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Colchicine's impact on the growth and chemical composition of *Withania somnifera l.* plant

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Abstract
The objective of this study investigated the effect of colchicine on growth and chemical composition of *Withania Somnifera* plant. Colchicine is currently used to improve crops and chemical compounds. The colchicine concentrations were 0.2, 0.3, and 0.5% for periods of 12, 24, and 36 hours. The results of both years showed that colchicine was significant in reducing plant growth parameters (plant height, leaf area, and fresh weight) compared with the control plants. Colchicine showed a significant increase in (branch number, dry weight, root length, root branches, root weight, and root diameter) compared to control plants. While it caused variation in the number of leaves, flowers, and fruits. Furthermore, colchicine at 0.5% application increased the total contents of phenols, but a slight adverse effect occurred with the total content of alkaloids. Moreover, the effect of time showed variation in results during the two years on the morphological and chemical responses of *Withania Somnifera L.* We recommend the use of colchicine to produce a high yield of the plant and elevate the therapeutic potency of the plant.

Keywords: Ashwagandha, colchicine, phenols, alkaloids, plant growth.

1. Introduction

Ashwagandha (*Withania Somnifera l.*) belongs to the Dunal family Solanaceae, it is a green shrub [1] commonly known as Ashwagandha, and it is an important perennial. The species is also known as 'Winter cherry' ***[2-3].*** Plant species have immense therapeutic uses in traditional as well as modern systems of medicine [4]. Ashwagandha was mentioned by Sage Atreya over 4000 years ago [5]. Ashwagandha is an erect, evergreen, branched shrub that attains a height of 30-60 cm. It grows well in dry and arid soil [6] with a preference for acid soil [7]. In India, *W. somnifera* is referred to as the "Indian Ginseng" [8]. Many parts of the plant have been used to treat a variety of diseases by practitioners of the conventional medical system for millennia. Ashwagandha has long been recognized as a potent rejuvenator, general health tonic, and treatment for a wide range of ailments [9-11]. Withania somnifera root contains flavonoids, alkaloids, steroid and many active functional ingredients [12]. The major biochemical constituents of *W. somnifera* are steroidal alkaloids and lactones, collectively known as withanolides [13]. Withanolides are structurally similar to the active constituents in Panax ginseng, known as ginsenosides [14]. Colchicine is one of the physical mutagens used in the field of mutation breeding [15]. It has been used at different times to inhibit mitosis in a wide variety of plants by interfering with the orientation and structure of the mitotic fibers and spindle [16].colchicine, an alkaloid extracted from the seeds or corms of the autumn *Crocus Colchicum autumnal L.* [17-18]. The objective of this study was to investigate the effect of colchicine treatments on improving the quantitative and qualitative growth characteristics of *withania sominefra L.* plant, including its chemical composition.

2. Material and methods

2.1. Experimental material and methods

The seeds used in the present investigation was obtained from the National Research Center in Dokki.
Dry seeds of Ashwagandha were soaked in three concentrations 0.2, 0.3 and 0.5 % of colchicine. 12, 24 and 36 hours were three different durations of exposure to each applied concentrations, in addition to control plants. The recommended dose of NPK (25-25-0) [19] was applied every 60 days throughout the growing seasons. The seeds of each treatment and duration were grown in the summer during two season. The experiment was designed with a completely randomized design with 12 replicates; each replicate contains 12 plants.
Seeds were sown on the trays with a suitable mixture (clay soil) of agricultural soil and wellyard manure. The seedlings were ready for transplanting a month after planting.

2.2. Vegetative and growth parameters and chemical content estimation

The nine morphological characters were rescored on the plot basis for each treatment: plant height, leaf area the third leaf from the bottom, number of leaves, number of branches, number of flowers and fruit, root length, fresh weight of plant, root diameter and root weight. The fresh and dry weights of each root for each plant in the treatments were recorded, and chemical

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contents of phenols. The contents of total phenols were calculated by using the Folin–Ciocalteu reagent with some alteration by using gallic acid as a standard curve and then assessed at 765 nm using the spectrophotometer [20]. Contents of alkaloids were measured according to Shamsa et al. (2008) [21].

2.3 Statistical Analysis

The data were statistically analyzed according to the statistical analysis system user's guide, (SAS, 1998). Separation among means (P> 0.05) was carried out by using Duncan Multiple test [22].

3. Results

Colchicine is used to develop new cultivars with novel phenotypic and cytogenetic characters is suitable for aromatic and ornamental plants [23].

3.1 Effect of colchicine concentrations on the Morphological characters

In the present study, morphological characters were employed to investigate the effects of different concentrations and period of exposure to colchicine on Ashwagandha grown in two seasons. The data in Tables (1 and 2) showed significant differences in these characters.

In the first season (2021), the mean performance of plant height was 91.33 cm which was treated with colchicine at 0.5 %. While, 59.61 cm height was for the shortest plants which were treated with 0.2 % colchicine. During the second year (2022), the tallest plants (78.83cm) belonged to control plants (untreated), while the shortest plants (41.94) were treated with colchicine at (0.2 %). The effect of the mutation appears clearly in the second year, which demonstrates the effect of colchicine on reducing plant height. As for time, in the first year the highest reading (92.66 cm) was recorded during treatment with colchicine at 36 hours. In the second year the highest reading (64.67 cm) was recorded during treatment with colchicine at 12 hours.

During the first year (2021) leaf area results were recorded the highest value (17.37cm) in the control plants (untreated) and the lowest rate (9.43 cm) the plants were treated with 0.5 % During the second year (2022) the highest value (15.64 cm) in the control plants and the lowest value (8.57 cm) the plants were treated with 0.5 % .The effect of colchicine was significant in reducing leaf area. But as for time in the first and second years, the highest reading were (15.21 and 12.54) respectively, at 24 hours.During the first year (2021) branch number results recorded the highest values (3.77 and 3.44) in the plants treated with 0.5 % and 0.3 % colchicine. During the second year (2022) the highest values recorded were (3.53 and 3.39) in the plants treated with 0.3 % and 0.5 % colchicine. As for time, the effect of colchicine on the number of branches during the first year appeared different from the second year during the first year, the largest result (3.49) was recorded in the plants treated with colchicine for 24 hours. During the second year, the largest result (3.15) was recorded in the plants treated with colchicine at 36 hours.

During year one (2021) leaf number results produced the highest number of leaves (89.00) in the control plants (untreated) and the lowest number (30.00) in the plants treated with 0.2% colchicine. In year two (2022) the leaf results produced the highest number of leaves (71.04) belonging to the treatment 0.3% colchicine, while the lowest value was (49.43) belonging to the treatment 0.2 % colchicine. The highest impact result in time during the first season and second season (81.08 and 58.32) respectively, was in treatment with soaking within 36 hours.

In (2021) the largest number of flowers (9.66) was obtained from this plants which were treated with colchicine at 0.5 %. In (2022) the largest number of flowers (10.00) was obtained from the control plants. The effect of colchicine appeared on fruit number during the first year after 24 hours of soaking, and it was (46.91).but during the second year after 36 hours of soaking and it was (29.91). However, the highest impact result in time during the first season and second season (7.08 and 7.87), respectively, was in treatment with soaking within 12 hours. In (2021) the largest number of fruit (64.44) was obtained from this plants which were treated with colchicine at 0.5%. The lowest number of fruit (0.44) was obtained from this plants which were treated with colchicine at 0.2 %. In (2022) the largest number of fruit (31.33) was obtained from the control plants. The lowest number of fruit (11.78) was obtained from this plants which were treated with colchicine at 0.2%.

In the first year (2021) the heaviest fresh weight (106.04 g) was obtained from control plants. The least fresh weight (36.70 g) was recorded from the plants treated with colchicine at 0.2%. In the second year (2022) the heaviest fresh weight (237.32 g) was obtained from control plants. .The least fresh weight (93.93 g) was recorded from the plants treated with colchicine at 0.2%. During year one (2021) the highest dry weight (87.63 g) was obtained from control plants. The lowest dry weight (29.80 g) was recorded from the plants treated with colchicine at 0.2%. In second year (2022) the highest dry weight (197.32 g) was obtained from this plants which were treated with colchicine at 0.5%.

The lowest dry weight (69.95 g) was recorded from the plants treated with colchicine at 0.2%. The heaviest fresh weight through the first year (2021) (90.46) was obtained from the time of soaking 24 hours, but through the second year (2022) 242.66 was obtained from the time of soaking 36 hours. The heaviest dry weight through the first year (2021) 69.60 was obtained from the time of soaking 24 hours, but through the second year (2022) it was 171.20 from the time of soaking 36 hours.

As shown in Table (1) and Table (2) colchicine categorically has affected roots. In year one the longest effect (29.66 cm) belonged to the control plants, whereas in year two the longest effect (26.35 cm) belonged to the plants treated with 0.5 % colchicine. The greatest values (23.33 and 23.77) were recorded, respectively, in the plants during the soaking time of 24 hours. In the first year (2021), the maximum number of root branches (9.09) was obtained from the plants treated with colchicine at 0.3%. The lowest number of root branches (3.66) was obtained from the plants treated with colchicine at 0.2%. In year two (2022) the maximum number of root branches (7.28) was obtained from the plants treated with colchicine at 0.3%. A lower number of root branches (4.21) was obtained from the plants treated with colchicine at 0.2%. The greatest results (6.08 and 6.47) were recorded, respectively, in the plants during the soaking time of 12 hours.

During the first year (2021) the heaviest root fresh weight (16.70 g) was obtained from the plants treated with colchicine at 0.3%. During the second year (2022) the heaviest root fresh weight (27.36 g) was obtained from the plants treated with colchicine at 0.5%. The highest result in the first year (15.68 g) was recorded at 24 hours, and the highest result in the second year (23.69 g) was recorded at 36 hours.

The results during the first year (2021) showed an increase in the dry root yield rate in concentrated treatments of 0.3% and 0.5% colchicine that were recorded (15.18 and 11.39), respectively, whereas in year two The results showed an increase in the dry root yield rate in concentrated treatments of 0.3% and 0.5 % too, recorded (22.19 and 25.22) respectively. The greatest values in the first year (2021) and in year two (2022) were obtained from 24 hours, which were (13.22) and (21.40) respectively.

Treatment with colchicine produced thicker roots over two years. From Table (2) in first year (2021) the maximum thickness was (1.60 cm) obtained from the control plants, but in year two (2022) the maximum thickness was (1.57 cm) obtained from the plants treated with colchicine at 0.3%. While the maximum thickness in the first year (1.55 cm) was obtained from 24 hours, but in year two (2022) the maximum thickness (1.33 cm) was obtained from 36 hours.

Table 1: Effect of Colchicine concentrations on morphological characters *of withania somnifera l* plants during (2021, 2022) years.

	Colchicine concentration							
	Y1 (2021)				**Y2 (2022)**			
	0%	0.2%	0.3%	0.5%	0%	0.2%	0.3%	0.5%
Plant height (cm)	83.00 **A**	59.61 **C**	78.00**B**	91.3 **A**	78.83 **B**	41.94 **D**	58.66 **C**	61.77 **C**
Leaf area (cm)	17.37 **A**	15.04 **B**	13.5**C**	9.43**D**	15.64**B**	13.06 **C**	11.71 **D**	8.57 **D**
Branches number/plant	2.33 **B**	2.33 **B**	3.44 **A**	3.77 **A**	2.67B	2.37 **B**	3.53**A**	3.39**A**
Leaves number /plant	89.00 **A**	30.00 **D**	57.78 **C**	79.34**B**	52.33 **C**	49.43**D**	71.04**B**	54.60**C**
Fruit number/plant	38.00 **B**	0.44 **E**	4.88 **E**	64.44**A**	31.33 **B**	11.78**D**	12.22**D**	25.11**C**
Flower number / plant	7.00 **B**	5.33 **C**	2.78 **E**	9.66 **A**	10.00 **A**	2.83**E**	4.00 **D**	2.05 **E**
Fresh weight (g) / plant	106.04**D**	36.70 **G**	83.98**F**	92.19**E**	237.32**A**	93.93**E**	154.82**C**	220.56**B**
dry weight (g)/ plant	87.63 **D**	29.80 **F**	61.02 **E**	73.97**E**	162.74**B**	69.95**E**	133.65 **C**	197.32**A**
Root length(cm)	29.66 **A**	16.11 **E**	21.11 **D**	20.94 **D**	25.25 **B**	20.62 **D**	24.05 **C**	26.35 **B**
N. root branches/plant	4.66 **D**	3.66 **E**	9.09 **A**	5.88 **C**	5.68 **C**	4.21 **D**	7.28 **B**	5.70 **C**
Root fresh weight(g)	13.72 **C**	6.09 **D**	16.70 **B**	12.67 **C**	19.16 **B**	11.54 **C**	24.35 **A**	27.36 **A**
Root dry weight (g)	9.03 **C**	4.69 **D**	15.18 **A**	11.39 **B**	16.29 **C**	10.47 **D**	22.19 **B**	25.22 **A**
Root diameter / plant (cm)	1.60 **A**	1.17 **A**	1.54 **A**	1.20 **A**	1.15 **A**	0.96 **B**	1.57 **A**	1.27 **A**

Y1: year one, Y2: year two. Meanings with the same letter in the same column are not significantly different.

Table 2: Effect of soaking time on morphological characters *of withania somnifera l* plants during (2021, 2022) years.

	Time (hours)					
	Y1 (2021)			**Y2 (2022)**		
	12	**24**	**36**	**12**	**24**	**36**
Plant height (cm)	65.70 **B**	75.58 **B**	92.66 **A**	64.67 **B**	64.37 **B**	51.87 **C**
leaf area (cm)	13.64 **B**	15.21 **A**	12.66 **B**	9.24 **C**	12.54 **B**	7.45 **C**
Branches number/plant	2.91 **A**	3.49 **A**	2.49 **B**	2.98 **B**	2.59 **B**	3.15 **A**
Leaves number /plant	63.42 **B**	47.58 **D**	81.08 **A**	55.00 **C**	57.23 **B**	58.32 **B**
Fruit number/plant	21.49 **B**	46.91**A**	12.41 **C**	13.58 **C**	15.99 **C**	29.91 **B**
Flower number /plant	7.08 **A**	6.66 **A**	4.83 **B**	7.87**A**	3.28 **C**	3.00 **C**
fresh weight (g) /plant	87.71**D**	90.46 **D**	61.02**E**	120.51**C**	194.29**B**	242.66**A**
dry weight (g) /plant	69.08 **E**	69.60 **E**	50.64 **F**	95.55 **D**	155.99 **B**	171.20**A**
Root length(cm)	22.75 **A**	23.33 **A**	19.79 **B**	22.23 **A**	23.77 **A**	23.04 **A**
N. root branches/plant	6.08 **A**	5.65 **B**	5.75 **B**	6.47 **A**	5.19 **B**	5.49 **B**
Root fresh weight (g)	13.00 **B**	15.68 **B**	8.21 **C**	14.86 **B**	23.26 **A**	23.69 **A**
Root dry weight (g)	10.74 **B**	13.22 **B**	6.25 **C**	13.01 **B**	21.40 **A**	21.23 **A**
Root diameter /plant (cm)	1.22 **A**	1.55 **A**	1.36 **A**	1.14 **B**	1.25 **A**	1.33 **A**

Y1: year one, Y2: year two.Meanings with the same letter in the same column are not significantly different.

3.2 Effect of colchicine on chemical composition of withania Somnifera plant.

From Table (3) and (4) the result of the effects of the chemical composition on the plant is shown The change in the amount of alkaloid production was ineffective, but the minor differences will be mentioned .

During the first and second years The highest content of alkaloids was obtained from control plants (0.017, 0.016 mg/100g) This means that colchicine had a negative effect on the roots' production of alkaloids. As shown in Table (4) the effect of time, a similar effect was recorded (0.014 mg/100g) in the first season for the three times of soaking, the same significant result, and there were no differences. While in the second year, small differences were recorded, and they were the highest (0.016 mg/ 100g F.W. (The reading was related to the soaking treatment for 12 hours. The highest content of phenols in both years was obtained from those plants treated with colchicine at 0.5 % (260.78 and 264.77 mg/ 100g F.W.), respectively.

While the results were different in the first year from the second with respect to time, as the first year achieved the highest amount of phenols) 212.66 mg/ 100g F.W.(during the 24-hour soaking time. In the second year, the effect of the 12-hour soaking treatment was the most influential on the value of phenols formed, as it achieved (172.29 mg/ 100g F.W.).

Table 3: Effect of Colchicine concentrations on chemical characters of *withania somnifera l* plant.

Colchicine concentrations	Total alkaloids (mg/100g F.W.)		Total phenols (mg/100g F.W.)	
	Y1 **(2021)**	Y2 **(2022)**	Y1 **(2021)**	Y2 **(2022)**
0%	0.017 **A**	0.016 **A**	119.05 **D**	110.54 **D**
0.2%	0.012 **B**	0.013 **B**	165.41 **C**	114.51 **D**
0.3%	0.014 **A**	0.013 **B**	246.69 **B**	159.04 **C**
0.5%	0.013 **B**	0.014 **A**	260.78 **A**	264.77 **A**

Y1: year one, Y2: year two.

Meanings with the same letter in the same column are not significantly different

Table 4: Effect of soaking time on chemical characters of *withania somnifera l* plant.

Time	Total alkaloids (mg/100g F.W.)		Total phenols (mg/100g F.W.)	
	Y1 **(2021)**	Y2 **(2022)**	Y1 **(2021)**	Y2 **(2022)**
12	0.014 **A**	0.016 **A**	183.35 **C**	172.29 **C**
24	0.014 **A**	0.014 **A**	212.66 **A**	134.52 **E**
36	0.014 **A**	0.013 **B**	197.93 **B**	166.32 **D**

Y1: year one, Y2: year two.Meanings with the same letter in the same column are not significantly different.

4. Discussion

The present results showed that colchicine had a strong effect on the height of the plant during the two years and led to a clear decrease and shortening of the height during the second year because that appears clearly during the second year in a higher manner than in the first year. The reduction in plant height is consistent with several of the results. Colchicine causes slowed mitotic divisions and cell divisions in larger cells with more chromosomes. Total plant height was lower in treatment compared with control, and this was due to shorter internodal distances. Several other researchers on *Dendranthema indicum* [24].on Solanaceae crops [25], on *Gladiolus grandiflorus* [26], on *Physalis peruviana* [27] pointed out that colchicine treatment had reduced the plant height. It is compatible with the results of our study.

The leaf area numbers during the two years in the control plants recorded the highest value .Visual examination revealed the greatest differences in leaf area between the treated plant and the control. Specifically, the leaves of treated plants were thicker and shorter than those of control plants. Moreover, treated plants grew slower than control plats. Similar results have been reported by [26] on *Gladiolus grandiflorus.*

Treatments 0.3 and 0.5% recorded the highest number of branches during the two years. Also, in other research, the use of colchicine has been associated with increased branching, or bushy habit [28-29] and [30] showed that applying colchicine to sesame (*Sesame indicum L*) increased the number of branches. In this study, the number of branches increased with an increase in incubation times and concentrations of colchicine. This result was similar to with the study of *Datura stramonium* L [31] the maximum number of branches was observed in the treatment containing 0.5g/48 h.

The highest leaf rates were at a concentration of 0.3% in the second year compared to the first year It was proven on *Phalaenopsis amabilis* [32], and that resulted in an improvement in the number of treated leaves compared to control plants.

The control plants in the second year had the highest number of flowers and fruits .*Withania somnifera* flowers decreased in the colchicine-treated plant. It is possible that the plant that was treated with colchicine had less pollen fertility because of multivalent association during chromosomal synopsis and the imbalanced gamete synthesis that followed. Meiotic process anomalies can also be the cause of low pollen grain viability. The viability of auto is affected by other genetic factors [33] .similar observations were earlier on French bean *Phaseolus vulgaris L* [34], on Japanese pear [35], on pomegranate [36-37] on *Trollius chinensis.* Delayed flowering [38] on *Withania sominefra l.*

The maximum leaf dry weight (g) belongs in year one to control plants, but in year two it belongs to the treatment Colchicine 0.5%. Based on Duncan multi-test (at level 5%), the minimum dry weight belonged to the control treatment. The results on *Datura stramonium L*. [39] that agreed with the results of our study. Important differences appeared clearly in the second year from the first year in root length, root thickness, root fresh weight, root dry weight, and root diameter obtained from colchicine

treatments, especially 0.3 and 0.5% colchicine, compared to the control. In this study, the treated plantlets consistently showed longer root lengths, and thicker roots than control. Similar trends were observed in the study on *Dendrobium ofcinaleo* [40] and on *Dendrobium cariniferum* [41] .On the other hand, thicker roots belong to treated plants [42-44].

In addition, in our study, the chemical analysis indicated significant results. After testing the influence of treated plants on alkaloid biosynthesis the results have been shown no differences in the alkaloid spectrum were found between the roots of control and treated plants, which agreed with the result on *D. stramonium* [45]. Alkaloid Production in control and treated Plants of Datura stramonium. Treatment with colchicine achieved the greatest benefit from increasing the content of phenols through treatment at a concentration of 0.5%. The total phenols of the plant due to the stress caused by treatment with colchicine. Our results are largely consistent with previous findings, where the results for phenolics and total antioxidant capacity were influenced by colchicine treatment [46].

However, this new study of Withania somnifera treated with colchicine showed an increased amount of phenols than the control plant. Therefore, the increase in the level of phenolics in 0.5% of plants treated with colchicine showed significant results. Moreover, the study confirms that treatment with colchicine increases the phenolic content of Withania somnifera, which is responsible for its medicinal value (anti-inflammatory activity). Therefore, our study is a valuable one to discover what is related to the colchicine-induced variation. In addition, a higher root yield was obtained than that obtained in the control treatment, and this also enables us to benefit from intensive research in obtaining a higher yield from the plants.

5. Conclusions

Our research shows that colchicine can produce greater results in *Withania somnifera L.* plants. Colchicine can lead to changes in the morphological, physiological, and biochemical levels of the plant. Additionally, colchicine cause stress in the plant, leading to higher levels of antioxidants, thus raising the therapeutic power of the plant. The transformed plant, with its high yield and chemicals of therapeutic importance, will be useful for the pharmaceutical industry and herbal drug formulations.

As a result, our research is critical in determining the plant's additional medicinal properties in relation to colchicine.

6. Conflict of interest

The authors declare that they have no competing interests.

7. Formatting of funding sources

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8. References

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