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Effect of chitosan foliar spray on plant growth parameters in Ashwagandha

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Abstract
Withania somnifera is a pantropic medicinal plant native to western and central India. The effect of application of chitosan as foliar spray in Ashwagandha (*Withania somnifera* (L.) Dunal) on growth parameters was studied in this experiment. Seeds primed with 5 mg mL^{-1} chitosan solution for 4 h were used to study the effect of foliar application of chitosan in Ashwagandha. These seedlings were raised for 30 days and then transplanted imposing different treatments, *viz*., chitosan and chitosan nanoparticles spray, each at three different concentrations, i.e., 1 mg mL^{-1}, 2.5 mg mL^{-1} and 5 mg mL^{-1}, primed seeds control and absolute control. The foliar spray treatments were given at transplanting, 15 days after transplanting and 45 days after transplanting. It was observed from the study that plants treated with foliar spray of chitosan at 2.5 mg mL^{-1} (T_2) were better than other treatments in most of the growth parameters. With respect to plant height, collar girth, leaf length, leaf breadth and leaf area at 75 days after transplanting, these plants recorded the values 108.20 cm, 2.62 cm, 15.03 cm, 10.87 cm and 114.74 cm^2 respectively. However, the number of branches and flowering branches was highest in the treatments T_1 (1 mg mL^{-1} chitosan) and T_5 (2.5 mg mL^{-1} chitosan nanoparticles) followed by T_2. Hence, the study showed the positive effect of chitosan on plant growth promotion as most of the parameters showed better values with respect to control treatment.

Keywords: Ashwagandha, plant growth parameters, chitosan, nanoparticles

1. Introduction
Medicinal plants have been in use for a long time in traditional medicine. *Withania somnifera* Dunal, known as ashwagandha, is an important medicinal plant used in Ayurveda. It is one of the important medicinal crops in many states of India. The root extract of this plant is widely used as tonic and for treating various illnesses. Due to the widespread usage of this herb as a general tonic in ailments related to weaknesses, it is known as 'Indian ginseng'. It is a member of the Solanaceae family and is also known by the names Indian Winter Cherry and Asgandh. Two species *viz*., *Withania somnifera* (L.) Dunal and *Withania coagulans* (L.) Dunal are found growing in the wild across the country. It is a resilient perennial plant that can withstand drought. According to Mir *et al*. (2012) [15], the plant is a 30-150 cm tall, indeterminately flowering, erect, evergreen shrub. They can be produced on plots which are unsuitable for the growth of other crops as they do not require continuous irrigation. As a result, they do not interfere with the cultivation of other economically important plants (Singh and Kumar, 1998). The maturity of the plant is indicated by the drying of the leaves and the ripening of the berries giving them a red colour (Kothari *et al*., 2003) [12].
Chitosan is a natural, safe and cost-effective biopolymer easily extracted from fungal cell wall and crustacean shells, obtained by the deacetylation of chitin, a long chain polymer of N-acetyl-glucosamine (Domard and Domard, 2002) [15]. According to Park and Kim (2010) [17], it is non-toxic, biocompatible, and biodegradable. The metabolite profile of chitosan is known to boost plant development processes and induce defence responses in plants. The use of these biostimulants in the cultivation of medicinal plants aims to enhance the secondary metabolite synthesis and increase the biomass output (Rafiee *et al*., 2016) [20]. Chitosan enhances crop production through its bioactivities of biodegradability, growth stimulation, enhancement of nutrient uptake, improvement in chlorophyll content, chloroplast enlargement, and antibacterial characteristics (Hadrami *et al*., 2010) [9]. Therefore, it can be used as foliar spray, seed coating, seedling root dip soil enrichment or as supplement into plant tissue media (Pichyangkuraa and Chadchawanb, 2015) [19].

Algam *et al.* (2010) [1] observed the enhancement in growth of tomato plants on application of chitosan. Foliar spray of chitosan on tomato plants increased the vegetative parameters like plant height, number of branches, number of leaves and plant biomass (El-Tantawy, 2009) [6]. The use of chitosan nanoparticles significantly increased the leaf area, leaf colour, number of grains per spike, grain yield, and harvest index in barley when applied as soil and foliar applications at concentrations of 60 and 90 ppm in three stages (Behboudi *et al*., 2018) [3]. The effect of application of chitosan in agriculture as foliar spray has not yet been studied in the crop, Ashwagandha. Therefore, the present investigation was carried out to determine the effect of application of chitosan as foliar spray in Ashwagandha (*Withania somnifera* (L.) Dunal) on its growth parameters.

2. Materials and Methods

The experiment was carried out in the Department of Plantation, Spices, Medicinal and Aromatic Crops, College of Agriculture, Vellayani, Thiruvananthapuram, Kerala during May 2020 to October 2020. The Ashwagandha seeds sourced from Anand Agriculture University were used in the study. Field experiment was conducted in completely randomized (CRD) design with eight treatments in three replications under protected conditions (rain shelter).

Seeds primed with 5 mg mL^{-1} chitosan solution for 4 h were used in the study. The primed seeds were sown in protrays and thirty-day old seedlings were transplanted to grow bags containing sand, soil and farmyard manure in the ratio 1:1:1. Chitosan was applied as foliar spray at 30 days after sowing (at transplanting), 45 days after sowing (15 days after transplanting) and 75 days after sowing (45 days after transplanting), at specified concentrations in equal volumes to all treatments (according to the growth stage of the crop). Spray volumes of 50 mL, 100 mL and 200 mL per plant were taken for foliar spray at transplanting, 15 days after transplanting and 45 days after transplanting. The various chitosan foliar spray treatments have been given in Table 1.

Three plants from each replication of each treatment were tagged as observational plants. The plant growth parameters were recorded at 15 days, 45 days (50 percent flowering) and 75 days (berry maturation stage) after transplanting. Observations were recorded for plant height, number of leaves, collar girth, leaf length, leaf breadth, leaf area, number of branches and number of flowering branches. Plant height was determined by taking the height of the plant from the base of the shoot to the tip and expressed in centimetres (cm). The total number of leaves at different growth stages from each of the observational plants were counted and the mean values were recorded to record the leaf number. The circumference of the stem at the collar region was measured by using the thread and scale method to estimate the collar girth and specified in centimetres. The length and breadth of leaves (5 numbers) was measured and average leaf length and breadth were calculated in each observational plant. The leaf area was calculated by multiplying the average leaf length with leaf breadth and further multiplying the result with the constant 0.7028 as described by Patidar *et al.* (1990) [18]. The total number of branches arising from the main branch and the number of branches bearing flowers in each observational plants were counted and the mean values were recorded.

3. Results

Application of chitosan foliar spray showed variation among the treatments in various plant growth parameters, which have been described below and diagrammatically represented in Fig.1 and Fig 2.

3.1 Plant height

The plant height recorded in various treatments at different growth stages of Ashwagandha is tabulated in Table 2. Plant heights at 15 and 45 days after transplanting were significantly higher in the treatment T_6 (chitosan nanoparticles at 5 mg mL^{-1}) with values of 13.23 cm and 66.92 cm respectively. Plants treated with foliar spray of chitosan at 2.5 mg mL^{-1} (T_2) recorded the highest plant height (108.20 cm) followed by T_6 (94.20 cm) at 75 days after transplanting. The control plants (T_8) recorded a significantly lower plant height than other treatments at 15 days after transplanting. Plants of the treatment T_4 recorded the lowest plant heights at 45 days after transplanting (49.00 cm). The same treatment recorded the lowest plant height (75.30 cm) at 75 days after transplanting which was on par with T_7 (best priming treatment) and T_8 (control).

3.2 Number of leaves

Foliar application of chitosan in Ashwagandha resulted in variation in the number of leaves at different growth stages, as given in Table 3. At 15 days after transplanting, the highest number of leaves was observed in the treatment T_6 (7.33). The highest number of leaves at 45 days after transplanting was observed in T_1 (1 mg mL^{-1} chitosan) with 39.17 leaves. The treatments T_2 (2.5 mg mL^{-1} chitosan) and T_6 were also on par with it, having 35.17 and 37 leaves, respectively. T_2 recorded significantly higher number of leaves than all other treatments at 75 days after transplanting, having 130.39 leaves at this stage. At 15 days after transplanting, T_3 recorded the lowest number of leaves (5.33). Plants of the treatment T_4 (1 mg mL^{-1} chitosan nanoparticles) recorded the lowest number of leaves at 45 after transplanting (23.17 leaves). The lowest number of leaves at 75 days after transplanting was observed in T_7 (Primed Seeds) and was observed to be on par with the control.

3.3 Collar girth

Collar girth in Ashwagandha showed significant variation among treatments. The effect of foliar application of chitosan on collar girth have been given in Table 4. T_6 recorded the highest collar girth at 15 days after transplanting (1.22 cm). At 45 days after transplanting, the treatments T_5 (2.5 mg mL^{-1} chitosan nanoparticles) and T_6 were observed to have the highest collar girth of 2.37 cm each. The treatment T_5 recorded the highest collar girth at 75 days after transplanting (2.72 cm) which was on par with T_2 (2.62 cm). Plants of the treatment T_4 recorded the lowest collar girths at all the stages of observation.

3.4 Leaf length

Leaf length in Ashwagandha showed variation among the treatments throughout the growth period. The effect of foliar spray of chitosan on leaf length is presented in Table 5. Throughout the period of observation, T_6 was observed to have significantly higher leaf length. These plants recorded

leaf lengths of 7.73 cm, 14.70 cm and 16.10 cm at 15, 45 and 75 and days after transplanting, respectively. The treatments T_2 and T_3 were found to be on par with T_6 at 75 days after transplanting (15.03 cm each). Leaf lengths at 15 and 45 days after transplanting was the lowest in T_4. The lowest leaf lengths were observed in T_5 and T_7 at 75 days after transplanting, and these were on par with the control.

3.5 Leaf breadth

Leaf breadth of Ashwagandha plants with respect to chitosan foliar spray showed significant variation among the treatments, as shown in Table 6. Plants of the treatment T_5 had the highest leaf breadth at 15 days after transplanting, bearing 5.32 cm broad leaves, and it was on par with T_6 which had a leaf breadth of 5.3 cm. Plants of the treatment T_2 recorded significantly highest leaf breadth of 9.8 cm at 45 days after transplanting. These plants were observed to have the highest leaf breadth at 75 days after transplanting also, measuring 10.87 cm. Leaf breadths in the treatments T_5 and T_6 were also found to be on par with T_2. At 45 and 75 days after transplanting, T_4 showed lower leaf breadth which found to be on par with control.

3.6 Leaf area

Ashwagandha plants showed variation in leaf area among the treatments throughout the growth period. The leaf area recorded in various treatments at different growth stages of Ashwagandha have been tabulated in Table 7. T_6 recorded higher leaf area in all stages of observation. At 15 days after transplanting, T_6 (28.81 cm^2) was observed to be on par with T_5 (27.95 cm^2); while at 45 and 75 days after transplanting, T_6 (leaf areas of 95.73 cm^2 and 116.01 cm^2 respectively) was observed to be on par with T_2 (leaf areas of 97.01 cm^2 and 114.74 cm^2 respectively). T_4 recorded the lowest leaf areas at 15 and 45 after transplanting. The lowest leaf area at 75 days after transplanting were observed in the control plants.

3.7 Number of branches

Chitosan foliar spray significantly influenced the number of branches in Ashwagandha. The results have been shown in Table 8. Branching was not observed at 15 days after transplanting. Plants of the treatment T_1 recorded the highest number of branches at 45 and 75 days after transplanting having 6.67 and 8.81 branches, respectively. The lowest number of branches at 45 and 75 days after transplanting (3.17 and 6.62) was observed in T_4, which was lower than the control.

3.8 Number of flowering branches

Similar trend as in number of branches was observed in case of number of flowering branches also. The effect of foliar application of chitosan on the number of flowering branches have been given in Table 9. There were no flowering branches in any of the treatments at 15 days after transplanting. The treatment T_1 recorded significantly higher number of flowering branches (5.67) than all other treatments at 45 days after transplanting. The highest number of flowering branches at 75 days after transplanting (7.42) was observed in the treatment T_5, which was on par with T_1, having 7.32 flowering branches. At 45 days after transplanting, T_4 recorded the lowest number of flowering branches than all other treatments. At 75 days after transplanting, T_7 recorded the lowest number of flowering branches which was on par with T_4.

Table 1: Chitosan foliar spray treatments

Treatment Name	Treatment Spécifications
T_1	Chitosan 1 mg mL^{-1}
T_2	Chitosan 2.5 mg mL^{-1}
T_3	Chitosan 5.0 mg mL^{-1}
T_4	Chitosan nanoparticles 1 mg mL^{-1}
T_5	Chitosan nanoparticles 2.5 mg mL^{-1}
T_6	Chitosan nanoparticles 5.0 mg mL^{-1}
T_7	Best priming treatment from Exp. 1.1
T_8	Water spray (control)

Table 2: Effect of foliar application of chitosan on plant height

Treatment	Plant height (in cm)		
	15 DAT	45 DAT	75 DAT
T_1 (Ch 1 mg mL^{-1})	11.32 ± 0.48^{c}	54.42 ± 0.68^{e}	84.47 ± 5.33^{de}
T_2 (Ch 2.5 mg mL^{-1})	10.62 ± 0.08^{de}	59.32 ± 0.28^{c}	108.20 ± 6.06^{a}
T_3 (Ch 5 mg mL^{-1})	11.08 ± 0.13^{cd}	58.30 ± 0.74^{cd}	87.47 ± 0.91^{cd}
T_4 (Ch NP 1 mg mL^{-1})	10.12 ± 0.44^{e}	49.00 ± 1.74^{f}	75.30 ± 1.91^{f}
T_5 (Ch NP 2.5 mg mL^{-1})	10.32 ± 0.40^{e}	54.47 ± 0.90^{e}	95.13 ± 6.37^{b}
T_6 (Ch NP 5 mg mL^{-1})	13.23 ± 0.31^{a}	66.92 ± 0.24^{a}	94.20 ± 4.16^{bc}
T_7 (Best priming treatment)	10.32 ± 0.40^{e}	65.40 ± 0.70^{b}	78.93 ± 0.25^{ef}
T_8 (Control)	9.00 ± 0.09^{f}	57.15 ± 0.53^{d}	81.10 ± 2.16^{def}
CD	0.551	1.464	7.036
S.Em (±)	0.184	0.488	2.347
CV (%)	2.89	1.456	4.614

Table 3: Effect of foliar application of chitosan on number of leaves

Treatment	Number of leaves		
	15 DAT	45 DAT	75 DAT
T_1 (Ch 1 mg mL^{-1})	5.83 ± 0.29^{de}	39.17 ± 3.75^{a}	94.83 ± 10.13^{b}
T_2 (Ch 2.5 mg mL^{-1})	6.67 ± 0.29^{bc}	35.17 ± 1.53^{abc}	130.39 ± 17.68^{a}
T_3 (Ch 5 mg mL^{-1})	5.33 ± 0.29^{e}	32.83 ± 2.75^{bc}	85.61 ± 9.32^{bc}
T_4 (Ch NP 1 mg mL^{-1})	6.17 ± 0.29^{cd}	23.17 ± 2.08^{e}	71.72 ± 3.73^{cd}
T_5 (Ch NP 2.5 mg mL^{-1})	5.83 ± 0.29^{de}	26.50 ± 5.57^{de}	71.11 ± 3.56^{cd}
T_6 (Ch NP 5 mg mL^{-1})	7.33 ± 0.29^{a}	37.00 ± 1.00^{a}	75.28 ± 9.89^{cd}
T_7 (Best priming treatment)	6.50 ± 0.50^{bc}	30.50 ± 3.78^{cd}	54.60 ± 5.73^{e}
T_8 (Control)	7.00 ± 0.50^{ab}	24.00 ± 3.12^{e}	62.56 ± 3.36^{de}
CD	0.612	5.62	15.848
S.Em (±)	0.204	1.875	5.286
CV (%)	5.582	10.459	11.337

Table 4: Effect of foliar application of chitosan on collar girth (in cm)

Treatment	Collar girth (in cm)		
	15 DAT	45 DAT	75 DAT
T_1 (Ch 1 mg mL^{-1})	0.97 ± 0.08^{d}	1.88 ± 0.15^{c}	2.14 ± 0.07^{d}
T_2 (Ch 2.5 mg mL^{-1})	0.97 ± 0.08^{d}	2.10 ± 0.13^{b}	2.62 ± 0.09^{ab}
T_3 (Ch 5 mg mL^{-1})	1.15 ± 0.05^{ab}	2.25 ± 0.10^{ab}	2.28 ± 0.05^{c}
T_4 (Ch NP 1 mg mL^{-1})	0.82 ± 0.03^{e}	1.88 ± 0.10^{c}	2.05 ± 0.06^{d}
T_5 (Ch NP 2.5 mg mL^{-1})	1.03 ± 0.08^{cd}	2.37 ± 0.08^{a}	2.72 ± 0.07^{a}
T_6 (Ch NP 5 mg mL^{-1})	1.22 ± 0.03^{a}	2.37 ± 0.08^{a}	2.56 ± 0.05^{b}
T_7 (Best priming treatment)	1.17 ± 0.03^{ab}	2.12 ± 0.08^{b}	2.52 ± 0.05^{b}
T_8 (Control)	1.12 ± 0.03^{bc}	2.23 ± 0.13^{ab}	2.72 ± 0.05^{a}
CD	0.093	0.189	0.109
S.Em (±)	0.031	0.063	0.036
CV (%)	5.123	5.068	2.573

Table 5: Effect of foliar application of chitosan on leaf length (in cm)

Treatment	Leaf length (in cm)		
	15 DAT	45 DAT	75 DAT
T_1 (Ch 1 mg mL^{-1})	6.53±0.08^{de}	12.35±0.30^{e}	13.67±0.91^{b}
T_2 (Ch 2.5 mg mL^{-1})	6.40±0.05^{ef}	14.08±0.21^{b}	15.03±0.47^{a}
T_3 (Ch 5 mg mL^{-1})	6.85±0.10^{c}	13.53±0.58^{c}	15.03±0.70^{a}
T_4 (Ch NP 1 mg mL^{-1})	6.32±0.08^{f}	12.18±0.10^{e}	13.10±0.56^{b}
T_5 (Ch NP 2.5 mg mL^{-1})	7.48±0.10^{b}	12.57±0.34^{de}	12.73±0.70^{b}
T_6 (Ch NP 5 mg mL^{-1})	7.73±0.03^{a}	14.70±0.26^{a}	16.10±0.30^{a}
T_7 (Best priming treatment)	6.62±0.13^{d}	12.37±0.16^{e}	12.73±0.90^{b}
T_8 (Control)	7.57±0.08^{a}	12.93±0.25^{d}	13.67±0.42^{b}
CD	0.147	0.532	1.132
S.Em (±)	0.049	0.177	0.377
CV (%)	1.222	2.347	4.667

Table 6: Effect of foliar application of chitosan on leaf breadth (in cm)

Treatment	Leaf breadth (in cm)		
	15 DAT	45 DAT	75 DAT
T_1 (Ch 1 mg mL^{-1})	4.40±0.05^{c}	8.33±0.10^{d}	8.75±0.35^{bc}
T_2 (Ch 2.5 mg mL^{-1})	4.90±0.18^{b}	9.80±0.18^{a}	10.87±0.40^{a}
T_3 (Ch 5 mg mL^{-1})	4.28±0.08^{cd}	8.88±0.25^{c}	9.32±0.63^{b}
T_4 (Ch NP 1 mg mL^{-1})	4.17±0.21^{d}	8.18±0.03^{d}	8.78±0.55^{bc}
T_5 (Ch NP 2.5 mg mL^{-1})	5.32±0.15^{a}	9.40±0.09^{b}	10.82±0.42^{a}
T_6 (Ch NP 5 mg mL^{-1})	5.30±0.08^{a}	9.27±0.36^{b}	10.25±0.38^{a}
T_7 (Best priming treatment)	4.78±0.15^{b}	8.47±0.03^{d}	9.08±0.34^{b}
T_8 (Control)	4.75±0.05^{b}	8.28±0.13^{d}	8.18±0.34^{c}
CD	0.23	0.309	0.76
S.Em (±)	0.077	0.103	0.254
CV (%)	2.801	2.023	4.619

Table 7: Effect of foliar application of chitosan on leaf area (in cm^2)

Treatment	Leaf area (in cm^2)		
	15 DAT	45 DAT	75 DAT
T_1 (Ch 1 mg mL^{-1})	20.20±0.27^{d}	72.34±2.64^{cd}	83.95±4.52^{c}
T_2 (Ch 2.5 mg mL^{-1})	22.04±0.70^{c}	97.01±2.81^{a}	114.74±2.39^{a}
T_3 (Ch 5 mg mL^{-1})	20.62±0.29^{d}	84.42±1.22^{b}	98.50±9.39^{b}
T_4 (Ch NP 1 mg mL^{-1})	18.50±1.12^{e}	70.07±0.79^{d}	80.79±4.33^{c}
T_5 (Ch NP 2.5 mg mL^{-1})	27.95±0.42^{a}	83.02±2.59^{b}	96.86±7.96^{b}
T_6 (Ch NP 5 mg mL^{-1})	28.81±0.58^{a}	95.73±4.03^{a}	116.01±5.87^{a}
T_7 (Best priming treatment)	22.23±0.33^{c}	73.59±0.90^{cd}	81.31±7.05^{c}
T_8 (Control)	25.26±0.30^{b}	75.30±2.25^{c}	78.48±3.01^{c}
CD	0.99	4.142	10.417
S.Em (±)	0.33	1.382	3.475
CV (%)	2.465	2.938	6.413

Table 8: Effect of foliar application of chitosan on number of branches

Treatment	Number of branches	
	45 DAT	75 DAT
T_1 (Ch 1 mg mL^{-1})	6.67±0.15^{a}	8.81±0.48^{a}
T_2 (Ch 2.5 mg mL^{-1})	5.17±0.21^{c}	6.81±0.30^{bc}
T_3 (Ch 5 mg mL^{-1})	4.67±0.21^{d}	6.96±0.21^{bc}
T_4 (Ch NP 1 mg mL^{-1})	3.17±0.15^{f}	6.62±0.20^{c}
T_5 (Ch NP 2.5 mg mL^{-1})	4.83±0.15^{d}	8.46±0.50^{a}
T_6 (Ch NP 5 mg mL^{-1})	5.67±0.15^{b}	6.94±0.10^{bc}
T_7 (Best priming treatment)	4.00±0.10^{e}	6.68±0.16^{bc}
T_8 (Control)	4.00±0.20^{e}	7.17±0.11^{b}
CD	0.293	0.516
S.Em (±)	0.098	0.172
CV (%)	3.544	4.081

Table 9: Effect of foliar application of chitosan on number of flowering branches

Treatment	Number of flowering branches	
	45 DAT	75 DAT
T_1 (Ch 1 mg mL^{-1})	5.67±0.15^{a}	7.32±0.32^{a}
T_2 (Ch 2.5 mg mL^{-1})	4.17±0.21^{c}	5.82±0.56^{bc}
T_3 (Ch 5 mg mL^{-1})	3.63±0.15^{d}	6.20±0.26^{b}
T_4 (Ch NP 1 mg mL^{-1})	2.17±0.15^{f}	5.66±0.41^{bc}
T_5 (Ch NP 2.5 mg mL^{-1})	3.83±0.15^{d}	7.42±0.40^{a}
T_6 (Ch NP 5 mg mL^{-1})	4.67±0.15^{b}	5.83±0.27^{bc}
T_7 (Best priming treatment)	3.00±0.10^{e}	5.55±0.34^{c}
T_8 (Control)	3.00±0.20^{e}	6.29±0.29^{b}
CD	0.28	0.638
S.Em (±)	0.094	0.213
CV (%)	4.301	5.889

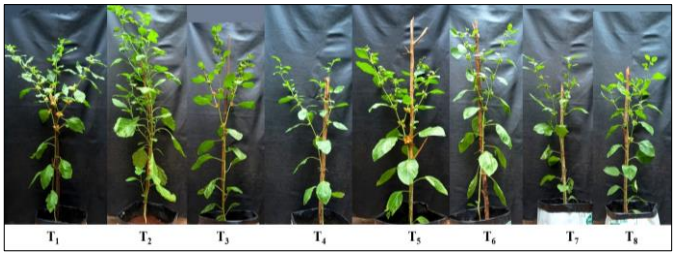

Fig 1: Ashwagandha plants treated with chitosan foliar spray at 75 DAT

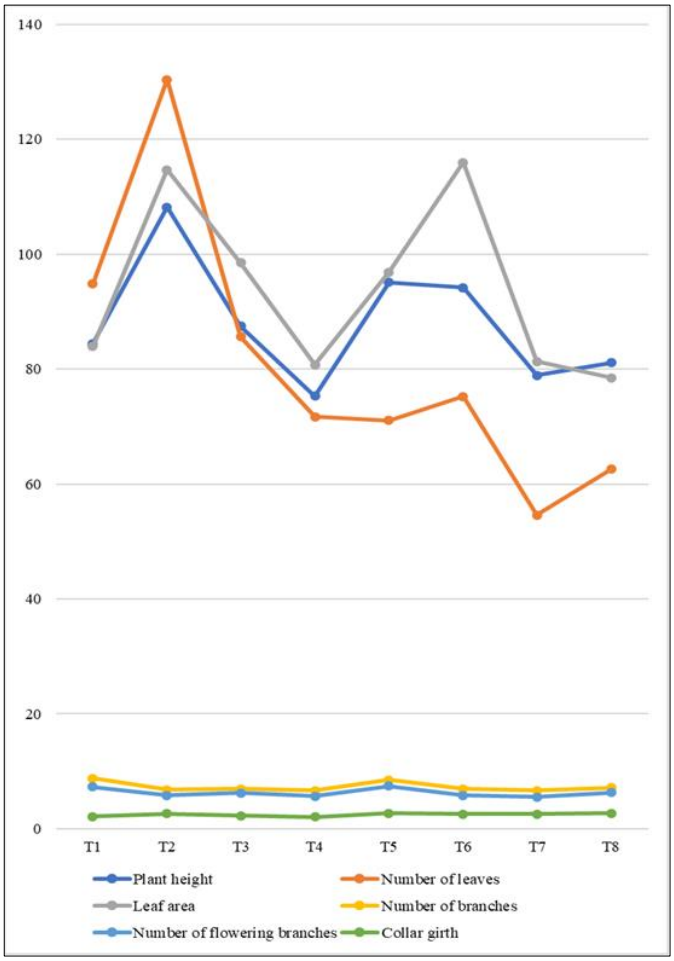

Fig 2: Effect of chitosan foliar spray on plant growth parameters in Ashwagandha at 75 DAT

4. Discussion

In the study, it was observed that all the treatments with chitosan gave higher values with respect to plant growth parameters except collar girth, number of branches and flowering branches. However, chitosan 2.5 mg mL^{-1} (T_2) was observed to give significantly higher values with respect to all plant growth parameters except number of branches and flowering branches. In case of number of branches and flowering branches, T_2 followed T_1 statistically. At higher concentration of chitosan, *i.e.,* 5 mg mL^{-1} (T_3), most of the growth parameters were observed to be lower than T_2. The number of branches and flowering branches in T_3 however, was observed to be on par with T_2.

The treatment T_5 (chitosan nanoparticles 2.5 mg mL^{-1}) was observed to give higher values over the control with respect to plant height, number of leaves, leaf area, number of branches and number of flowering branches, whereas T_4 (chitosan nanoparticles 1 mg mL^{-1}) was observed to give lower values compared to control with respect to plant height, number of branches and number of flowering branches. The priming treatment with chitosan (T_7) was observed to give on par values with the control with all the plant growth parameters except number of flowering branches at 75 days after transplanting.

With respect to plant height, collar girth, leaf length, leaf breadth and leaf area at 75 days after transplanting, the best treatment, T_2 recorded the values 108.20 cm, 2.62 cm, 15.03 cm, 10.87 cm and 114.74 cm^2 respectively. There was variation among the treatments in the plant growth parameters studied. The plant height ranged from 75.3 cm to 108.2 cm, number of leaves ranged from 54.60 to 130.39 leaves, collar girth ranged from 2.05 cm to 2.72 cm among the treatments; the leaf length, leaf breadth and leaf area ranged from 12.73 cm to 16.1 cm, 8.18 cm to 10.87 cm 78.48 to 116.01 to cm^2. and the number of branches and flowering branches ranged from 6.62 to 8.81 and 5.55 to 7.32 branches respectively at 75 days after transplanting of the crop. These findings are similar to the study by Khanna *et al*. (2014) [11] where variations were recorded for plant height, branches, leaf length and leaf width. The plant height of Withania accessions ranged from 37.78 cm (AGB-025) to 135.82 cm (AGB-002). AGB-002 also recorded the maximum leaf length and leaf width of 9.6 cm and 5.8 cm respectively. The potential of cultivation of Ashwagandha as a medicinal crop in Jaffna district of Sri Lanka was studied by Shanmugaratnam *et al*. (2013) [22] who observed that *W. somnifera* is an evergreen shrub reaching growing to a height of 118±32.40 cm. Kubsad *et al*. (2009) [14] conducted a field experiment at Agricultural Research Station, Annigeri (Karnataka) where they observed that leaf area in Ashwagandha increased linearly from transplanting to 90 days after transplanting.

Chitosan foliar spray has been reported to enhance the plant growth parameters in other crops also. According to Algam and Elwagia (2015) [2], tomato, a solanaceous crop, when treated with 5 mg mL^{-1} of chitosan as a seed treatment and 5 mg mL^{-1} of foliar spray showed increased plant height by 16.88, percent. Another study by Hassnain *et al*. (2020) [10] in tomato found that foliar application of chitosan at 100 mg L^{-1} resulted in maximum average plant height (80.74 cm), average number of leaves (104.19) and leaf area (81.05 cm^2) at its maximum growth stage compared to other treatments. A report in chilli pepper stated that chitosan spray at 50 ppm increased the plant height and other vegetative parameters (Kramchote and Suwor, 2022) [13]. Chen *et al.* (2023) [4] in their study observed that spraying of 0.5 - 1.0 percent chitosan effectively increased the leaf area and plant height of *Pinellia ternata*, a medicinal and ornamental herb of the Araceae family. Nithin *et al*. (2020) [16] studied the effect of chitosan application on strawberry under naturally ventilated polyhouse. They found that foliar application of chitosan increased vegetative growth characteristics *viz*., plant height, number of trifoliate leaves per plant and leaf area compared to water spray. The effect of application of chitosan nanoparticles has also been studied on other crops. Geries *et al*. (2020) [7] observed that the maximum values of growth characteristics like plant height, number of leaves per plant and leaf area in onion were obtained by soaking the seedlings with nano chitosan at the rate of 75 ppm. Seed priming with chitosan has also proved to enhance the growth parameters in a number of crops. The use of foliar spray of chitosan (200 ppm) along with seed priming with chitosan (2%) in *Lablab purpureus* was found to be significantly superior in context with plant height when compared with rest of the treatments (Godase *et al*., 2023) [8].

5. Conclusions

It can be concluded that foliar application of chitosan in Ashwagandha has a positive effect on plant growth promotion as most of the parameters showed better values in comparision to control treatment.

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7. References

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