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Allelopathic Influence of *Withania somnifera* Leaf Extracts on the Germination and Seedling Growth of Wheat Genotypes

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Abstract

The experiment was conducted from February to March, 2023 in Lab-02, Department of Plant Physiology and Biochemistry, Bihar Agricultural University, Sabour, Bhagalpur, Bihar, India, to study the allelopathic properties of Ashwagandha on germination and seedling growth of wheat. The allelopathic influence of *Withania somnifera* leaf extracts on the germination and seedling growth of four wheat genotypes (HD2967, HD2285, WH147 and WL711) was systematically investigated. The study revealed that aqueous leaf extracts of *Withania somnifera* significantly inhibited seed germination and seedling growth across all genotypes. The degree of inhibition varied among the genotypes, with the susceptible genotypes (WH147 and WL711) exhibiting a greater reduction in germination percentage and Germination Relative Index (GRI) compared to the tolerant genotypes (HD2967 and HD2285). Physiological observations demonstrated a progressive decline in root length, shoot length, and root and shoot dry weights with increasing concentrations of the leaf extract. The biochemical analysis showed a marked reduction in sugar and protein content in wheat seedlings treated with the leaf extract, while a stimulatory effect was observed on total amino acid content. These findings suggest a strong inhibitory effect of *Withania somnifera* leaf extracts on the overall physiological and biochemical parameters of wheat seedlings, particularly in susceptible genotypes. This study highlighted the allelopathic potential of *Withania somnifera* and its possible implications for wheat crop productivity and management strategies, especially in the context of weed control and crop compatibility in intercropping systems.

Keywords: *Withania somnifera*, allelopathy, wheat genotypes, seed germination

1. Introduction

Withania somnifera (commonly known as Ashwagandha) is a prominent medicinal plant widely recognized for its multi-health benefits (Kaur et al., 2022). It has been traditionally used as an aphrodisiac, liver tonic, anti-inflammatory agent, and astringent (Sprengel et al., 2025). In modern medicine, Ashwagandha has gained attention for its potential to treat a variety of ailments, including bronchitis, asthma, ulcers, emaciation, insomnia, arthritis, high cholesterol levels, and senile dementia. Its rich medicinal properties are attributed to the diverse bioactive compounds it contains, making it a subject of extensive scientific investigation (Choudhary et al., 2017; Birla et al., 2019; Ng et al., 2019). The bioactive compounds are predominantly concentrated in the plant's leaves and roots, which are frequently employed to produce a range of medicinal preparations (Srivastava et al., 2018). In recent years, the industrial supply of the plant has struggled to keep up with a 30% increase in demand for its raw materials, namely leaves and roots (Afewerky et al., 2021), resulting in a growing disparity between market supply and demand. The consistent availability of these raw materials relies heavily on both their quality and quantity, which are significantly affected by nutrient availability as well as soil physical properties such as porosity, moisture, and pH (Isah, 2019).

Wheat (*Triticum aestivum* L.), belonging to the Poaceae family, is a significant cereal crop extensively grown for its grains, which serve as a staple food and are also utilized in producing various processed products (Majeed et al., 2017). Weeds have a significant impact on the germination and growth of wheat and other crops due to their strong competition for resources and their allelopathic properties. Allelopathy allows weeds to influence the development and germination potential of neighbouring plants. These weeds release various allelochemicals that can affect other plants in multiple ways, either inhibiting or promoting their germination and growth (Majeed et al., 2017; Muhammad et al., 2014). Previous studies have reported that aqueous extracts of sunflower reduced wheat and maize germination, along with radicle and plumule length, and dry biomass (Muhammad et al., 2014). In contrast, lower concentrations of Chenopodium album and sugarcane extracts were found to stimulate wheat seedling growth (Majeed et al., 2017; Majeed et al., 2012). Common weeds such as Melilotus officinalis, Avena fatua, and Polypogon hissaricus frequently infest wheat fields in many countries.

Beginning in the 1960s during the "Green Revolution," the extensive use of chemical fertilizers led to a significant increase in food production to address the demands of a growing population (Rehman et al., 2023). Nevertheless, the presence of agrochemical residues in food products has caused both health problems and environmental pollution (De et al., 2021). These negative effects have highlighted the need for sustainable agricultural practices that can maintain high productivity without compromising environmental and human health. In this context, allelopathy emerges as a natural and eco-friendly alternative. Allelopathy is a biological phenomenon in which an organism releases biochemicals, known as allelochemicals, that influence the growth, survival, and reproduction of other organisms in its vicinity. These allelochemicals are introduced into the environment through root exudation, leaching from stems and leaves, or decomposition of plant material. Previous studies (Rizvi and Rizvi, 1992) have documented the significant role of allelochemicals in shaping plant interactions. Mathela (1994) highlighted that secondary metabolites, such as flavonoids, glycosides, steroids, and diterpenoids, found in medicinal and aromatic plants, contribute to their allelopathic activity.

Given the significant potential of allelopathy in ecological interactions and agricultural applications, the present study was designed to assess the effect of aqueous leaf extracts of Withania somnifera on physiological and biochemical changes during seed germination and seedling growth. This study aimed to provide insights into the allelopathic properties of Ashwagandha, contributing to our understanding of its role in plant-plant interactions and its potential applications in sustainable agriculture.

2. Materials and Methods

The experiment was conducted from February to March, 2023 in Lab-02, Department of Plant Physiology and Biochemistry, Bihar Agricultural University, Sabour, Bhagalpur, Bihar. The collected leaves were shade-dried for 10 days, ground into a fine powder using a grinder, and sieved. For the preparation of extracts, 20 g of leaf powder was soaked in 100 ml of distilled water for 24 h to obtain a 20% stock solution. This stock solution was further diluted with distilled water to prepare 4%, 8%, and 12% concentrations. Seeds of *Triticum aestivum* were surface-sterilized by soaking in 0.2% mercuric chloride for 2 min, followed by thorough washing with deionized water. The seeds were then germinated in sterilized germination boxes lined with blotting paper, using distilled water as the control and leaf extracts at 4%, 8%, and 12% concentrations. The germination boxes were maintained at 25±2°C in a BOD incubator under controlled conditions, with three replications for each treatment. The Germination Relative Index (GRI) was calculated using the formula $\Sigma Xn(h-n)$. The Vigour Index (VI) was determined using the formula Germination percentage×Seedling length. After seven days of growth, the seedlings were harvested for biochemical analysis.

3. Results and Discussion

3.1. Seed germination and seedling growth

The germination percentage of wheat seeds after seven days of seedling growth, presented in Table 1. The increasing concentration of aqueous leaf extracts of *Withania somnifera* resulted in reduced germination due to the release of allelochemicals, which disrupted water uptake, enzyme activity, and hormonal balance. The genotypes HD2967, HD2285, WH147, and WL711 exhibited a significant decline in germination percentage. The maximum reduction was observed in the susceptible genotypes (WH147 and WL711) compared to the tolerant ones (HD2967 and HD2285). The Germination Rate Index (GRI) and Vigour Index (VI) of all test genotypes decreased as the concentration of leaf extracts increased, affecting enzymatic functions, water absorption, and hormonal activity. The interaction between treatments and genotypes was statistically significant ($p<0.05$). Comparable results in germination and seedling growth with increasing aqueous leaf extract concentrations have been reported in various crops (Prasad et al., 2011).

3.2. Root and shoot length

Root and shoot lengths progressively decreased with increasing leaf extract concentrations (Table 1). The highest values for root and shoot length were recorded in the control treatment (distilled water). Tolerant genotypes (HD2967 and HD2285) exhibited greater root and shoot length than susceptible ones (WH147 and WL711) at 4.0%, 8.0%, and 12.0% extract concentrations. The reduction in root and shoot length was attributed to the inhibitory effects of the leaf extract, which impeded cell division, water uptake, and nutrient absorption. Similar findings were reported by Mandal et al. (2003, 2005). A similar trend was observed in Withania somnifera, where susceptible genotypes showed a more significant reduction in root and shoot length than tolerant ones.

3.3. Root and shoot dry weight

The dry weight of roots and shoots significantly declined as the concentration of *Withania somnifera* leaf extract increased. The inhibitory effects of the extract were likely due to disruptions in cell division, water uptake, enzyme

Table 1: Influence of *Withania somnifera* leaf aqueous extracts on seed germination and biomass of wheat seedlings after seven days

Concentration	Genotypes	Germination (%)	GRI	Vigour index	Shoot length (cm)	Root length (cm)	Shoot dry weight (mg)	Root dry weight
Control (0%)	HD2967	100.0	300.6	2491.6	13.3	14.7	149.6	86.6
	HD2285	98.6	298.9	2743.6	14.6	14.4	145.6	85.1
	WH147	100.0	300.6	2587.6	13.6	13.4	144.6	85.8
	WL711	98.6	298.2	2759.7	14.7	14.5	145.1	84.5
	Mean	99.3	299.5	2645.6	14.05	14.2	146.2	85.5
4%	HD2967	97.2	278.9	2226.9	12.5	11.6	139.5	77.4
	HD2285	95.6	276.6	2437.3	13.6	13.2	133.6	74.6
	WH147	93.9	270.2	2264.3	11.2	10.5	130.1	72.8
	WL711	92.2	265.6	2252.0	12.0	11.0	128.6	71.5
	Mean	94.7	272.8	2295.1	12.3	11.5	132.9	74.0
8%	HD2967	92.1	262.8	1968.6	11.6	10.8	131.0	72.6
	HD2285	90.5	259.1	2140.7	12.6	12.1	127.3	69.6
	WH147	88.8	244.8	1820.0	9.8	9.1	122.3	66.2
	WL711	85.5	240.1	1883.2	10.2	9.7	120.5	63.0
	Mean	89.2	251.7	1953.1	11.05	10.4	125.3	67.8
12%	HD2967	88.7	236.7	1662.2	10.2	9.3	118.4	63.9
	HD2285	87.0	230.4	1788.4	11.0	10.3	114.3	59.7
	WH147	84.4	198.7	1418.0	7.8	7.3	104.3	51.8
	WL711	77.0	194.0	1434.1	7.8	7.6	99.9	48.7
	Mean	84.2	214.9	1575.6	9.2	8.6	109.0	56.0
	CD (p=0.05)							
	Stress (S)	0.4	13.7	112.3	0.6	0.7	5.2	1.1
	Genotypes (G)	0.4	13.7	112.3	0.6	0.7	5.2	1.1
	Interaction (S×G)	0.9	27.5	224.7	1.3	1.4	10.3	2.2

activity, and nutrient absorption, resulting in reduced biomass accumulation. Within each concentration level, the highest root and shoot dry weight values were recorded for the tolerant wheat genotypes, followed by the susceptible ones (Table 1). The percentage reduction in biomass was more pronounced in WH147 and WL711 than in HD2967 and HD2285. A significant inhibitory effect was observed on seedling dry weight as the leaf extract concentration increased. The effects of the extracts on all four genotypes were statistically significant (p<0.05). The pronounced suppression of root and shoot growth in this study aligned with previous findings (Kaur and Rao, 1998), which reported similar inhibitory effects of plant leaf extracts. The maximum biomass reduction occurred in the susceptible genotypes.

3.4. Biochemical parameters

The levels of reducing and non-reducing sugars in wheat seedlings were higher in the control group than in those treated with aqueous leaf extracts. Additionally, sugar content was found to be greater in tolerant genotypes compared to susceptible ones (Table 2). With an increase in the concentration of *Withania somnifera* leaf extract, the sugar content in different wheat genotypes declined. The minimum sugar content was recorded in susceptible genotypes due to enzymatic inhibition and interference with carbohydrate metabolism, leading to reduced starch hydrolysis. Similar findings were reported by Mandal et al. (2003, 2005), who observed a decrease in sugar content in wheat seedlings with increasing leachate concentration of Dalbergia sissoo, Acacia lenticularis, Bombax ceiba, and Populus deltoides. Padhy et al. (2000) also reported a decline in sugar content in finger millet seedlings when exposed to increasing concentrations of Eucalyptus leachates.

Table 2: Influence of aqueous leaf extracts of *Withania somnifera* on carbohydrate and protein metabolism of seven-day-old wheat seedlings

Concentration	Genotypes	Reducing sugar (mg g.d.w^{-1})	Non-reducing sugar (mg g.d.w^{-1})	Total Sugar (mg g.d.w^{-1})	Soluble protein (mg g.d.w.$^{-1}$)	Free amino acids (mg g.d.w.$^{-1}$)
Control (0%)	HD2967	25.4	29.8	54.1	39.2	20.5
	HD2285	25.3	29.8	53.9	39.1	20.6
	WH147	25.4	29.3	54.9	39.0	20.4
	WL711	25.3	29.1	55.0	39.1	20.3
	Mean	25.35	29.5	54.5	39.1	20.4
4%	HD2967	23.9	27.3	50.7	36.1	29.9
	HD2285	23.6	27.2	50.3	35.7	30.1
	WH147	21.2	25.9	49.8	34.3	28.8
	WL711	20.9	26.0	49.7	33.9	29.2
	Mean	22.4	26.6	50.1	35.0	29.5
8%	HD2967	22.1	25.2	46.9	34.0	28.6
	HD2285	21.7	25.2	46.5	33.6	28.5
	WH147	17.6	18.4	35.6	31.2	26.6
	WL711	17.3	18.2	35.1	30.8	26.4
	Mean	19.7	21.7	41.0	32.4	27.52
12%	HD2967	19.7	21.7	41.1	30.1	27.8
	HD2285	19.4	21.3	40.3	29.8	27.7
	WH147	13.7	16.8	30.2	25.0	25.5
	WL711	13.2	16.5	29.5	24.5	25.4
	Mean	16.5	19.0	35.3	27.3	26.6
	CD (p=0.05)					
	Stress (S)	0.9	0.9	0.7	0.8	0.8
	Genotypes (G)	0.9	0.9	0.7	0.8	0.8
	Interaction (S×G)	1.8	1.9	1.4	1.6	1.6

Regarding soluble total proteins in seven-day-old wheat seedlings, both tolerant and susceptible genotypes exhibited a decreasing trend with increasing leaf extract concentration. However, the protein content remained higher in tolerant genotypes compared to susceptible ones (Table 2). A high concentration of *Withania somnifera* leaf extract might reduce soluble protein levels due to the activation of proteolytic enzymes and interference with protein synthesis. A linear decline in protein content with increasing leaf extract concentration was also observed by Mandal et al. (2003). The reduction in protein content was accompanied by an increase in free amino acids. Ali and Mandal (2009) similarly reported that disturbances in protein metabolism could leads to decreased protein levels.

The free amino acid content exhibited an increasing trend at each leaf extract concentration across all four wheat genotypes, with the highest values recorded in tolerant genotypes at 12% leaf extract concentration. The increase in free amino acids due to *Withania somnifera* leaf extract might help tolerant genotypes cope with stress by acting as antioxidants, supporting growth, and aiding in stress-response protein synthesis. In contrast, susceptible genotypes, having weaker defense mechanisms, produced fewer amino acids under stress conditions. The interaction between treatments and genotypes for protein and total amino acids was highly significant (p<0.05).

The laboratory experiment conducted in this study indicated that *Withania somnifera* exerted an allelopathic effect on seed germination and seedling growth in different wheat genotypes. However, further studies, including chemical analysis of toxic compounds present in leaf leachates and field experiments, are necessary before drawing definitive conclusions on the allelopathic impact of *Withania somnifera*.

4. Conclusion

Withania somnifera (Ashwagandha) leaf extracts significantly inhibited wheat germination and seedling growth, with WH147 and WL711 genotypes showing higher sensitivity. The reduction in sugar and protein content, along with increased amino acids, highlighted adverse biochemical responses.

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