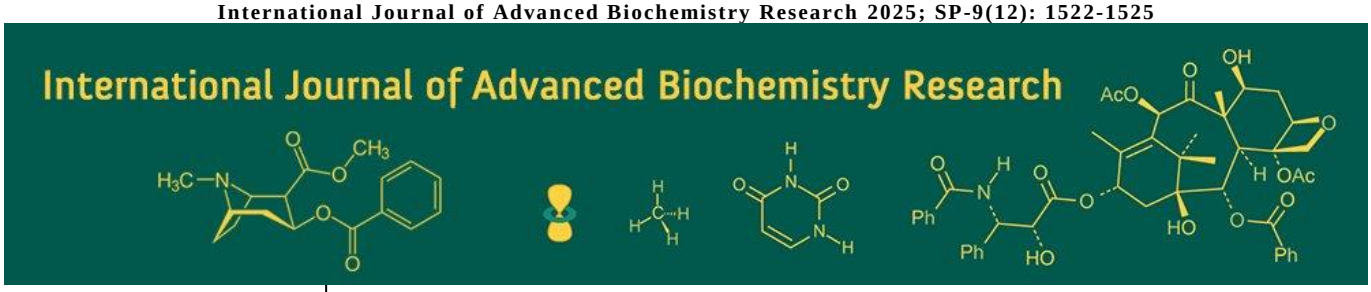

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Vaishnavi Gawande
Ph.D. Scholar, Department of Plant Physiology, Post Graduate Institute, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola, Maharashtra, India

Dr. SB Amarshettiwar
Professor, CAS, Department of Plant Physiology, Post Graduate Institute, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola, Maharashtra, India

Dr. MP Moharil
Associate Professor, Biotechnology Centre, Department of Agricultural Botany, Post Graduate Institute Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola, Maharashtra, India

Dr. TH Rathod
Head Centre Department of Agricultural Botany, Post Graduate Institute, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola, Maharashtra, India

Dr. SB Deshmukh
Associate Cotton Breeder, Cotton Research Unit, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola, Maharashtra, India

Corresponding Author:
Vaishnavi Gawande
Ph.D. Scholar, Department of Plant Physiology, Post Graduate Institute, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola, Maharashtra, India

Physiological response of IAA, chitosan and chitosan-based indole acetic acid nanoparticles on morpho-physiological traits of cotton (*Gossypium hirsutum* L.)

Vaishnavi Gawande, SB Amarshettiwar, MP Moharil, TH Rathod and SB Deshmukh

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Abstract
Cotton is an economically important fibre crop whose growth and productivity are influenced by hormonal balance, nutrient uptake, and stress regulation. Chitosan-based nanoparticles have emerged as an efficient nano-biopolymer technology for enhancing plant growth due to their slow-release behavior and ability to improve nutrient absorption, hydration status, and metabolic activity. The present investigation entitled "Physiological Response of Chitosan-Based Indole Acetic Acid Nanoparticles in Cotton" was conducted during Kharif 2022-2023 at the Department of Agricultural Botany, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola, to evaluate the effect of chitosan, IAA, and chitosan-based IAA nanoparticles on morphological and physiological growth attributes of cotton. The experiment comprised ten treatments with foliar sprays of auxin, chitosan, and varying concentrations of chitosan-based IAA nanoparticles. Results revealed that treatment 25 ppm auxin (T_2) recorded significantly maximum plant height, leaf area, leaf area index, and dry matter accumulation, while 75 ppm chitosan (T_3) resulted in the highest relative water content at harvest. Conversely, the lowest values for all parameters were obtained under the control (T_1). While twice-applied auxin at 25 ppm primarily enhanced morphological traits, nanoparticle treatment was more effective in improving yield and yield-attributing characters, with chitosan (75 ppm) further contributing to improved water retention and stress tolerance.

Keywords: Cotton, chitosan-based indole acetic acid nanoparticles, auxin, indole acetic acid, morphology, physiology, foliar spray, growth attributes

Introduction
Cotton (*Gossypium hirsutum* L.) is one of the world's most important fibre crops, cultivated extensively for its lint, seed, oil, and by-products that support several agro-industrial sectors. As a major raw material for the textile industry, cotton contributes significantly to rural employment, national income, and export earnings. India is the only country where all four cultivated cotton species *G. hirsutum*, *G. arboreum*, *G. herbaceum* and *G. barbadense* are grown commercially, with *G. hirsutum* occupying the majority of the cultivated area and contributing more than 90% of the national production (Chen *et al*., 2007).

The productivity of cotton is influenced by physiological processes such as photosynthesis, transpiration, nutrient uptake, and hormonal balance. In particular, the regulation of plant hormones plays a crucial role in vegetative and reproductive development. Chitosan is a natural, biodegradable, non-toxic polysaccharide derived from chitin, the second most abundant organic polymer found in crustacean shells, insect exoskeletons, and fungal cell walls. Over the last two decades, chitosan has gained significant attention in agriculture due to its beneficial effects on plant growth, immunity, and physiological regulation.

Chitosan enhances plant growth by: improving nutrient uptake due to its chelating ability, inducing antioxidant enzyme activity, increasing chlorophyll content, improving water-use efficiency, modulating stomatal conductance and enhancing resistance to biotic and abiotic stresses.

Chitosan forms a semi-permeable film on leaf surfaces, thereby reducing transpiration and improving the plant's water retention capacity. Several studies have demonstrated increased RWC, reduced oxidative stress and improved leaf hydration in chitosan-treated plants (Lee et

al., 1999) [2]. Environmental constraints, nutrient imbalances, and stress conditions frequently hinder cotton's physiological performance. Foliar nutrition and hormonal regulation have therefore emerged as valuable strategies to modulate plant metabolism, enhance growth attributes, and improve yield components. Auxins, particularly IAA, have been shown to increase cell elongation by modifying cell wall plasticity through the acid growth hypothesis. Auxin application enhances proton pump activity, increases cell wall loosening enzymes, and promotes turgor-driven expansion. Additionally, auxin plays a crucial role in vascular tissue differentiation which ensures efficient transport of water and nutrients. Improved vascular functioning directly influences physiological processes like photosynthesis and assimilate translocation.

Nanotechnology has revolutionized agriculture by enabling the development of nano-formulations of fertilizers, pesticides, and plant growth regulators. Nanoparticles offer unique properties such as: higher surface area to volume ratio, enhanced penetration into plant tissues, controlled release of active ingredients, better stability and bioavailability and reduced loss due to drift, volatilization, or degradation. Chitosan nanoparticles (CS-NPs) are particularly advantageous due to their biocompatibility, environmental safety, and exceptional delivery efficiency. When hormones like IAA are encapsulated within chitosan nanoparticles, they gain improved protection from degradation and enhanced cellular uptake, allowing for slow and sustained release into plant tissues. This results in increased biological activity even at low concentrations (Pereira *et al*., 2019) [3]. Considering the importance of chitosan-based hormones in improving crop growth, the present research was undertaken to assess the morpho-physiological response of cotton to chitosan, auxin, and chitosan-based IAA nanoparticles.

Materials and Methods

The study was carried out during Kharif 2022-2023 at the Research Field of the Department of Agricultural Botany, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola. The cotton variety PDKV-JKAL-116 BGII was used. Chitosan-based IAA nanoparticles were prepared using laboratory-grade chitosan and Indole Acetic Acid.

Ten treatments were evaluated: T_1: Control (water spray); T_2: Auxin 25 ppm (two sprays); T_3: Chitosan 75 ppm (two sprays); T4: Chitosan-based IAA NPs 25 mg/L (one spray); T_5: Chitosan-based IAA NPs 2.5 mg/L (two sprays); T6: Chitosan-based IAA NPs 5 mg/L (two sprays); T7: Chitosan-based IAA NPs 10 mg/L (two sprays); T8: Chitosan-based IAA NPs 15 mg/L (two sprays); T9: Chitosan-based IAA NPs 20 mg/L (two sprays); T10: Chitosan-based IAA NPs 25 mg/L (two sprays). Data on plant height, leaf area, LAI, dry matter, and RWC were recorded at standard intervals. The experiment was laid out in a Randomized Block Design (RBD) with three replications with recommended spacing of 90 cm × 60 cm. Sprays were applied using a hand-held knapsack sprayer with a fine nozzle to ensure uniform leaf coverage. Spray schedule: First spray: At the onset of flowering and Second spray: During boll development (for treatments requiring two sprays). Spraying was done in early morning to avoid rapid evaporation.

Observations Recorded

Measurements were collected from five randomly selected plants per plot at various growth stages. Growth Parameters such as Plant height (cm) at 30, 60, 90, 120 and 150 DAS; Leaf area (dm² plant^{-1}). Calculated using formula by Ashley *et al*. (1963) at 30, 60, 90, 120 and 150 DAS; Leaf Area Index (LAI)Computed using Watson's (1952) [5] formula at 30, 60, 90, 120 and 150 DAS; Dry matter accumulation (g plant^{-1}) Plants were dried to constant weight at 70 °C; Relative Water Content (RWC%) Measured using Barrs & Weatherley (1962) [6] method.

Statistical Analysis

Data were analyzed using analysis of variance (ANOVA) as described by Panse and Sukhatme (1954) [7]. Critical Difference at 5% level was used for mean comparison.

Results and Discussion

Auxin at 25 ppm (T_2) significantly improved plant height, leaf area, leaf area index, and dry matter accumulation compared to the control. The enhanced growth may be attributed to increased cell elongation and improved metabolic activity. Chitosan at 75 ppm (T_3) recorded the highest relative water content at harvest, suggesting its role in enhancing plant osmotic adjustment and water retention.

1. Plant Height (cm)

Plant height data was considered non-significant at 30 DAS based on data from 2022-2023. Similarly, at 60 DAS, the data on plant height was deemed non-significant. Data showed that plant height varied significantly starting at 90 DAS.

According to the data, the significantly highest plant height (118.10 cm) of cotton was recorded with treatment T_2 (25 ppm auxin) at 90 DAS, and this was statistically at par with T_3 (75 ppm chitosan) (109.20 cm) and T_6 (5 mg l^{-1} of chitosan based IAA NPs) (104.72 cm) at 90 DAS. So, treatments T_2, T_3, and T_6 were found to be at par with each other. However, minimum plant height (90.1 cm) was recorded in treatment T_1 (control).

At 120 DAS, significantly maximum plant height (142.82 cm) was recorded in treatment T_2 (25 ppm auxin) to rest of all other treatments under study except T_3 (75 ppm chitosan) (132.00 cm), T_6 (5 mg l^{-1} of chitosan based IAA NPs) (130.12 cm), and T_5 (2.5 mg l^{-1} of chitosan based IAA NPs) (128.65 cm). Treatments T_2, T_3, T_6, and T_5 were found at par with each other. On the other hand, minimum plant height (109.10 cm) was recorded in treatment T_1 (control).

At 150 DAS, a similar trend was observed; maximum and significantly superior plant height was recorded in treatment T_2 (25 ppm auxin) (149.18 cm), followed by T_3 (75 ppm chitosan) (138.55 cm), T_6 (5 mg l^{-1} of chitosan based IAA NPs) (137.89 cm), and T_5 (2.5 mg l^{-1} of chitosan based IAA NPs) (135.41 cm) when compared with treatment T_1 (control). Treatments T_2, T_3, T_6, and T_5 were found at par with each other. On the other hand, minimum plant height (115.34 cm) was recorded in treatment T_1 (control).

Auxin increases endogenous hormone levels, stimulates meristematic activity and improves vascular functioning, resulting in improved assimilate translocation and stem elongation.

2. Leaf Area (dm² plant^{-1})

Data regarding 2022-23 stated that, at 30 DAS, the data regarding leaf area per plant was found to be nonsignificant. Similarly, at 60 DAS, the data regarding leaf area per plant were found non significant. Data showed a significant difference in leaf area per plant from 90 DAS onwards.

According to the data, the significantly maximum leaf area (50.03 dm²) of cotton was recorded with treatment T_2 (25 ppm auxin) at 90 DAS, and this was statistically at par with T_3 (75 ppm chitosan) (45.00 dm²) at 90 DAS. So, treatments T_2 and T_3 were found at par with each other. On the other hand, minimum leaf area (34.12 dm²) was recorded in treatment T_1 (control).

At 120 DAS, the maximum leaf area (68.15 dm²) was recorded in treatment T_2 (25 ppm auxin) to rest of all other treatments under study except T_3 (75 ppm chitosan) (63.44 dm²). Treatments T_2 and T_3 were found at par with each other. However, minimum leaf area (47.65 dm²) was recorded in treatment T_1 (control).

At 150 DAS, maximum and significantly superior leaf area was recorded in treatment T_2 (25 ppm auxin) (54.90 dm²) followed by T_3 (75 ppm chitosan) (52.19 dm²), T_6 (5 mg l^{-1} of chitosan based IAA NPs) (52.17 dm²), and T_5 (2.5 mg l^{-1} of chitosan based IAA NPs) (50.09 dm²) when compared with treatment T_1 (control). Treatments T_2, T_3, T_6, and T_5 were found at par with each other. However, minimum leaf area (35.63 dm²) was recorded in treatment T_1 (control).

Auxin promotes cell expansion and division in leaves, resulting in larger lamina size.

Chitosan also contributes by enhancing chlorophyll content and improving nutrient uptake.

3. Leaf Area Index (LAI)

Data regarding 2022-23 stated that, at 30 DAS, the data regarding leaf area index per plant was found non significant. Similarly At 60 DAS, the data regarding leaf area index per plant was found non significant. Data showed a significant difference in leaf area index per plant from 90 DAS onwards.

According to the data, the significantly maximum leaf area index (1.235) of cotton was recorded with treatment T_2 (25 ppm auxin) at 90 DAS, and this was statistically at par with T_3 (75 ppm chitosan) (1.111) at 90 DAS. So, treatments T_2 and T_3 were found at par with each other. However, the minimum leaf area index (0.842) was recorded in treatment T_1 (control).

At 120 DAS, significantly maximum leaf area index (1.683) was recorded in treatment T_2 (25 ppm auxin) to rest of all other treatments under study except T_3 (75 ppm chitosan) (1.567). Treatments T_2 and T_3 were found at par with each other. On the other hand, the minimum leaf area index (1.177) was recorded in treatment T_1 (control).

At 150 DAS, the maximum and significantly superior leaf area index was recorded in treatment T_2 (25 ppm auxin) (1.356), followed by T_3 (75 ppm chitosan) (1.289), T_6 (5 mg l^{-1} of chitosan based IAA NPs) (1.288), and T_5 (2.5 mg l^{-1} of chitosan based IAA NPs) (1.237) when compared with treatment T_1 (control). Treatments T_2, T_3, T_6, and T_5 were found at par with each other. However, the minimum leaf area index (0.880) was recorded in treatment T_1 (control).

Improved LAI in auxin-treated plants may be due to increased leaf expansion and better canopy structure.

4. Dry Matter Accumulation (g plant^{-1})

Dry matter accumulation reflects the crop's overall photosynthetic performance.

According to data from 2022-2023, dry matter production data was regarded as non-significant at 30 DAS. In the precise same way, the dry matter production data was assessed as non-significant at 60 DAS. Data demonstrated a notable variation in dry matter production commencing at 90 DAS.

Table 1: Effect of different concentrations of chitosan, IAA and chitosan based IAA nanoparticles on plant height (cm plant^{-1}) at different growth stages of cotton.

Treatments	plant height (cm plant^{-1})					leaf area (dm^2 plant^{-1})					leaf area index				
	30 DAS	60 DAS	90 DAS	120 DAS	150 DAS	30 DAS	60 DAS	90 DAS	120 DAS	150 DAS	30 DAS	60 DAS	90 DAS	120 DAS	150 DAS
T1 (Control (Water spray)	27.26	72.70	90.10	109.1	115.34	5.77	19.76	34.12	47.65	35.63	0.142	0.488	0.842	1.177	0.88
T_2 (Auxin spray as referenced doses Sprayed twice)	24.12	67.19	118.1	142.82	149.18	4.61	20.49	50.03	68.15	54.9	0.114	0.506	1.235	1.683	1.356
T_3 (Chitosan spray as referenced doses Sprayed twice)	29.55	71.31	109.2	132.00	138.55	5.47	21.85	45	63.44	52.19	0.135	0.539	1.111	1.567	1.289
T_4 (25 mg l^{-1} of chitosan based IAA NPs Sprayed once.)	23.00	66.86	95.94	117.17	123.04	5.32	25.63	36.95	51.22	40.62	0.131	0.633	0.912	1.265	1.003
T_5 (2.5 mg l^{-1} of chitosan based IAA NPs Sprayed twice.)	26.62	70.55	102.44	128.65	135.41	4.27	23.15	42.13	59.45	50.09	0.105	0.572	1.04	1.468	1.237
T_6 (5 mg l^{-1} of chitosan based IAA NPs Sprayed twice.)	25.00	65.75	104.72	130.12	137.89	4.81	24.71	43.51	60.52	52.17	0.119	0.61	1.074	1.494	1.288
T_7 (10 mg l^{-1} of chitosan based IAA NPs Sprayed twice.)	28.65	68.06	100.31	122.38	130.01	5.78	22.25	41.98	57.45	47.48	0.143	0.549	1.037	1.419	1.172
T_8 (15 mg l^{-1} of chitosan based IAA NPs Sprayed twice.)	23.65	66.86	99.49	121.38	129.07	4.72	22.97	40.12	55.32	45.2	0.117	0.567	0.991	1.366	1.116
T_9 (20 mg l^{-1} of chitosan based IAA NPs Sprayed twice.)	29.12	70.02	97.63	119.11	126.88	5.97	24.05	39.45	53.15	42.14	0.147	0.594	0.974	1.312	1.04
T_{10} (25 mg l^{-1} of chitosan based IAA NPs Sprayed twice)	27	69.13	93.12	113.61	118.97	5.18	22.65	35.08	49.1	37.85	0.128	0.559	0.866	1.212	0.935
SEm±	-	-	4.78	5.95	6.43	-	-	2.03	2.47	2.21			0.05	0.061	0.054
CD or LSD	-	-	14.22	17.69	19.12	-	-	6.03	7.36	6.59			0.148	0.181	0.162
F-Test (p=0.05)	NS	NS	S	S	S	NS	NS	S	S	S	NS	NS	S	S	S

Table 2: Effect of different concentrations of chitosan, IAA and chitosan based IAA nanoparticles dry matter (g) and RWC (%) at different growth stages of cotton.

Treatments	Dry matterc (g)					RWC (%)	
	30 DAS	60 DAS	90 DAS	120 DAS	150 DAS	50% flowering	At harvest
T_1 (Control (Water spray)	6.53	53.57	143.21	199.75	180.98	75.65	54.8
T_2 (Auxin spray as referenced doses Sprayed twice)	5.7	46.81	191.94	264.94	231.57	70.46	62.35
T_3 (Chitosan spray as referenced doses Sprayed twice)	8.49	49.38	183.27	255.9	223.35	78.69	67.91
T_4 (25 mg l^{-1} of chitosan based IAA NPs Sprayed once.)	6.93	57.57	156.51	215.51	189.7	74.88	57.77
T_5 (2.5 mg l^{-1} of chitosan based IAA NPs Sprayed twice.)	7.59	54.48	170	237.95	216.12	69.92	66.6
T_6 (5 mg l^{-1} of chitosan based IAA NPs Sprayed twice.)	8.23	50.92	172.14	238.46	220.85	72.72	65.5
T_7 (10 mg l^{-1} of chitosan based IAA NPs Sprayed twice.)	5.7	55.33	167	230.57	215.54	67.23	63.41
T_8 (15 mg l^{-1} of chitosan based IAA NPs Sprayed twice.)	7.22	55.5	166.57	227.56	211.25	71.4	61.6
T_9 (20 mg l^{-1} of chitosan based IAA NPs Sprayed twice.)	7.06	60.36	164.64	224.64	206.41	76.82	60.52
T_{10} (25 mg l^{-1} of chitosan based IAA NPs Sprayed twice)	6.07	40.56	149.43	206.99	188.79	68.49	57.24
SEm±	-	-	7.79	11.5	9.8	-	1.85
CD or LSD	-	-	23.16	34.17	29.13	-	5.52
F-Test (p=0.05)	NS	NS	S	S	S	NS	S

According to the data, the significantly highest dry matter production (191.94 g) of cotton was recorded with treatment T_2 (25 ppm auxin) at 90 DAS, and this was statistically at par with T_3 (75 ppm chitosan) (183.27 g), T_6 (5 mg l^{-1} of chitosan based IAA NPs) (172.14 g), and T_5 (170.00 g) at 90 DAS. So, treatments T_2, T_3, T_6, and T_5 were found at par with each other. However, minimum dry matter production (143.21 g) was recorded in treatment T_1 (control).

At 120 DAS, significantly maximum dry matter production (264.94 g) was recorded in treatment T_2 (25 ppm auxin) to rest of all other treatments under study except T_3 (75 ppm chitosan) (255.90 g), T_6 (5 mg l^{-1} of chitosan based IAA NPs) (237.95 g), and T_5 (2.5 mg l^{-1} of chitosan based IAA NPs) (237.95 g). Treatments T_2, T_3, T_6, and T_5 were found at par with each other. However, minimum dry matter production (199.75 g) was recorded in treatment T_1 (control).

At 150 DAS, maximum and significantly superior dry matter production was recorded in treatment T_2 (25 ppm auxin) (231.57 g), followed by T_3 (75 ppm chitosan) (223.35 g), T_6 (5 mg l^{-1} of chitosan based IAA NPs) (220.85 g), T_5 (216.12 g) (2.5 mg l^{-1} of chitosan based IAA NPs), T_7 (10 mg l^{-1} of chitosan based IAA NPs) (215.54 g), and T_8 (15 mg l^{-1} of chitosan based IAA NPs) (211.25 g) when compared with treatment T_1 (control). However, minimum dry matter production (180.98 g) was recorded in treatment T_1 (control).

Auxin significantly enhanced biomass accumulation due to enhanced photosynthesis, respiration and metabolite transport. Chitosan also improved dry matter due to its positive effects on stomatal regulation and water use efficiency.

5. Relative Water Content (RWC%)

Data regarding relative water content during 2022-23, significantly maximum (67.91%) was recorded in treatment T_3 (75 ppm chitosan) to rest of all treatments under study except T_5 (66.60%) (2.5 mg l^{-1} of chitosan based IAA NPs), T_6 (65.50) (5 mg l^{-1} of chitosan based IAA NPs) and T_7 (10 mg l^{-1} of chitosan based IAA NPs) (63.41 %). Treatments T_3, T_5, T_6 and T_7 were at par with each other. However, minimum relative water content (54.80%) was found in treatment T_1 (control) followed by treatment T_4 (57.77%) when compared with other treatments.

Conclusion

Based on the results of the 2022-23 investigation, it can be concluded that:

- Auxin (25 ppm) significantly improved plant height, leaf area, LAI and biomass accumulation.
- Chitosan (75 ppm) effectively enhanced the plant's water retention capacity.
- Chitosan-based IAA nanoparticles showed promising but moderate improvements in morpho-physiological traits.
- Control treatment exhibited the poorest performance in all parameters.

Thus, the foliar application of 25 ppm auxin and 75 ppm chitosan are recommended for improving physiological performance and vigour of cotton.

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