme [9].

3. Results and Discussion

3.1 Integrated interpretation of phenological responses

The phenological observations showed a clear regulator-specific response. TIBA at 50 ppm required the minimum number of days for 50% germination, 50% flowering, physiological maturity and physical maturity, indicating a consistent acceleration of crop development. In contrast, the higher IBA concentration tended to prolong the duration required to attain comparable phenological stages. The

response is consistent with the role of TIBA as an inhibitor of polar auxin transport, through which developmental balance and the relative expression of vegetative and reproductive growth may be altered [11, 12]. Earlier work cited in Ashwagandha also reported that plant growth regulators can modify floral initiation and maturity-related events [18, 21]. An important feature of the present data is that the intermediate TIBA concentration was more effective than both the lower and higher concentrations. This concentration-dependent pattern suggests that the response was not simply proportional to the amount of regulator applied. Plant growth regulators frequently produce optimum responses within a restricted concentration range, beyond which stimulation may decline or inhibitory effects may become dominant [3, 11]. Therefore, the present findings support the identification of an effective physiological dose rather than the assumption that a higher concentration necessarily produces a stronger response.

3.2 Leaf area development and canopy expansion

Leaf area is a major determinant of the assimilatory surface available for interception of solar radiation and subsequent dry-matter production. Significant treatment differences were evident during the early and middle stages of crop development, while differences became non-significant at 135 DAS and harvest. IBA at 100 ppm produced the greatest leaf area during the early stages, whereas CCC at 250 ppm produced the highest value at 105 DAS. The disappearance of statistical differences toward maturity indicates convergence of canopy size among treatments during the final phase of crop development. The superiority of IBA at 100 ppm during the early growth period agrees with the established developmental role of IBA-derived auxin in plant growth and organ development [3, 4]. Earlier studies on Ashwagandha and other crops have also reported growth stimulation and improved vegetative development under appropriate concentrations of IBA [17, 18, 21]. In the present experiment, however, increasing IBA concentration from 100 to 150 or 200 ppm did not improve canopy development, again demonstrating that the response was concentration dependent. The mid-season advantage of CCC at 250 ppm suggests a different physiological mode of action. CCC is widely used as a growth regulator that modifies vegetative development and can alter stem elongation, canopy architecture and chlorophyll status [5, 14]. The present response indicates that moderate CCC concentration may have supported canopy maintenance during the period of maximum vegetative activity without producing the stronger growth restriction expected at excessive doses.

3.3 Leaf area index and canopy-level response

The LAI results closely complemented the leaf-area response but provided a canopy-level interpretation by relating assimilatory surface to the ground area occupied by the crop. Significant treatment differences were observed during the first three growth intervals. IBA at 100 ppm produced the highest LAI during 45–75 and 105–135 DAS, while CCC at 250 ppm was superior during 75–105 DAS. The final interval showed no significant difference among treatments. The changing treatment ranking across growth stages is particularly important. It indicates that no single regulator dominated canopy performance throughout the

complete crop cycle. Instead, IBA at 100 ppm was more closely associated with rapid establishment and later canopy persistence, whereas CCC at 250 ppm expressed its strongest response during the active middle phase. Similar growth-stage dependence has been recognized in crop growth analysis, where leaf development and assimilatory performance are influenced by both developmental age and environmental conditions ^[6-8].

3.4 Leaf area duration and persistence of the assimilatory surface

Leaf area duration integrates canopy magnitude with the length of time over which the leaf surface is maintained. Thus, LAD provides a useful indication of the persistence of the photosynthetically active canopy ^[6, 7]. In the present study, IBA at 100 ppm recorded superior LAD during the early intervals, whereas the treatment advantage shifted during later stages. TIBA at 50 ppm recorded the highest numerical LAD during 135 DAS to harvest, although the final-stage differences were non-significant. The increase in LAD up to the middle-to-late crop period followed by a decline toward harvest corresponds to the normal sequence of canopy establishment, maximum persistence and progressive senescence. Since the treatment differences were strongest before maturity, the results suggest that plant growth regulators primarily altered the timing and persistence of canopy development rather than maintaining a permanent separation among treatments until harvest.

3.5 Specific leaf area, crop growth rate and relative growth rate

Specific leaf area, crop growth rate and relative growth rate did not differ significantly among treatments. These non-significant responses are important because they demonstrate that changes in visible canopy development and chlorophyll status were not necessarily accompanied by statistically detectable differences in all integrated growth indices. SLA reflects the relationship between leaf area and leaf dry matter, whereas CGR and RGR integrate the outcome of several simultaneous processes, including photosynthesis, respiration, dry-matter partitioning and developmental stage ^[6-8]. Accordingly, the absence of significant treatment effects for these indices should not be interpreted as evidence that the regulators had no physiological effect. Rather, it indicates that treatment-induced variation in canopy size and chlorophyll status was not sufficiently consistent to produce significant differences in integrated biomass accumulation rates under the experimental conditions. This distinction between component traits and composite growth indices is a central principle of classical growth analysis ^[6, 7]. The control CGR value of 0.04 at 105–135 DAS has been retained in the final dataset as corrected by the authors.

3.6 Chlorophyll dynamics and physiological status

Chlorophyll content showed one of the clearest treatment responses. IBA at 100 ppm maintained the highest SPAD values from 75 DAS through harvest. Chlorophyll values generally increased from 45 DAS to a maximum around 105 DAS and then declined sharply at 135 DAS and harvest. The temporal pattern is consistent with progressive canopy development followed by physiological ageing and senescence. The sustained SPAD advantage under IBA at

100 ppm complements the favourable leaf-area and LAI responses under the same treatment. Together, these observations suggest that the treatment improved not only canopy size during key stages but also the physiological status of the leaves. Nevertheless, SPAD values are an indirect estimate of chlorophyll status and should not be interpreted as a direct measurement of photosynthetic rate. Future work combining chlorophyll estimation with gas exchange or chlorophyll fluorescence would provide a stronger mechanistic explanation. CCC also produced measurable chlorophyll responses, consistent with earlier reports that growth-regulating compounds can influence pigment status and vegetative development ^[5, 14]. However, the present data indicate that IBA at 100 ppm was more consistently associated with high chlorophyll readings across successive observations.

3.7 Overall physiological interpretation

Taken together, the results reveal three distinct response patterns. TIBA at 50 ppm primarily accelerated phenological progression. IBA at 100 ppm promoted stronger canopy development and maintained comparatively high chlorophyll status. CCC at 250 ppm expressed its strongest influence during the middle phase of canopy development. These findings demonstrate that the three regulators should not be considered interchangeable; each altered a different dimension of crop development. The concentration effect was equally important. The lowest IBA concentration tested was generally more favourable for canopy-related traits than the higher IBA concentrations, while the intermediate TIBA concentration produced the strongest phenological acceleration. Likewise, CCC at 250 ppm was more favourable for selected physiological traits than higher CCC concentrations. Such patterns reinforce the importance of dose optimization in growth-regulator research. Because the present manuscript focuses specifically on phenology and physiological growth analysis, growth and yield attributes reported elsewhere from the same experiment are not reproduced as primary results here. The current interpretation therefore provides a distinct physiological perspective on the experiment and avoids duplication of previously reported outcome variables.

3.8 Relationship between canopy development and physiological activity

The combined behaviour of leaf area, LAI, LAD and SPAD values provides a more complete interpretation than any individual variable alone. Leaf area describes the size of the assimilatory surface at a particular observation, whereas LAI relates that surface to the land area occupied by the crop. LAD further incorporates the duration for which the canopy is maintained. Consequently, a treatment may exhibit an advantage in early leaf expansion but lose that advantage later, while another treatment may become more prominent during the period of maximum canopy activity. Such stage-dependent responses are consistent with the principles of crop growth analysis described by Watson and later growth-analysis literature ^[6, 8, 10, 11]. In the present study, IBA at 100 ppm was consistently associated with favourable early canopy development and higher chlorophyll readings during much of the observation period. The relationship between these responses is physiologically plausible because leaf expansion determines the potential

radiation-intercepting surface, while chlorophyll status provides an indication of the photosynthetic pigment environment within that canopy. Nevertheless, SPAD readings should be interpreted as an indirect estimate of chlorophyll status rather than as a direct measurement of photosynthetic rate. Studies of Ashwagandha under different management systems have similarly reported coordinated changes in leaf area, growth indices and pigment concentrations [24].

3.9 Concentration-dependent response to plant growth regulators

A major outcome of the experiment is that the strongest response was not always obtained at the highest concentration. IBA at 100 ppm was generally more favourable for several canopy-related traits than the higher IBA concentrations, TIBA at 50 ppm was most consistently associated with accelerated phenological development, and CCC at 250 ppm showed its most distinct response during the active middle stage. This pattern supports the concept that plant growth regulation involves an optimum response range rather than a simple linear increase with dose [3, 5, 20].

Comparable concentration-dependent responses have been reported in Ashwagandha and other crop systems. Kukreti *et al.* observed differential growth and biomass responses to growth-regulator treatments in *Withania somnifera* [21], while Barathkumar and Manivannan reported that combinations of biofertilizers and PGRs could alter growth, yield and alkaloid content [23]. Recent field work has also continued to demonstrate that foliar PGR application can modify biomass, productivity and quality-related traits in Ashwagandha [25]. The present results therefore add physiological evidence to the broader observation that PGR responses are dependent on dose and developmental context.

3.10 Phenology in relation to the crop production cycle

The earlier attainment of phenological stages under TIBA at 50 ppm is important because the developmental calendar of Ashwagandha determines the duration available for vegetative growth, flowering and subsequent maturation. The consistent acceleration observed across several phenological events suggests that the treatment influenced the overall developmental progression rather than a single isolated stage. Research on Ashwagandha cultivation indicates that harvest maturity and crop duration are important considerations for root production and quality, while seasonal conditions can modify plant productivity and secondary-metabolite accumulation [17, 26]. However, earlier phenological progression should not automatically be interpreted as superior for every production objective. A faster transition toward maturity may be desirable where crop duration is limiting, whereas prolonged vegetative activity may support greater biomass accumulation under other circumstances. The present experiment therefore demonstrates that PGR selection should be linked to the intended agronomic outcome. TIBA at 50 ppm showed the strongest phenological acceleration, whereas IBA at 100 ppm was more consistently associated with canopy development and chlorophyll status.

3.11 Interpretation of non-significant growth indices

The absence of significant treatment effects for SLA, CGR

and RGR deserves particular attention. Growth indices are derived variables that integrate changes occurring over a defined time interval. As a result, variation in photosynthesis, respiration, dry-matter allocation and plant size can partially offset one another when expressed as a single composite index [6, 8]. A non-significant CGR or RGR result does not therefore negate significant treatment effects on component traits such as leaf area or chlorophyll status.

This interpretation is supported by physiological work in Ashwagandha, where management treatments have produced differential responses among individual growth indices and pigment variables [24]. In the present study, the significant responses in canopy traits occurred mainly during early and middle stages, while treatment differences became less distinct as the crop approached maturity. The convergence among treatments at later stages may have reduced the magnitude of differences expressed in interval-based indices. Thus, repeated observations are essential for identifying the period during which physiological regulation is most pronounced.

3.12 Relevance to Ashwagandha improvement and future research

Ashwagandha improvement involves the simultaneous consideration of genotype, agronomy and physiological performance. Genetic studies have documented considerable variation in morphological and chemical traits, while multi-environment evaluations have demonstrated the importance of genotype $\times$ environment interaction for yield and quality [18, 19, 27]. Molecular and genetic-diversity studies further emphasize the availability of diverse germplasm that can be exploited for crop improvement [28, 29]. The present results add a crop-management dimension by showing that physiological expression can also be modified through exogenous growth regulation. Future studies should integrate repeated physiological measurements with direct photosynthetic measurements, chlorophyll fluorescence, and nutrient status and validated biochemical estimation of withanolides. Such an approach would help determine whether treatments that improve canopy traits also produce consistent changes in medicinal quality. Recent studies have linked seasonal and developmental variation with changes in productivity, photosynthetic pigments, gene expression and withanolide accumulation [26, 30]. Integrating these measurements under field conditions would provide a stronger mechanistic basis for developing precise PGR recommendations.

3.13 Practical interpretation

Under the conditions of the present experiment, the results suggest three practical response profiles. TIBA at 50 ppm was associated primarily with accelerated phenological progression. IBA at 100 ppm was associated with stronger canopy development and maintenance of higher chlorophyll status across important growth stages. CCC at 250 ppm was associated with favourable mid-season canopy performance. These findings should be considered treatment-specific responses under the soil, climate and management conditions of Mandsaur and should be validated across additional seasons and locations before being translated into broad agronomic recommendations.

Table 1: Treatment details used in the experiment.

Treatment	Plant growth regulator	Concentration
T ₁	IBA	100 ppm
T ₂	IBA	150 ppm
T ₃	IBA	200 ppm
T ₄	TIBA	40 ppm
T ₅	TIBA	50 ppm
T ₆	TIBA	60 ppm
T ₇	CCC	250 ppm
T ₈	CCC	500 ppm
T ₉	CCC	750 ppm
T ₁₀	Water spray	Control

Table 2: Effect of plant growth regulators on phenological development of Ashwagandha

Treatment	50% germination	50% flowering	Physiological maturity	Physical maturity
T ₁ IBA 100	12.67	76.33	135.67	154.0
T ₂ IBA 150	13.67	79.0	138.67	157.0
T ₃ IBA 200	13.67	79.33	139.0	159.67
T ₄ TIBA 40	13.0	76.33	134.0	156.67
T ₅ TIBA 50	11.33	72.67	131.0	151.33
T ₆ TIBA 60	12.33	75.33	132.33	153.67
T ₇ CCC 250	12.33	75.33	133.67	152.0
T ₈ CCC 500	13.67	78.0	136.0	158.67
T ₉ CCC 750	13.33	76.0	133.67	154.67
T ₁₀ Control	13.67	77.0	137.67	155.33
S.Em.±	0.5	0.97	1.15	1.32
CD at 5%	1.49	2.87	3.42	3.92

Table 3: Effect of plant growth regulators on leaf area (cm² plant⁻¹)

Treatment	45 DAS	75 DAS	105 DAS	135 DAS	Harvest
T ₁ IBA 100	527.33	1505.0	1731.0	2265.67	1845.0
T ₂ IBA 150	511.67	1501.67	1618.33	2020	1803.33
T ₃ IBA 200	510	1306	1520.67	2077.33	1834
T ₄ TIBA 40	492.67	1166	1420.33	2020	1732.67
T ₅ TIBA 50	440	1269.67	1649.33	2216.67	1953.67
T ₆ TIBA 60	445	1434	1542	1902	1716.67
T ₇ CCC 250	444.33	1489	1852.67	1936	1786
T ₈ CCC 500	473	1489.67	1535.67	2061.33	1974
T ₉ CCC 750	450.67	1205	1624.67	1452.33	1935
T ₁₀ Control	510.33	1436	1565.67	2067.67	2017.33
S.Em.±	15.74	45.9	73.96	53.75	231.98
CD at 5%	46.76	136.38	306.36	NS	NS

Table 4: Effect of plant growth regulators on leaf area index

Treatment	45-75 DAS	75-105 DAS	105-135 DAS	135 DAS-Harvest
T ₁ IBA 100	1.69	2.7	3.33	3.43
T ₂ IBA 150	1.68	2.6	3.03	3.19
T ₃ IBA 200	1.51	2.36	3.0	3.26
T ₄ TIBA 40	1.38	2.16	2.87	3.13
T ₅ TIBA 50	1.42	2.43	3.22	3.48
T ₆ TIBA 60	1.57	2.48	2.87	3.02
T ₇ CCC 250	1.61	2.78	3.16	3.1
T ₈ CCC 500	1.64	2.52	3.0	3.36
T ₉ CCC 750	1.38	2.36	2.56	2.82
T ₁₀ Control	1.62	2.5	3.03	3.4
S.Em.±	0.04	0.06	0.08	0.2
CD at 5%	0.12	0.18	0.25	NS

Table 5: Effect of plant growth regulators on leaf area duration (cm² day⁻¹)

Treatment	45-75 DAS	75-105 DAS	105-135 DAS	135 DAS-Harvest
T ₁ IBA 100	30.49	48.54	69.43	61.66
T ₂ IBA 150	30.2	46.8	61.88	57.35
T ₃ IBA 200	27.24	42.4	60.75	58.67
T ₄ TIBA 40	24.88	38.8	58.71	56.29
T ₅ TIBA 50	25.65	43.79	66.0	62.56
T ₆ TIBA 60	28.19	44.64	58.21	54.28
T ₇ CCC 250	29.0	50.13	67.05	55.83
T ₈ CCC 500	29.44	45.38	62.69	60.53
T ₉ CCC 750	24.84	42.45	52.23	50.81
T ₁₀ Control	29.2	45.03	61.98	61.28
S.Em.±	0.75	1.08	1.2	3.66
CD at 5%	2.22	3.2	NS	NS

Table 6: Effect of plant growth regulators on specific leaf area (cm² g⁻¹)

Treatment	45-75 DAS	75-105 DAS	105-135 DAS	135 DAS-Harvest
T ₁ IBA 100	390.54	293.19	285.21	274.02
T ₂ IBA 150	399.72	256.29	239.31	243.62
T ₃ IBA 200	340.57	257.19	255.59	235.71
T ₄ TIBA 40	313.88	233.28	248.49	237.08
T ₅ TIBA 50	392.88	241.93	268.41	264.68
T ₆ TIBA 60	381.46	237.07	218.93	235.42
T ₇ CCC 250	354.77	277.72	260.1	243.23
T ₈ CCC 500	371.44	279.14	264.29	268.08
T ₉ CCC 750	335.7	264.34	248.5	226.64
T ₁₀ Control	373.35	284.67	271.14	252.08
S.Em.±	28.46	15.58	12.78	15.47
CD at 5%	NS	NS	NS	NS

Table 7: Effect of plant growth regulators on crop growth rate

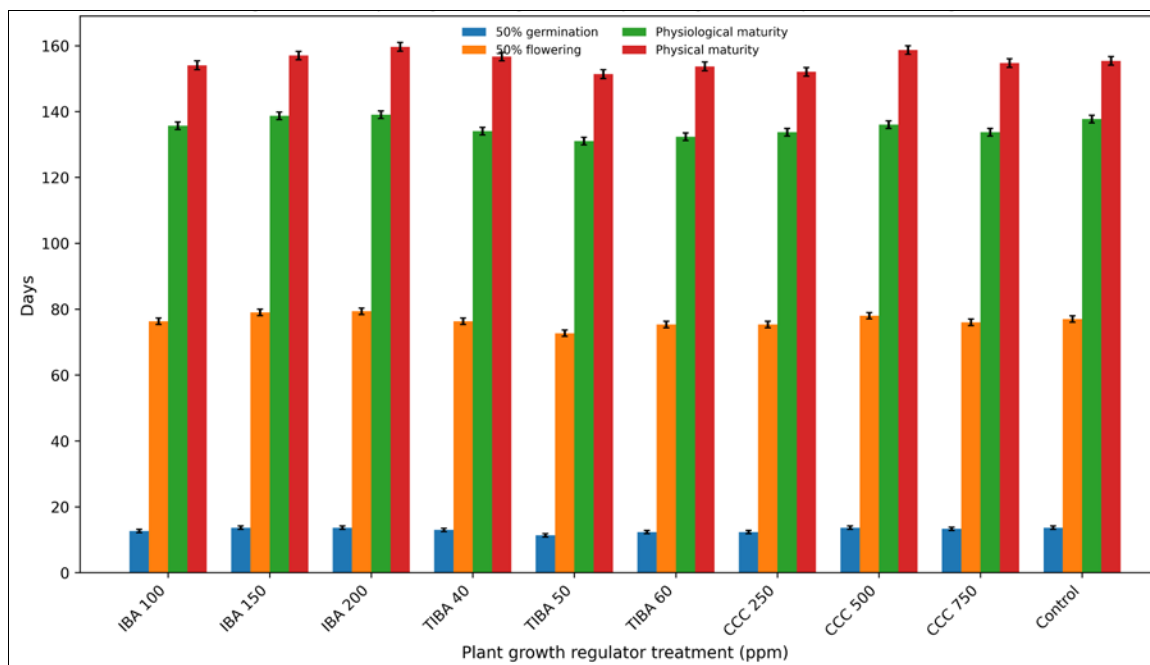
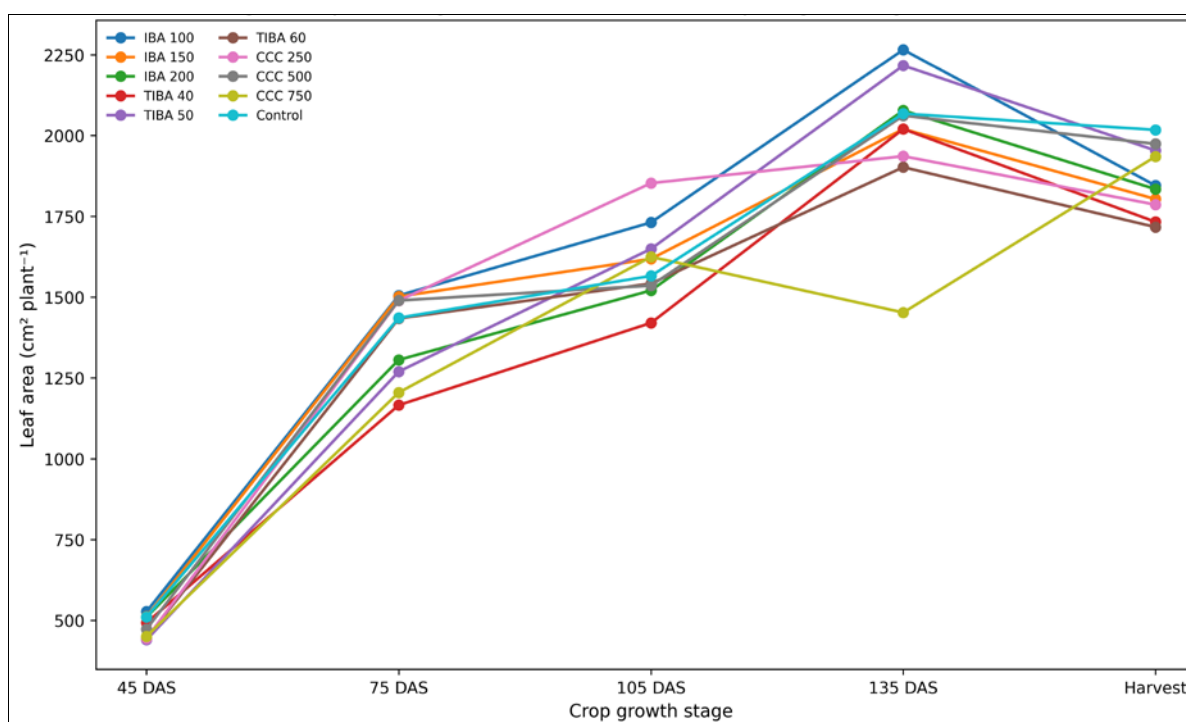
Treatment	45-75 DAS	75-105 DAS	105-135 DAS	135 DAS-Harvest
T ₁ IBA 100	0.48	0.14	0.04	0.04
T ₂ IBA 150	0.39	0.26	0.07	0.03
T ₃ IBA 200	0.37	0.22	0.1	0.04
T ₄ TIBA 40	0.41	0.17	0.06	0.04
T ₅ TIBA 50	0.5	0.16	0.06	0.07
T ₆ TIBA 60	0.5	0.11	0.11	0.08
T ₇ CCC 250	0.45	0.06	0.19	0.08
T ₈ CCC 500	0.39	0.14	0.14	0.04
T ₉ CCC 750	0.42	0.17	0.09	0.05
T ₁₀ Control	0.45	0.12	0.08	0.04
S.Em.±	0.06	0.05	0.03	As per corrected analysis
CD at 5%	NS	NS	NS	NS

Table 8: Effect of plant growth regulators on relative growth rate (g g⁻¹ day⁻¹)

Treatment	45-75 DAS	75-105 DAS	105-135 DAS	135 DAS-Harvest
T ₁ IBA 100	0.0646	0.0078	0.0018	0.0027
T ₂ IBA 150	0.0683	0.0151	0.0033	0.0024
T ₃ IBA 200	0.0529	0.0136	0.0045	0.0025
T ₄ TIBA 40	0.0554	0.0106	0.0028	0.0026
T ₅ TIBA 50	0.0736	0.0083	0.0028	0.0043
T ₆ TIBA 60	0.0617	0.0058	0.0048	0.0047
T ₇ CCC 250	0.0545	0.0035	0.009	0.0048
T ₈ CCC 500	0.0478	0.0092	0.0066	0.0027
T ₉ CCC 750	0.0554	0.0094	0.0041	0.003
T ₁₀ Control	0.0625	0.0069	0.004	0.028
S.Em.±	0.0058	0.0034	0.0014	0.0083
CD at 5%	NS	NS	NS	NS

Table 9: Effect of plant growth regulators on chlorophyll content (SPAD)

Treatment	45 DAS	75 DAS	105 DAS	135 DAS	Harvest
T ₁ IBA 100	66.79	77.52	82.67	44.67	20.0
T ₂ IBA 150	66.07	74.39	80.59	44.47	18.33
T ₃ IBA 200	58.71	63.71	66.24	37.42	14.0
T ₄ TIBA 40	67.83	71.81	74.82	42.0	18.0
T ₅ TIBA 50	64.61	71.49	74.39	40.8	17.67
T ₆ TIBA 60	55.7	62.05	65.38	36.17	13.33
T ₇ CCC 250	62.35	68.76	71.81	39.47	16.67
T ₈ CCC 500	58.75	65.83	69.33	38.33	14.17
T ₉ CCC 750	53.71	60.09	63.42	35.0	13.33
T ₁₀ Control	52.51	59.37	62.89	32.67	12.67
S.Em.±	2.57	1.86	3.98	2.12	0.86
CD at 5%	7.62	5.53	11.85	6.29	2.54

**Fig 1:** Effect of plant growth regulators on phenological development of Ashwagandha.**Fig 2:** Temporal changes in leaf area under different plant growth regulator treatments.

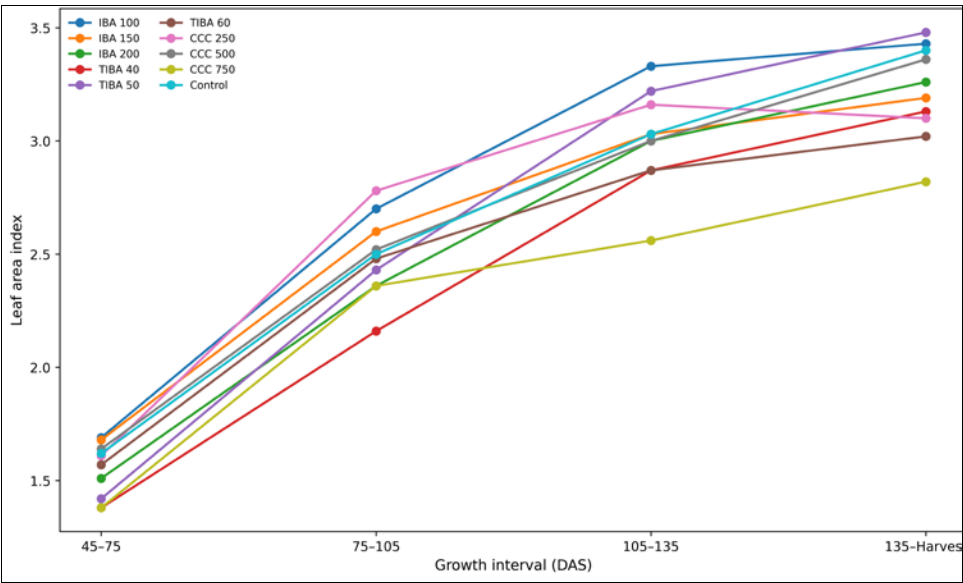


Fig 3: Effect of plant growth regulators on leaf area index during successive growth intervals.

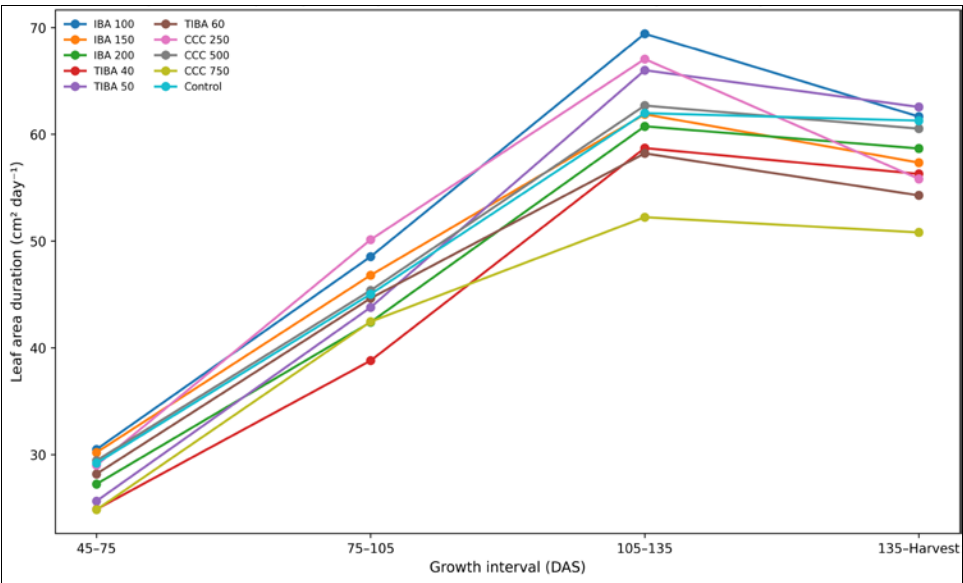


Fig 4: Effect of plant growth regulators on leaf area duration during successive growth intervals.

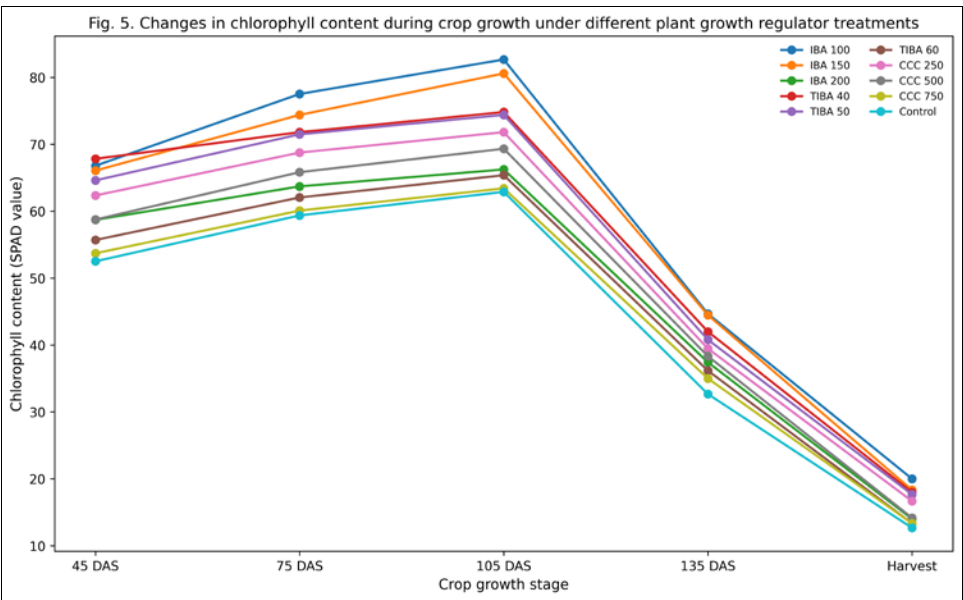


Fig 5: Changes in chlorophyll content during crop growth under different plant growth regulator treatments

5. Conclusion

The present study demonstrated that plant growth regulators produced distinct, concentration-dependent and growth-stage-specific responses in Ashwagandha. TIBA at 50 ppm consistently accelerated phenological development and required the minimum number of days for major developmental stages. IBA at 100 ppm was particularly effective in promoting early canopy development and maintaining higher chlorophyll status, whereas CCC at 250 ppm produced favourable responses during the active middle phase of crop growth. Leaf area, LAI, LAD and chlorophyll content showed significant treatment responses at selected stages, while SLA, CGR and RGR remained statistically non-significant. Overall, TIBA at 50 ppm may be useful where accelerated crop development is desired, while IBA at 100 ppm showed the most consistent advantage for canopy development and physiological leaf status under the conditions of this experiment.

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Conflict of Interest

The authors declare that there is no conflict of interest.

Author Contributions

Patidar Sachin conducted the field experiment and collected the original data. Meena KC contributed to planning and supervision of the research. Bepari Abdulrazaq contributed to data interpretation, manuscript organization and preparation. R. H. Vinoda contributed to scientific review and improvement of the manuscript. All authors reviewed and approved the final manuscript.

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