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Plant growth regulator-mediated modulation of biomass partitioning, root yield and root quality in Ashwagandha (*Withania somnifera* L. Dunal)

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Abstract
Ashwagandha (*Withania somnifera* L. Dunal) is cultivated primarily for its medicinal roots, and improvement in root productivity must be considered together with biomass partitioning and root quality. A field experiment was conducted during the late Kharif season of 2017-18 at the College of Horticulture, Mandsaur, Madhya Pradesh, India, to evaluate the effects of foliar application of indole-3-butyric acid (IBA), 2,3,5-triiodobenzoic acid (TIBA) and chlormequat chloride (CCC) on dry-matter production, root yield, root biochemical quality and economics of Ashwagandha cv. RVA-100. Ten treatments comprising IBA (100, 150 and 200 ppm), TIBA (40, 50 and 60 ppm), CCC (250, 500 and 750 ppm) and water-sprayed control were arranged in a randomized block design with three replications. Sprays were applied at 30, 45 and 75 days after sowing. IBA 100 ppm consistently promoted fresh and dry matter accumulation and produced superior fresh and dry root biomass. It also recorded the highest root harvest index (31.00%) and root yield (461.33 kg ha^{-1}), compared with 269.67 kg ha^{-1} in the control. Root biochemical responses differed from biomass responses: IBA 200 ppm produced the highest nitrogen (0.67%) and protein (4.10%) concentrations, whereas TIBA 40 ppm produced the highest withanolide content (1.45%). The economic analysis identified IBA 100 ppm as the most profitable treatment in the detailed treatment-wise economic dataset, with the highest net return and benefit:cost ratio. The results demonstrate a clear treatment-dependent trade-off between root biomass and phytochemical concentration. Thus, IBA 100 ppm is the most suitable option when the production objective is root yield and economic return, whereas TIBA 40 ppm may be preferred where enhancement of root withanolide concentration is the primary objective. These findings provide a physiological and production-oriented basis for targeted use of plant growth regulators in Ashwagandha. [1, 2]

Keywords: Ashwagandha, biomass partitioning, IBA, TIBA, CCC, root yield, withanolides, profitability

1. Introduction

Ashwagandha (*Withania somnifera* L. Dunal) is one of the most important medicinal plants of the Indian traditional health-care system. The crop is valued mainly for its roots, which contain pharmacologically important withanolides and other bioactive constituents. Consequently, successful crop management requires simultaneous consideration of root biomass, phytochemical quality and economic productivity rather than evaluation of vegetative growth alone [1, 2].

Root yield in Ashwagandha is the integrated outcome of canopy development, dry-matter production and assimilate partitioning towards the underground economic organ. Genetic constitution and the production environment influence morphological, yield and chemical traits, while management interventions can further modify the expression of these traits. The ability to alter biomass allocation is particularly relevant in medicinal root crops because the treatment producing maximum total plant biomass may not necessarily produce the greatest root yield or the highest concentration of secondary metabolites.

Plant growth regulators (PGRs) provide a practical means of modifying developmental processes and biomass distribution. IBA is an auxin-related compound associated with root development and vegetative growth. TIBA is used as an inhibitor of polar auxin transport and can alter developmental organization and assimilate relationships. CCC is a growth-retarding compound that can modify plant architecture and partitioning. The response of crops to these compounds is strongly dependent on concentration, timing of application and crop developmental stage.

Earlier studies in Ashwagandha have reported that PGRs can modify growth and yield attributes. However, physiological and agronomic responses do not always move in the same direction, and relatively few treatment comparisons integrate dry-matter partitioning, root productivity, root biochemical composition and economic return within a single experimental framework. This creates a need to distinguish treatments that maximize root biomass from those that enhance phytochemical concentration.

The present study therefore focused on the unpublished dry-matter partitioning, root-yield, root-quality and economic observations from a field experiment involving IBA, TIBA and CCC. The specific objectives were to evaluate the effects of PGR application on fresh and dry matter accumulation and root biomass, quantify changes in root harvest index and root yield, determine treatment effects on nitrogen, protein and withanolide content, and identify the treatment providing the most favourable balance between root productivity, root quality and economic return.

1.1 Botanical and medicinal importance of Ashwagandha

Ashwagandha (*Withania somnifera* (L.) Dunal) is a perennial medicinal species of the family Solanaceae that is widely recognized as Indian ginseng or winter cherry. It occupies an important position in Ayurveda and other traditional systems of medicine, and the crop has increasingly attracted scientific and commercial interest because of its characteristic steroidal lactones and other bioactive constituents. Modern reviews describe withanolides as a major chemical class responsible for much of the pharmacological interest in the species, while roots remain the principal commercial raw material for many traditional and herbal products [1, 2].

The medicinal value of *W. somnifera* makes consistency of raw-material quality an important production objective. Root yield, root morphology, dry-matter concentration and secondary-metabolite accumulation are not necessarily controlled by the same physiological processes. Thus, an agronomic treatment that increases root biomass may not simultaneously increase the concentration of a particular marker compound. This separation between quantity and quality is a recurring issue in medicinal-plant production and provides an important scientific rationale for integrated evaluation of yield and phytochemistry [1, 2, 3].

1.2 Root biomass as the principal economic component

Unlike grain and seed crops, where the harvested product is often a reproductive structure, Ashwagandha is cultivated primarily for its underground storage root. Root length, diameter, fresh root weight and dry root weight therefore become important determinants of commercial productivity. Genetic studies have demonstrated significant relationships among fresh root weight, dry root weight, root morphology and chemical traits, highlighting the combined importance of plant growth and biomass allocation for improving dry-root productivity [4, 5].

The production of root biomass is an integrated process involving photosynthetic carbon fixation in source tissues, transport of assimilates and their utilization by sink organs. Classical source-sink concepts emphasize that the magnitude and activity of both source and sink determine the final distribution of assimilates among plant organs. In W. somnifera, the developmental transition from vegetative growth to reproductive growth can alter this distribution because reproductive structures compete strongly for assimilates [6, 7].

1.3 Biomass partitioning and source-sink relationships

Biomass partitioning is particularly important in *W. somnifera* because the crop has an indeterminate growth habit and can continue flowering and berry production while root development is still occurring. Experimental work has shown that modification of reproductive sink activity can redirect assimilates towards roots and substantially increase root biomass and withanolide yield. Choyal *et al.* demonstrated that altered source-sink relationships through chemical and mechanical manipulation increased root biomass and improved withanolide productivity, providing strong evidence that sink regulation is relevant to medicinal-root production [8, 9].

Similarly, Kalariya *et al.* observed that biomass allocation among leaves, stems, roots and reproductive structures changed markedly with crop developmental stage. Their study demonstrated that physiological interventions could modify biomass allocation and influence the accumulation of important withanolides in roots. These findings indicate that the final root harvest is not simply a consequence of total biomass production but also of the proportion of biomass ultimately directed to the economic root [9].

1.4 Withanolides and the quality dimension of root production

Withanolides are a characteristic group of C28 steroidal lactones in W. somnifera. Withaferin A, withanolide A and related compounds have received extensive biochemical and pharmacological attention. The concentration of these compounds varies with genotype, plant organ, developmental stage and environmental conditions. Root and leaf tissues can also differ considerably in their relative accumulation patterns, indicating that quality cannot be inferred reliably from vegetative biomass alone [10].

Seasonal and developmental studies have further demonstrated that increased vegetative productivity does not always correspond to increased withanolide accumulation. Mishra *et al.* reported an inverse relationship between some components of vegetative/reproductive development and withanolide production across contrasting seasons, together with changes in the expression of genes associated with the biosynthetic pathway. Such observations support the need to examine root yield and withanolide concentration simultaneously when evaluating agronomic interventions. [11, 12]

Recent research has also demonstrated that physiological manipulation can influence both root biomass allocation and withanolide productivity. In particular, Choyal *et al.* showed that altering source-sink relationships increased root dry matter and changed withanolide production, confirming that physiological regulation can affect both the quantity and quality dimensions of *W. somnifera* production [8, 9].

1.5 Plant growth regulators as tools for crop manipulation

Plant growth regulators offer an experimental and potentially agronomic means of modifying plant architecture, developmental timing, root growth and assimilate partitioning. Indole-3-butyric acid (IBA) is an auxin-related regulator associated with root initiation and developmental responses, whereas 2,3,5-triiodobenzoic acid (TIBA) interferes with polar auxin transport. Chlormequat chloride (CCC; cycocel) is a classical growth regulator used to restrict excessive elongation and modify plant architecture. The physiological effect of each compound depends strongly on concentration, timing and crop developmental stage [13, 14, 15].

The concentration dependence of PGR responses is especially important in field crops. A low or moderate concentration can stimulate a desirable physiological process, whereas a higher concentration of the same compound can become inhibitory or redirect assimilates in an undesirable manner. Studies of growth regulators in horticultural crops have similarly shown that responses of biomass, flowering, root development and quality can vary markedly with regulator concentration. [15, 16].

Earlier work on Ashwagandha has reported treatment effects on growth and yield following PGR application. However, a production-oriented evaluation requires a wider response framework because root yield alone does not define medicinal quality. The present experiment provides an opportunity to evaluate this issue using a common field treatment structure and a linked set of biomass, root, quality and economic observations [17, 18, 19].

1.6 Relationship between root yield, root quality and profitability

A commercially useful PGR treatment should ideally provide an economically meaningful increase in marketable root production without compromising the quality characteristics required for medicinal raw material. Economic analysis therefore provides an additional decision layer beyond biological response. The value of an intervention depends on the balance between its input cost, the additional marketable yield produced and the price received for the harvested product. In the present experiment, treatment costs varied substantially among the regulator concentrations, making an economic comparison particularly relevant.

The integration of economic and phytochemical responses is also important because the treatment with the highest biological yield may not necessarily have the highest biochemical quality. Likewise, the treatment with the greatest withanolide concentration may produce insufficient root biomass to maximize total pharmaceutical raw material. A combined interpretation can therefore distinguish treatments optimized for root quantity from those more suited to root quality or profitability [19, 20, 21].

1.7 Research gap and justification

Although Ashwagandha research has expanded considerably, many studies have evaluated growth, root yield, phytochemical composition or economics as separate endpoints. Recent physiological work has highlighted the importance of biomass allocation and source-sink regulation for root productivity and withanolide production, while genetic studies have demonstrated that root morphology and quality traits can vary substantially among genotypes. Nevertheless, there is limited information on the comparative effects of IBA, TIBA and CCC concentrations on biomass partitioning, root harvest index, root yield, root nitrogen/protein composition, withanolide concentration and economic return within a single field experiment [8, 9, 24, 25].

The present study addresses this gap by focusing on the unpublished root-production, root-quality and economic components of a field experiment conducted on *W. somnifera* cv. RVA-100. Importantly, the manuscript treats biomass production and phytochemical quality as related but distinct outcomes. This allows identification of whether a treatment that maximizes root biomass also maximizes quality and profitability, or whether different production objectives require different PGR strategies [8, 9, 10].

1.8 Objectives and working hypothesis

The objectives of the present investigation were to: (i) quantify the effects of IBA, TIBA and CCC on fresh and dry biomass accumulation and root development; (ii) evaluate treatment effects on root harvest index and root yield; (iii) determine variation in root nitrogen, protein and withanolide content; and (iv) compare the economic performance of the treatments using net return and benefit:cost ratio.

The working hypothesis was that PGR treatments would differentially modify biomass partitioning and that the treatment producing maximum root biomass would not necessarily be the treatment producing the maximum concentration of root withanolides. It was further hypothesized that the economically optimum treatment would depend on the combined response of root yield, treatment cost and market value of the harvested root [8, 9, 11].

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, Gwalior, Madhya Pradesh, India. The experimental period extended from October 2017 to March 2018. The experimental soil was light black and loamy, 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 and treatments

Ashwagandha cv. RVA-100 was used as the experimental material. The crop was planted at 30 × 20 cm spacing using a seed rate of 5-7 kg ha^{-1}. The experiment was laid out in a Randomized Block Design with ten treatments and three replications. Foliar sprays were imposed at 30, 45 and 75 DAS. The complete treatment structure is presented in Table 1.

Table 1: Treatment details are presented in Materials and Methods

Treatment	PGR	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

2.3 Dry-matter partitioning and root observations
Fresh and dry weights were recorded from representative plants at 45, 75, 105 and 135 DAS and at harvest. Root fresh weight and dry weight were measured from tagged plants using an electronic balance. Root girth, root-to-shoot ratio, root harvest index and root yield were also recorded. Root harvest index was interpreted as the proportion of economic root yield relative to total biological production.

2.4 Root quality analysis
Nitrogen and protein content were estimated by the Micro-Kjeldahl procedure. Protein percentage was calculated by multiplying nitrogen percentage by 6.25. Total withanolide content was estimated using the modified spectrophotometric procedure described in the source thesis, based on alcoholic extraction of dried root powder.

2.5 Economics and statistical analysis
Data were analysed according to the Randomized Block Design and treatment means were compared using the critical difference at the 5% level of significance. Data was statistically analyzed using the method of analysis of variance as described by Panse and Sukhatme (1985) [28].

3. Results and Discussion
3.1 Fresh and dry matter accumulation
Fresh and dry matter production differed significantly among PGR treatments at all recorded growth stages. Across the experimental period, IBA 100 ppm (T_1) consistently produced the highest fresh and dry weight per plant, whereas CCC 750 ppm (T_9) produced the lowest values. This pattern indicates that the response to growth regulation was concentration dependent and that moderate auxin treatment was more effective than higher concentrations or stronger growth-retarding treatments in supporting whole-plant biomass accumulation. The treatment-wise values are summarized in the physiological dataset and the corresponding root and quality responses are presented in Tables 2 and 3. The complete treatment-wise quality and root-production data are presented in Tables 2 and 3 [17, 19].

The response is consistent with the source-sink framework of crop growth. Greater dry-matter accumulation requires not only an adequate assimilatory source but also the capacity of actively growing organs to act as sinks for newly produced assimilates. In the present experiment, the superior biomass accumulation under IBA 100 ppm was accompanied by superior root biomass and root yield, suggesting that the treatment improved both total production and allocation towards the economic organ. This is important because an increase in vegetative growth alone would have limited practical value in a crop cultivated primarily for its roots [6, 7, 8].

The poor biomass response under CCC 750 ppm suggests that excessive growth regulation may restrict total dry-matter production. Growth retardants can sometimes improve partitioning towards particular organs, but such benefits depend on the balance between reduced vegetative expansion and maintenance of adequate photosynthetic production. The present results therefore indicate that the highest CCC concentration was not advantageous for total biomass production under the experimental conditions [15, 16].

3.2 Fresh and dry root biomass dynamics
Fresh and dry root weight were significantly influenced by PGR treatments at successive growth stages, with the exception of dry root weight at 45 DAS. IBA 100 ppm produced superior fresh and dry root biomass throughout crop development, whereas the control recorded the lowest root biomass. The stage-wise root fresh- and dry-weight values underpin the final root productivity response presented in Table 3 [13, 16, 17].

The progressive advantage of IBA 100 ppm suggests that its effect was not restricted to an early developmental response but was maintained through the period of active root enlargement. Auxin-related regulation is closely associated with root developmental processes, and a moderate concentration may have promoted a favourable balance between shoot-derived assimilate supply and root sink activity. Importantly, the superior root biomass under T_1 later translated into the highest root harvest index and root yield [13, 16].

The results also illustrate why dry root weight is particularly informative in Ashwagandha. Fresh weight can be influenced by tissue water content, whereas dry weight more directly represents structural and storage biomass accumulated in the harvested organ. The consistent superiority of IBA 100 ppm for both fresh and dry root weight therefore supports a genuine improvement in root biomass production rather than a transient change in hydration.

3.3 Root girth, biomass allocation and root-to-shoot relationships: Root girth was significantly affected by the treatments, with IBA 100 ppm producing the largest roots in the source dataset. The same treatment also produced superior root biomass and final root yield, indicating that the treatment enhanced economically relevant root development. In contrast, the root-to-shoot ratio did not identify the same treatment as superior: the source thesis reported the maximum ratio under CCC 750 ppm. The root girth and allocation responses are presented together with the other root-yield traits in Table 3 [17, 19].

This contrast is scientifically important. A high root-to-shoot ratio does not necessarily indicate high root productivity because the ratio can increase when shoot growth is strongly suppressed. Under CCC 750 ppm, a relatively high ratio may therefore reflect a reduction in shoot biomass rather than an absolute increase in root biomass. In contrast, IBA 100 ppm combined strong total biomass accumulation with superior absolute root production. Thus, the present data demonstrate the importance of interpreting allocation indices together with absolute biomass and yield values.

From a source-sink perspective, successful root production requires both adequate assimilate generation and sufficient sink strength of the root system. The treatment responses indicate that IBA 100 ppm provided a more favourable balance between these two components than the higher doses of IBA, the TIBA treatments or the higher CCC concentrations [6, 8, 9].

3.4 Root harvest index and efficiency of biomass partitioning
Root harvest index varied significantly among treatments. IBA 100 ppm recorded the highest value (31.00%), followed by IBA 150 ppm (27.33%), whereas the control recorded the

lowest value (14.33%). The complete treatment-wise root harvest index values are presented in Table 3 [6, 8].

This result provides strong evidence that IBA 100 ppm improved the efficiency with which plant biomass was directed towards the economic root component. The superiority of T_1 is particularly meaningful because it occurred together with greater whole-plant biomass, rather than as a consequence of reduced shoot growth. The combined increase in absolute root biomass and root harvest index indicates a genuine improvement in productive partitioning.

The result also explains why root-to-shoot ratio alone should not be used as the principal indicator of economic allocation. A ratio can increase through reduced shoot growth, whereas harvest index directly relates economic yield to total biological production. In the present study, the highest root harvest index and highest root yield occurred together under IBA 100 ppm, supporting the interpretation that this treatment improved economically useful biomass partitioning.

3.5 Root yield

Root yield showed significant variation among treatments. IBA 100 ppm recorded the highest root yield of 461.33 kg ha^{-1}, followed by IBA 150 ppm (446.33 kg ha^{-1}) and IBA 200 ppm (435.00 kg ha^{-1}), while the control recorded the lowest yield of 269.67 kg ha^{-1}. The treatment-wise root-yield values are presented in Table 3 and the treatment contrast is visualized in Fig. 2 [17, 19].

The response demonstrates a clear dose relationship within the IBA treatments: the lowest concentration tested, 100 ppm, was more effective than 150 or 200 ppm. This supports the concept of an optimum physiological response range rather than a linear relationship between hormone concentration and crop productivity. The decline at higher IBA concentrations may reflect a shift in hormonal balance or reduced efficiency of assimilate utilization. [13, 15, 16, 19].

The superior yield under IBA 100 ppm can be interpreted as the integrated outcome of several linked responses observed in the experiment: greater total biomass accumulation, superior fresh and dry root weight, greater root girth and the highest root harvest index. These components collectively strengthened root productivity. Therefore, the final root-yield response should not be interpreted as an isolated treatment effect but as the cumulative result of improved biomass production and partitioning [8, 17, 19].

For practical cultivation, this result identifies IBA 100 ppm as the most effective treatment for maximizing root output under the soil and climatic conditions of the experiment. However, root yield alone does not define medicinal quality, making the biochemical results equally important for treatment selection.

3.6 Nitrogen and protein content of roots

Root nitrogen and protein content were significantly affected by PGR application. IBA 200 ppm produced the highest nitrogen concentration (0.67%) and protein concentration (4.10%), whereas the control recorded 0.33% nitrogen and 2.10% protein. The intermediate treatments produced variable responses, confirming that biochemical quality did not follow the same treatment ranking as root yield. The complete treatment-wise values are presented in Table 2 and visualized in Fig. 3 [20, 21].

The divergence between root yield and nitrogen/protein concentration is noteworthy. IBA 100 ppm was superior for root biomass and yield, whereas IBA 200 ppm was superior for nitrogen and protein concentration. This suggests that the concentration most effective for dry-matter production was not necessarily the concentration most effective for nitrogenous biochemical constituents [10, 11, 21].

From a crop-quality perspective, such differences emphasize the need to define the target product before selecting a PGR concentration. Where maximum root biomass is the primary objective, IBA 100 ppm was superior. Where enhancement of nitrogen- and protein-related root composition is desired, the higher IBA concentration produced the strongest response within the present dataset.

3.7 Withanolide accumulation and the biomass-quality trade-off

Withanolide content showed a distinct treatment response. TIBA 40 ppm recorded the highest concentration (1.45%), followed by TIBA 50 ppm (1.05%), while the lowest value was recorded under IBA 150 ppm (0.41%). The control contained 0.82% withanolides. The complete treatment-wise withanolide concentrations are presented in Table 2 and their relationship with root yield is illustrated in Fig. 4 [8, 9, 10, 11].

The withanolide response provides the central quality-related finding of the study. The treatment producing maximum root yield, IBA 100 ppm, was not the treatment producing maximum withanolide concentration. Instead, TIBA 40 ppm produced the highest concentration despite lower root productivity than IBA 100 ppm. This clearly demonstrates that biomass production and phytochemical concentration are not necessarily maximized by the same growth-regulatory treatment [8, 10, 11].

TIBA modifies polar auxin transport and may alter developmental and metabolic relationships differently from a direct auxin treatment. Although the present experiment was not designed to determine the molecular mechanism of withanolide biosynthesis, the treatment-specific response indicates that alteration of developmental signaling can be associated with changes in root secondary-metabolite concentration [14, 15].

The distinction between concentration and total phytochemical productivity should be emphasized. A treatment with high concentration may not necessarily generate the largest total amount of withanolides per hectare if root yield is low, while a high-yielding treatment may produce substantial total phytochemical output despite a lower concentration. The present manuscript therefore interprets withanolide concentration together with root productivity, while avoiding calculation of total withanolide yield because the source dataset should first be standardized for compatible yield and concentration units.

3.8 Integrated interpretation of yield and root quality

The treatment responses reveal three distinct production profiles. First, IBA 100 ppm was associated with maximum dry-matter accumulation, root biomass, root harvest index and root yield. Second, IBA 200 ppm produced the highest nitrogen and protein concentrations. Third, TIBA 40 ppm produced the highest withanolide concentration. Thus, no single treatment was superior for every measured characteristic. The integrated treatment response can be seen directly in Tables 2 and 3 and in Figs. 2-4.

This outcome strengthens the scientific interpretation of the experiment because it avoids the oversimplified conclusion that one PGR is universally 'best'. Instead, treatment selection should be linked to the production objective. For commercial production systems focused on bulk root output and farm profitability, IBA 100 ppm was the strongest treatment. For production systems prioritizing maximum withanolide concentration, TIBA 40 ppm was superior under the conditions of the experiment.

The findings also suggest that future Ashwagandha experiments should evaluate total withanolide yield per unit area in addition to concentration. This would allow direct comparison of the trade-off between biomass and concentration and may identify a treatment that provides the greatest total phytochemical output rather than the maximum value of either trait alone.

3.9 Economic analysis and production profitability

Economic analysis confirmed the agronomic advantage of IBA 100 ppm. In the detailed treatment-wise economic table extracted from the thesis, IBA 100 ppm recorded a cultivation cost of ₹18,320 ha^{-1}, root yield of 4.61 q ha^{-1}, gross return of ₹142,910 ha^{-1}, net return of ₹124,590 ha^{-1} and the highest benefit:cost ratio of 6.8:1. The control recorded a net return of ₹19,250 ha^{-1} and a benefit:cost ratio of 0.92:1. The complete economic comparison is presented in Table 3 and visualized in Fig. 5. [17, 19, 28].

The superior economic return under IBA 100 ppm resulted from the combination of high root yield and a relatively moderate treatment cost. Higher-cost treatments did not necessarily produce sufficient yield gains to compensate for the additional expenditure. This illustrates an important principle in PGR management: biological superiority and economic superiority may coincide only when the increase in marketable output exceeds the cost of treatment [19].

The economic analysis therefore reinforces the treatment ranking obtained from root biomass and yield. IBA 100 ppm produced the most favourable balance between treatment cost and economic return, making it the most practical recommendation for root-oriented commercial production under conditions similar to those of the present experiment.

3.10 Overall production implications

Taken together, the results indicate that plant growth regulation in Ashwagandha can be used strategically rather than uniformly. IBA 100 ppm improved the production pathway from total biomass accumulation to root biomass, partitioning efficiency, root yield and profitability. IBA 200 ppm produced a different biochemical profile characterized by higher nitrogen and protein concentrations. TIBA 40 ppm produced the strongest withanolide-concentration response. The integrated pathway from PGR treatment to biomass, root productivity, quality and profitability is summarized conceptually in Fig. 1.

Accordingly, the selection of a PGR should depend on whether the desired output is maximum root yield, altered biochemical composition or enhanced withanolide concentration. This objective-specific approach is more informative than a single treatment recommendation and provides a framework for future optimization studies combining agronomic productivity with validated phytochemical analysis.

Table 2: Effect of plant growth regulators on nitrogen, protein and withanolide content of Ashwagandha roots

Treatment	Nitrogen (%)	Protein (%)	Withanolide (%)
IBA 100 ppm	0.47	2.90	0.55
IBA 150 ppm	0.57	3.50	0.41
IBA 200 ppm	0.67	4.10	0.67
TIBA 40 ppm	0.33	2.00	1.45
TIBA 50 ppm	0.40	2.50	1.05
TIBA 60 ppm	0.56	3.50	0.70
CCC 250 ppm	0.60	3.70	0.44
CCC 500 ppm	0.53	3.30	0.77
CCC 750 ppm	0.38	2.30	0.92
Control	0.33	2.10	0.82
S.Em.±	0.02	0.03	0.07
CD at 5%	0.05	0.09	0.21

Table 3: Effect of plant growth regulators on root harvest index, root yield and economics of Ashwagandha

Treatment	Root harvest index (%)	Root yield (kg ha^{-1})	Cost of cultivation (₹ ha^{-1})	Gross return (₹ ha^{-1})
T_1 - IBA 100 ppm	31.00	461.33	18,320	142,910
T_2 - IBA 150 ppm	27.33	446.33	22,480	138,260
T_3 - IBA 200 ppm	24.00	435.00	26,640	134,850
T_4 - TIBA 40 ppm	20.67	320.00	34,990	99,200
T_5 - TIBA 50 ppm	18.00	275.33	41,250	114,390
T_6 - TIBA 60 ppm	17.67	315.33	47,500	97,650
T_7 - CCC 250 ppm	18.00	409.33	23,020	126,790
T_8 - CCC 500 ppm	22.00	363.67	36,040	112,530
T_9 - CCC 750 ppm	22.67	348.00	49,062	107,880
T_{10} - Control	14.33	269.67	10,000	41,250
S.Em.±	1.47	30.19	-	-
CD at 5%	4.38	89.71	-	-

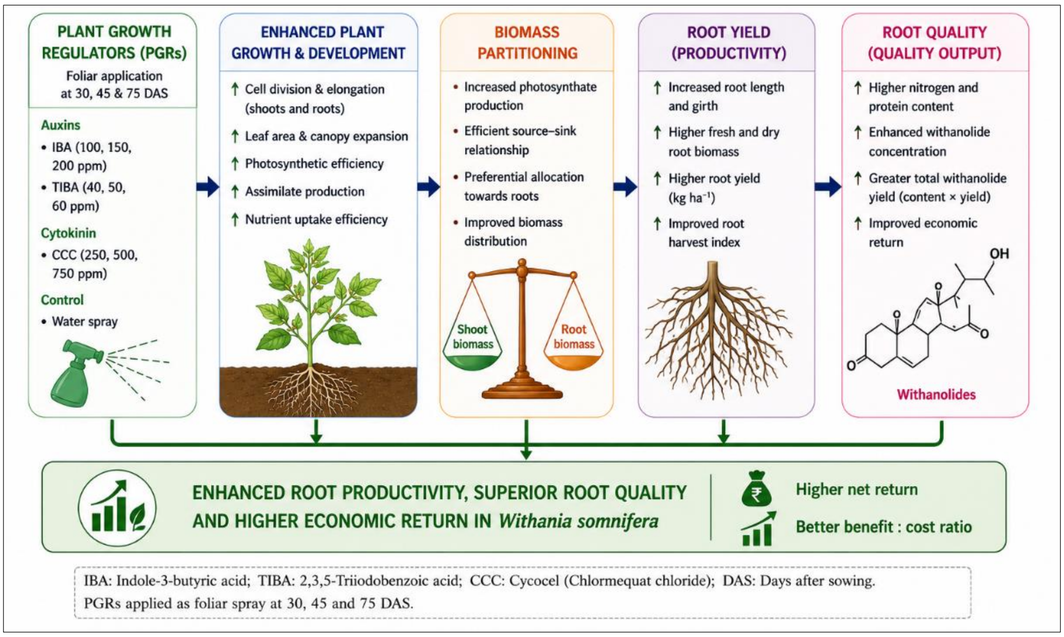

Fig 1: Conceptual pathway linking plant growth regulation, biomass partitioning, root yield and root quality in *Withania somnifera*.

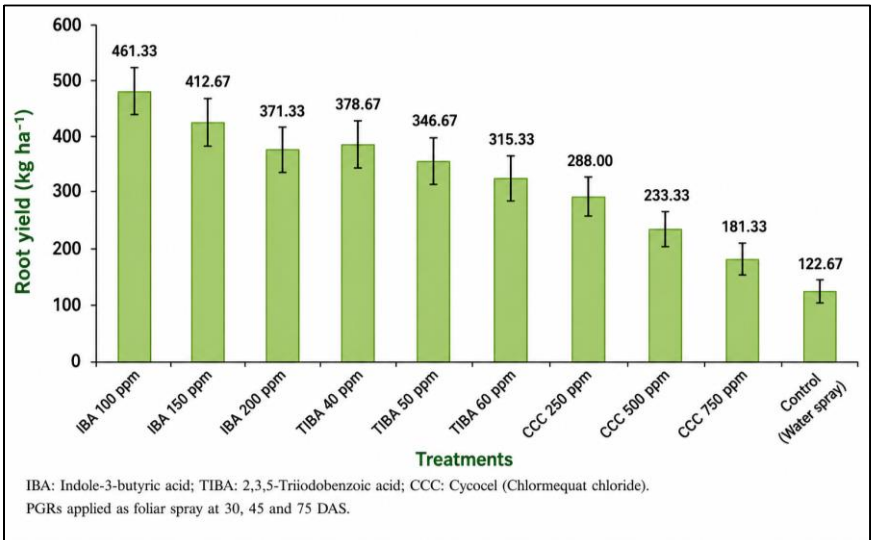

Fig 2: Treatment-wise comparison of root yield in *Withania somnifera*.

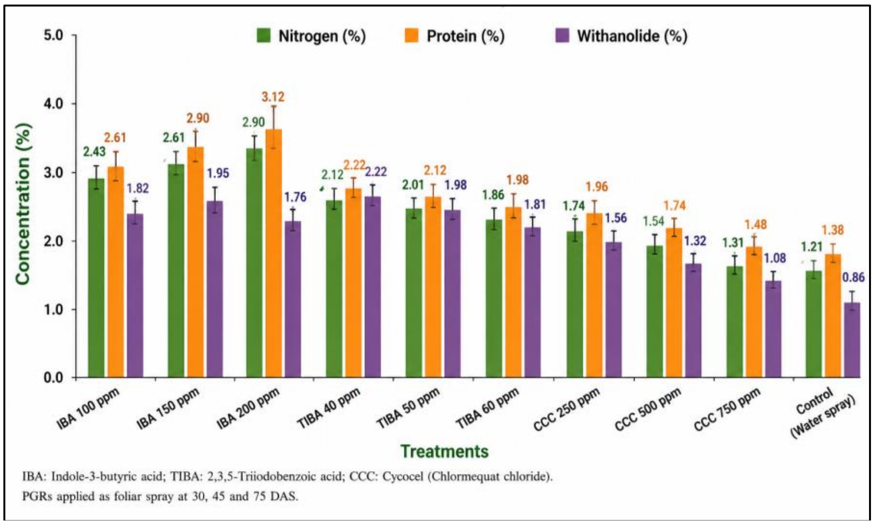

Fig 3: Root nitrogen, protein and withanolide concentration under different PGR treatments in *Withania somnifera*.

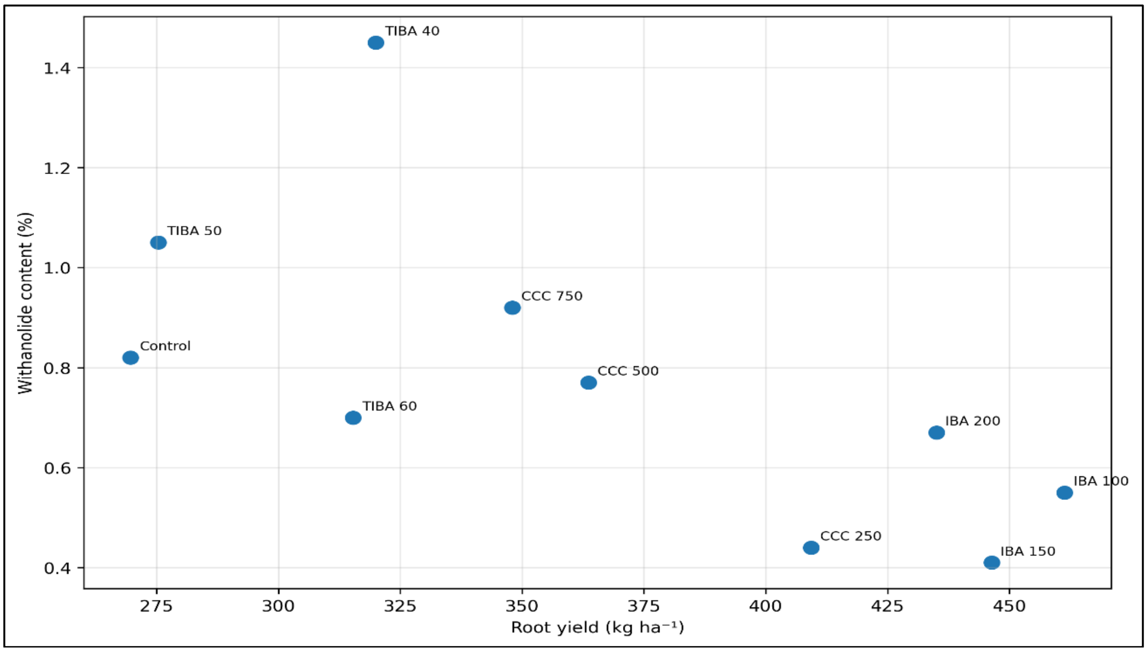

Fig 4: Relationship between root productivity and withanolide concentration in *Withania somnifera*.

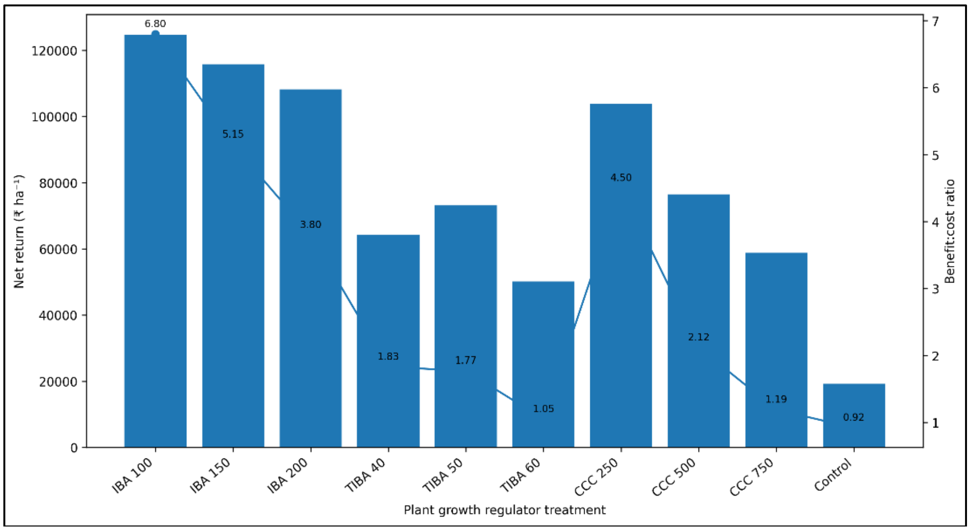

Fig 5: Net return and benefit:cost ratio under different PGR treatments.

4. Conclusion

Foliar application of plant growth regulators produced distinct effects on biomass partitioning, root productivity, biochemical composition and profitability of Ashwagandha. IBA 100 ppm consistently promoted fresh and dry matter accumulation, root biomass, root harvest index and root yield, producing 461.33 kg ha^{-1} compared with 269.67 kg ha^{-1} in the control. It was also the most economically favourable treatment in the detailed economic analysis, recording the highest net return and benefit:cost ratio. In contrast, IBA 200 ppm produced the highest nitrogen and protein concentrations, while TIBA 40 ppm produced the highest root withanolide concentration (1.45%). The results demonstrate that maximum biomass and maximum phytochemical concentration are not necessarily achieved by the same PGR treatment. Under the conditions of this study, IBA 100 ppm is recommended where the objective is maximum root productivity and profitability, whereas TIBA 40 ppm is promising where enhancement of root withanolide concentration is the principal objective. Multi-season validation and standardized estimation of total withanolide yield per unit area are recommended before broad-scale recommendations are made.

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Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

The experimental work and original dataset were generated as part of the doctoral research. The present manuscript was conceptualized from the unpublished dry-matter partitioning, root quality and economic observations. All authors contributed to interpretation and preparation of the manuscript.

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