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Spraying plant growth regulators alters morphological yield attributes, productivity and root withanolides in *Withania somnifera* (L.) Dunal

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Abstract
Ashwagandha (*Withania somnifera* (L.) Dunal), is a one of the most important medicinal plants of India and is widely used in Ayurveda for its adaptogenic, neuroprotective, and memory-enhancing properties. Its therapeutic value is attributed to active compounds such as alkaloids and withanolides. A field experiment was conducted during the winter season of 2022-2023 at the Herbal Garden, Department of Plant Physiology, JNKVV, Jabalpur, to assess the effect of foliar-applied plant growth regulators (PGRs) on crop morphology, biomass, and withanolide content. Results indicated that the leaf area index (LAI) was the highest when no growth regulator sprayed (only plain water spray). The maximum leaf biomass yield was found with spraying of IBA @ 50 ppm. The maximum stem, fruit, seed, and total biomass were observed under IBA @ 100 ppm. Root length as well as secondary root formation were also highest at IBA @ 100 ppm. Both IBA @ 100 ppm and NAA @ 100 ppm individually effective in enhances root yield. The maximum root yield 739.73 kg ha^{-1} was found with either of 100 ppm IBA or 100 ppm NAA. The highest harvest index (19.98%) was achieved with IBA @ 100 ppm and NAA @ 50 ppm (when root is used as economic yield), while 25% HI was recorded with IBA @ 50 ppm (when seed as economic yield). The maximum Withanolide A (0.067%) and Withanolide B (0.005%) contents were recorded under NAA @ 50 ppm and NAA @ 100 ppm, respectively. Corresponding yields were 40.65 kg ha^{-1} for Withanolide A and 3.48 kg ha^{-1} for Withanolide B. The study confirms that optimized PGR application improves biomass and bioactive content in ashwagandha, enhancing its therapeutic and cognitive value

Keywords: Ashwagandha, growth regulators, NAA, BA, IBA, alkaloids, withanolides, morphology

1. Introduction

Withania somnifera (L.) Dunal, commonly known as Ashwagandha or Indian Jinseng, is a highly valued medicinal plant belonging to the family *Solanaceae* (2n = 48). It is extensively cultivated in various regions of India, particularly in arid and semi-arid regions, with Madhya Pradesh contributing significantly to its commercial production over approximately 2000 hectares (Singh *et al*., 2011; Bhaure *et al*., 2014; Jana & Charan, 2018) [21, 8, 14]. Traditionally, both roots and leaves are utilized for their therapeutic properties, including anti-inflammatory, hepatoprotective, antibacterial, anti-tumor, hypotensive, immunosuppressive, and neurostimulant activities (Tiwari *et al*., 2014; Kushwaha *et al*., 2012; Mahima *et al*., 2012; Afewerky *et al*., 2021) [8, 18, 19, 1].

Ashwagandha is generally grown as a rainfed crop during the late *kharif* and *rabi* seasons, and its roots are the primary economic part used in Ayurvedic and modern medicine. In addition to essential primary metabolites like carbohydrates, proteins, and lipids, Ashwagandha synthesizes a wide array of secondary metabolites such as alkaloids, withanolides, flavonoids, lignans, and terpenoids, which hold significant pharmaceutical and nutraceutical value.

The biosynthesis of these secondary metabolites is often regulated by developmental cues and environmental stressors. Elicitors, whether biotic (e.g., microbial derivatives, plant extracts) or abiotic (e.g., plant growth regulators, heavy metals, light), act as signals that trigger physiological and metabolic responses, including the enhancement of secondary metabolite production (Radman *et al*., 2003; Ghorbanpour *et al*., 2014) [12, 20]. This process, known as elicitation, has emerged as a practical strategy to improve the yield of bioactive compounds in medicinal crops. Productivity result of any crop depends on numerous factors interacting throughout the life cycle of that crop.

Given the escalating demand for Ashwagandha in both domestic and global markets due to its proven pharmacological benefits, it is essential to explore agronomic and physiological interventions to boost its productivity and quality. While plant growth regulators (PGRs) have been widely reported to enhance morphological and physiological traits in various crops (Kumar *et al*., 2023) [17], limited studies have evaluated their role in improving the biomass and active phytochemical content of *Withania somnifera*. Therefore, the present study aims to assess the impact of PGRs on growth, yield, and withanolide accumulation in Ashwagandha under field conditions.

2. Materials and Methods

The experiment was conducted during the winter season of 2022-23 at the Herbal Garden, Department of Plant Physiology, Jawaharlal Nehru Agriculture University, Jabalpur. The study was laid out in a Completely Randomized Block Design (CRBD) comprising eight treatments, each replicated three times. The experimental site under the subtropical climatic region with clay loam soil texture characterized by low organic carbon content. Growbags were filled with a mixture of soil and Farmyard Manure (FYM) in a 2:1 ratio up to a height of 0.25 meters. Seeds of *Withania somnifera* (variety: Jawahar Ashwagandha 20) were sown in these growbags. Treatment sprays were applied at 25 and 55 days after transplanting (DAT). Data collected during the experiment were statistically analyzed from the methods described by Fisher (1967) [11]. Details of the treatments are provided in Table 1

Table 1: Details of Treatment and doses of PGRs used under the study.

Treatment	Treatment spray	Dose
T1	Control	-
T2	Naphthalene Acetic Acid (NAA)	50 mgL^{-1}
T3	Naphthalene Acetic Acid (NAA)	100 mgL^{-1}
T4	Benzyl Adenine (BA)	50 mgL^{-1}
T5	Benzyl Adenine (BA)	100 mgL^{-1}
T6	Indole Butyric Acid (IBA)	50 mgL^{-1}
T7	Indole Butyric Acid (IBA)	100 mgL^{-1}
T8	Indole Butyric Acid (IBA)	150 L^{-1}

3. Results and Discussion

The increasing medicinal significance of alkaloids and withanolides in pharmaceutical applications has amplified the cultivation of *Withania somnifera*. Plants synthesize alkaloids under stress conditions, providing defense against biotic and abiotic stress factors. To enhance the production of these phytochemicals, various plant growth regulators (PGRs) were evaluated through foliar sprays at different concentrations. Application of these PGRs not only influenced active compound production but also significantly impacted plant morphology, yield, and associated attributes.

3.1 Effect of PGRs on Morphological Traits

Morphological observations recorded during the Rabi season (sowing date: 17 October 2022) revealed substantial variation due to PGR applications administered at 25 and 55 days after transplanting (DAT). Plant height showed progressive increments until harvest maturity, significantly influenced by different PGR treatments. Maximum plant height was achieved with IBA @ 100 mg L^{-1}, while minimum growth was noted with NAA @ 50 mg and 100 mg L^{-1} individually. This result aligns with the known behavior of auxins, which stimulate plant growth at lower concentrations but inhibit growth at higher concentrations. The highest number of primary branches was recorded in treatments IBA @ 50, 100, 150 mg L^{-1} and NAA @ 100 mg L^{-1}, whereas BA @ 100 and 50 mg L^{-1} produced the fewest branches. The maximum secondary branches were achieved with BA @ 100 and 50 mg L^{-1} and NAA @ 50 mg L^{-1} individually. The number of leaves per plant varied significantly, more number of leaves with IBA @ 100 mg L^{-1}. Leaves function as the primary sites for photosynthesis and the biosynthesis of alkaloids and withanolides (Bhaure *et al*., 2014; Evans, 1989; Verma, 2002) [8, 10, 25]. An increase in leaf number potentially enhances biomass production, alkaloid, and withanolide synthesis, subsequently maximizing their yield if efficiently partitioned (Baraiya *et al*., 2012) [4]. The highest Leaf Area Index (LAI) was recorded in the control treatment (water spray), while the lowest LAI was noted with IBA @ 50 mg L^{-1} and BA @ 100 mg L^{-1}, statistically at par with each other.

3.2 Effect of PGRs on Yield and Yield Attributes

Stem biomass accumulation peaked at 1302.97 kg ha^{-1} with the application of IBA at 100 mg L^{-1}, significantly surpassing both the control treatment (water spray) and the BA treatment at 50 mg L^{-1}, which recorded the lowest biomass values of 654.37 and 708.75 kg ha^{-1}, respectively. Fruit biomass was highest (1672.46 kg ha^{-1}) under IBA @ 100 mg L^{-1} treatment, while the lowest fruit biomass ranged between 1069.58 to 1088.96 kg ha^{-1} in control, BA @ 100 mg L^{-1}, and IBA @ 150 mg L^{-1} treatments. Maximum seed biomass (686.62 kg ha^{-1}) was observed with IBA @ 100 mg L^{-1}, contrasting with minimum accumulation (379.17 kg ha^{-1}) in the control. Auxin at lower concentrations promotes flowering, fruit set, and seed yield. Root length progressively increased with plant maturity, reaching its maximum under IBA @ 100 mg L^{-1}, while the minimum was recorded with water spray and NAA @ 100 mg L^{-1} treatments individually. The application of IBA induces mild stress in aerial plant parts, stimulating root growth to counteract stress conditions. Root biomass production was highest (739.73-700.73 kg ha^{-1}) under NAA @ 100 mg L^{-1}, statistically comparable to IBA @ 100 mg L^{-1}, whereas the lowest biomass (509.17-540.83 kg ha^{-1}) was recorded under control, BA @ 50 mg L^{-1}, and IBA @ 150 mg L^{-1}. The IBA @ 100 mg L^{-1} treatment enhanced root yield by 45.28% compared to the control, attributed to delayed senescence and flowering, facilitating greater assimilate accumulation in roots (Bhaure *et al*., 2014; Barath Kumar *et al*., 2018) [8, 7]. Secondary root number per plant was maximum in treatments involving IBA @ 100 mg L^{-1}, BA @ 100 mg L^{-1}, and BA @ 50 mg L^{-1}, with minimum numbers under water spray. IBA promotes early and profuse secondary root formation across horticultural and forestry crops (Taiz and Zeiger, 2003; Bhaure *et al*., 2014) [22, 8]. Spraying of Withania root extract was very effective to managing weed in maximizing harvest index (22.52%) taking root as economic yield showing improvement in partitioning of assimilates towards economic sink, i.e., root (Mohare *et al*., 2023) [2]. Total biomass accumulation was highest with IBA @ 100 mg L⁻¹, resulting in a 56.89% increase over the control. The maximum harvest index (HI) (19.98%) for root yield was obtained with IBA @ 100 mg L⁻¹ and NAA @ 50 mg L⁻¹ individually, while for seed yield, maximum HI (25%) was recorded with IBA @ 50 mg L⁻¹ (Table 2).

Table 2: Effect of PGRs spray on estimated biomass yield attributes, Yield and HI (%) of *Withania somnifera*.

Treatment	Stem (kg/ha)	Root (kg/ha)	Leaves (kg/ha)	Fruit (kg/ha)	Seed (kg/ha)	HI1* (%)	HI 2* (%)
T_1(Water spray)	654.37	509.17	330.00	1069.58	379.17	19.21	14.31
T_2(NAA @ 50 ppm)	726.25	610.42	353.33	1125.12	479.17	19.88	15.60
T_3(NAA @ 100 ppm)	983.75	739.73	386.75	1190.33	451.42	18.16	10.86
T_4(BA @ 50 ppm)	708.75	529.10	285.67	1245.16	487.50	15.26	16.64
T_5(BA @ 100 ppm)	947.50	552.61	340.42	1071.13	533.75	16.24	17.37
T_6(IBA @ 50 ppm)	828.33	647.32	422.00	1285.00	624.58	18.15	24.57
T_7(IBA @ 100 ppm)	1302.97	700.73	259.17	1672.46	686.62	15.28	25.00
T_8(IBA @ 150 ppm)	723.33	540.83	344.58	1088.96	440.83	19.98	16.27
Sem±	22.116	14.185	12.015	17.511	11.815	0.727	0.466
CD (at 5%)	66.302	42.526	36.022	52.498	35.421	2.180	1.396

3.3 Effect of PGRs on Withanolide A and Withanolide B Content

The highest concentration of Withanolide A (0.067%) was obtained with NAA @ 50 mg L⁻¹, while Withanolide B peaked (0.005%) under NAA @ 100 mg L⁻¹ and IBA @ 150 mg L⁻¹ treatments individually. The highest Withanolide A yield (40.65 kg ha⁻¹) was also observed with NAA @ 50 mg L⁻¹, and the lowest under BA @ 50 mg L⁻¹ and IBA @ 50 mg L⁻¹ treatments. Withanolide B yield reached a maximum (3.48 kg ha⁻¹) with NAA @ 100 mg L⁻¹ and minimum yields under water spray and IBA @ 50 mg L⁻¹ treatments. The alkaloid assimilation was first initiated in the leaves (source) and subsequently transported to the roots (sink) via the stem (partitioning channel), as the highest percentage of alkaloids was noted initially in the leaves at 45 DAS, whereas the roots recorded the lowest alkaloid percentage at the same stage. Alkaloids synthesized in the leaves were progressively translocated towards the roots, as evidenced by an increase in alkaloid percentage in the roots with advancing crop age up to 105 DAS (50% fruiting stage). The total alkaloid accumulation in the roots continued to increase up to 135 DAS (first fruit maturity stage). However, the partitioning efficiency of alkaloids towards the roots was impaired due to enhanced retention of alkaloids within the transport channel (stem) from 45 DAS (tertiary branch initiation) to 105 DAS (50% fruiting stage) in *Withania somnifera* (Baraiya *et al*., 2005) [6]. Withanolides, like alkaloids, are secondary metabolites synthesized in response to environmental stress. Foliar sprays of synthetic auxins presumably mimic stress conditions, thereby enhancing the biosynthesis of Withanolide A and B in the cultivated crop (Table 3).

Table 3: Effect of PGRs spray on percentage (%) and yield (kg ha-1) of Withanolide A and Withanolide B in roots of *Withania somnifera*.

Treatment	Withanolide A (%)	Withanolide B (%)	Withanolide A (kg ha^{-1})	Withanolide B (Kg ha^{-1})
T_1(Water spray)	0.066	0.003	33.44	1.27
T_2(NAA @ 50 ppm)	0.067	0.003	40.65	1.83
T_3(NAA @ 100 ppm)	0.049	0.005	35.88	3.48
T_4 (BA @ 50 ppm)	0.043	0.003	22.91	1.69
T_5 (BA @ 100 ppm)	0.065	0.004	35.86	2.43
T_6 (IBA @ 50 ppm)	0.036	0.002	23.43	1.34
T_7(IBA @ 100 ppm)	0.000	0.003	0.01	2.03
T_8(IBA @ 150 ppm)	0.000	0.005	0.01	2.70
Sem±	0.0001	0.0001	0.798	0.059
CD (at 5%)	0.0002	0.0002	2.392	0.176

4. Conclusion

The foliar application of NAA at 100 mg L⁻¹ and IBA at 100 mg L⁻¹ individually proved most effective in enhancing root biomass, with values ranging from 700.73 to 739.73 kg ha⁻¹. Among these, IBA at 100 mg L⁻¹ also resulted in the greatest root length (18.56 cm) and highest number of secondary roots (9.25). Additionally, both IBA concentrations (50 and 100 mg L⁻¹) were equally effective in optimizing the Harvest Index, achieving values between 18.15% and 19.88%, emphasizing the economic importance of root yield. In terms of phytochemical accumulation, the highest concentration of Withanolide A (0.067%) was observed with NAA at 50 mg L⁻¹, while the peak concentration of Withanolide B (0.005%) was recorded with NAA at 100 mg L⁻¹ and was comparable to IBA at 150 mg L⁻¹. Notably, the maximum Withanolide A yield (40.65 kg ha⁻¹) and Withanolide B yield (3.48 kg ha⁻¹) were achieved with foliar sprays of NAA at 50 mg L⁻¹ and 100 mg L⁻¹, respectively.

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