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COLD PLASMA-INDUCED MODULATION OF GROWTH AND PHYTOCHEMICAL COMPOSITION IN ASHWAGANDHA AND CAPSICUM

Agriculture Science

Ramya Siddagiri Msc., Department of Biological and Chemical Sciences, Mohan Babu University, Tirupati

Muppuru Kesavulu Professor, Dr. D. Y. Patil Biotechnology and Bioinformatics Institute, Tathawade, Pune

Sivaranjani Pakala* Assistant Professor, Department of Chemistry and Biochemistry, Jain University, Bengaluru *Corresponding Author

ABSTRACT

Cold atmospheric plasma (CAP) has recently gained attention as an ecofriendly technique for improving seed germination and plant growth. Plasma, recognized as the fourth state of matter, is rich in reactive species capable of inducing subtle modifications on the seed surface without damaging internal tissue integrity. Operating at ambient temperatures, cold atmospheric plasma (CAP) has recently gained significant attention for diverse agricultural applications. In this study CAP treatment was applied to seeds of ashwagandha (withaniasomnifera) and capsicum (capsicum annuum) to examine its effect on germination and early seedling development. Seeds were exposed to plasma for different durations (0.5, 5, 15 and 30 minutes) along with untreated control seeds. After treatment, germination and early growth parameters were observed. The results demonstrated that plasma-treated seeds exhibited enhanced germination and improved seedling vigor compared to the untreated control. Seedlings derived from treated seeds also showed slightly increased growth during the early developmental stages, indicating a positive influence of plasma exposure. Qualitative phytochemical analysis confirmed the presence of key secondary metabolites, including alkaloids, flavonoids, phenols, tannins, terpenoids, and glycosides, in both treated and untreated samples. Notably, variations in color intensity observed during the assays suggested differences in the relative abundance of these compounds, with certain plasma treatments eliciting stronger responses. In addition, important bioactive compounds such as withanolides in ashwagandha were detected. Overall, the findings suggest that cold atmospheric plasma (CAP) is a simple, effective, and sustainable approach for enhancing seed germination, early plant growth, and potentially influence bioactive compound levels in medicinal and agricultural crops.

KEYWORDS

Cold Plasma Treatment, Ashwagandha, Capsicum and Phytochemical Analysis.

1 INTRODUCTION

Plants are complex biochemical systems that produce diverse primary and secondary metabolites essential for survival and environmental adaptation, many of which possess significant nutritional and pharmacological importance for human health [1]. In recent years there has been a growing shift toward plant-based bioactive compounds owing to their therapeutic potential, reduced side effects and sustainability [2]. Among important medicinal and agricultural plants, *withaniasomnifera*(Ashwagandha) and *capsicum annuum* (capsicum) both belonging to the solanaceae family are of particular interest [3,4].

Plasma is considered the fourth state of matter distinct from solid, liquid and gases. It is formed when sufficient energy is supplied to a gas leading to ionization and the formation of electrons, ions and reactive species. Cold atmospheric plasma (CAP) is a nonthermal form of plasma generated at atmospheric pressure and near room temperature, making it highly suitable for various applications, including medicine, material processing and agriculture. Unlike thermal plasma in which all particles exist at high temperatures, CAP is characterized by high energy electrons while the bulk gas remains relatively cool. This unique property allows CAP to interact with biological systems without causing thermal damage [5, 6, 7].

CAP interacts with plants through a combination of physical, chemical, and biological mechanisms. At the physical level, plasma treatment modifies the seed surface by increasing its roughness and wettability, thereby enhancing water uptake and gas exchange, which are essential for seed germination [8]. Chemically, CAP generates a diverse range of reactive oxygen and nitrogen species (RONS), including hydroxyl radicals (OH), hydrogen peroxide (H_2O_2), superoxide ions (O_2^-), nitric oxide (NO), and ozone (O_3), which play a crucial role in mediating plant responses. These reactive species interact with cellular components, leading to controlled oxidation processes and activation of redox signaling pathways [9]. Biologically, RONS influence key cellular processes such as membrane permeability, enzyme activity, and gene expression. They also regulate redox signaling, thereby promoting seed germination, plant growth, and associated biochemical responses. Furthermore, these reactive molecules act as signaling agents that activate important pathways, including mitogen-activated protein kinase (MAPK) cascades, calcium signaling, and hormone-mediated responses, which collectively contribute to plant growth and stress tolerance [10, 11, 12]. CAP treatment also enhances the activity of antioxidant enzymes such as superoxide dismutase, catalase and peroxidase which help maintain cellular balance and improve stress resistance. Furthermore, CAP stimulates secondary metabolite production by activating pathways such as phenolpropanoid and terpenoid metabolism, leading to increased synthesis of phenols, flavonoids and withanolides [13, 14]. In addition, CAP exhibits antimicrobial properties by reducing microbial load on seed surface through oxidative damage and membrane disruption [15, 16, and 17].

Hence, the present study aims to address these existing gaps by evaluating the effects of cold atmospheric plasma (CAP) on both a medicinal plant, *Withaniasomnifera* (ashwagandha), and an economically important crop, *Capsicum annuum* (capsicum). Although CAP has emerged as a promising tool in agriculture, most studies have focused on major cereals, with limited attention given to plants possessing distinct metabolic pathways [18]. Ashwagandha and capsicum, despite belonging to the same family (*Solanaceae*), differ significantly in their phytochemical composition, producing withanolides and capsaicinoids, respectively, which may lead to differential responses under plasma treatment [19, 20]. Moreover, the lack of standardized plasma treatment parameters, particularly in dielectric barrier discharge (DBD) systems, and the limited exploration of phytochemical variations further highlight the need for comprehensive studies. In addition, comparative evaluation under different growth conditions, such as controlled germination systems and soil environments, remains underexplored. Therefore, this study integrates seed germination, plant growth, and qualitative phytochemical analysis within a single experimental framework to better understand the influence of CAP on plant development and secondary metabolite accumulation, thereby contributing to its potential application in sustainable agriculture and medicinal plant research.

2. MATERIALS AND METHODS

2.1. Plant Material

Certified seeds of *Withaniasomnifera* (ashwagandha) and *Capsicum annuum* (capsicum) were procured and used for the study. Uniform, healthy, and undamaged seeds were carefully selected to ensure experimental consistency and reliability.

2.2. Cold Atmospheric Plasma (CAP) Treatment

Cold atmospheric plasma (CAP) treatment of the seed samples ashwagandha and capsicum were carried out at the High Voltage

Engineering Laboratory, Department of Electrical Engineering, Indian Institute of Science (IISc), Bengaluru, under the guidance of Prof. B. S. Rajinikanth. CAP treatment was carried out using a dielectric barrier discharge (DBD) plasma system operated at atmospheric pressure and near room temperature. The system was powered by a high voltage alternating current source with a frequence of 80Hz and an applied voltage in the range of 17.4-18Kv, enabling stable and consistent plasma generation.

The CAP treatment for seeds of Ashwagandha and Capsicum were evenly spread in a single layer and positioned at a fixed distance of approximately 1-2 cm from the plasma electrode to ensure uniform exposure. The seeds were then subjected to plasma for different durations 0.5, 5, 15 and 30 minutes while a set of untreated seeds was maintained as the control. All experimental parameters were carefully kept constant throughout the study to maintain consistency and reproducibility.

The plasma generated under the conditions contain a mixture of reactive oxygen and nitrogen species (RONS) along with charged particles and free radicles. These reactive species primarily interact with the outer surface of the seeds, bringing about subtle physiochemical changes such as improved surface wettability and permeability. As a result, treated seeds may exhibit enhanced water uptake and activation of metabolic processes associated with germination. Importantly, these surface level modifications occur without causing damage to the internal seed structure, making CAP a safe and effective approach for seed treatment [21, 22].

2.3. Seed Sowing Under Tray and Soil Conditions

Treated and untreated (control) seeds of *Withaniasomnifera* and *Capsicum annuum* were sown under two different conditions to enable comparative analysis. For the germination tray setup, seeds were sown in sterile cocopeat, which provided a uniform growth medium with adequate aeration, moisture retention, and minimal microbial interference. This controlled environment facilitated consistent germination and allowed accurate assessment of the direct effects of plasma treatment on early seedling development.

In contrast, seeds sown in soil were exposed to natural environmental conditions, including variable moisture levels, microbial activity, and nutrient availability, thereby representing more realistic cultivation conditions. Both experimental setups were maintained at a temperature of 25 ± 2 °C, and adequate moisture was ensured through regular watering. Successful seedling establishment in both systems confirmed seed viability and enabled reliable comparison of germination behavior, growth parameters, and overall plant performance across treatments. This dual approach provided a comprehensive evaluation of cold atmospheric plasma (CAP) treatment under both controlled and practical conditions.

2.4. Germination Assay

Germination was recorded daily for both treated and control groups of *Withaniasomnifera* and *Capsicum annuum* under both experimental conditions. A seed was considered germinated upon the emergence of the radicle with a length of ≥2 mm. The number of germinated seeds was counted for each treatment, including control and plasma-treated samples. Germination percentage was calculated based on the total number of seeds sown, following standard procedures recommended by the International Seed Testing Association [23] (ISTA, 2015).

2.5. Seedling Growth and Vigour Index

Seedling growth parameters were assessed 20 days after sowing. Root length, shoot length, and total seedling length of Withaniasomnifera and Capsicum annuum were measured for all treatment groups, including the control. Observations were recorded from three independent replicates (n = 3) to ensure accuracy and reproducibility. The seedling vigour index (SVI) was calculated according to the method described by Abdul-Baki and Anderson (1973) [24].

2.6. Qualitative Phytochemical Analysis for Ashwagandha and Capsicum

Qualitative phytochemical analysis was performed to detect the presence of major bioactive compounds in methanolic leaf extracts of *Withaniasomnifera* and *Capsicum annuum*. Screening of *W. somnifera* included alkaloids, flavonoids, terpenoids, phenols, tannins, glycosides, quinones, carbohydrates, and amino acids, along with a specific test for withanolides (steroidal lactones). In *C. annuum*, the analysis comprised alkaloids, flavonoids, terpenoids, phenols, tannins, glycosides, quinones, carbohydrates, amino acids, proteins, resins, and cellulose using standard qualitative protocols.

Fresh leaf samples from both plasma-treated and control plants were collected and thoroughly washed with distilled water to remove surface contaminants. Approximately 5g of fresh tissue was finely chopped and homogenized in methanol using a mortar and pestle. The homogenate was transferred to a conical flask and subjected to extraction with intermittent shaking to ensure efficient recovery of phytoconstituents. The extract was then filtered through Whatman No. 1 filter paper to obtain a clear filtrate, which was used for subsequent phytochemical screening. Wet extraction using methanol was employed as it facilitates efficient extraction of thermolabile and polar compounds while minimizing degradation [25, 26].

Qualitative phytochemical screening of *Withaniasomnifera* and *Capsicum annuum* was performed using standard protocols to identify major bioactive constituents. All assays were conducted in accordance with established procedures described by Harborne (1998) [27], Trease and Evans (2002) [28], and Mirjalili et al. (2009) [29].

3. RESULTS AND DISCUSSION

3.1. Plant Selection and Seed Selection

The *Withaniasomnifera* and *Capsicum annuum*, although belonging to the same *Solanaceae* family, exhibit distinct metabolic pathways and phytochemical profiles, characterized by the production of withanolides and capsaicinoids, respectively. These differences make them ideal candidates for investigating species-specific responses to external treatments. Cold atmospheric plasma (CAP), a non-thermal and eco-friendly technology, has gained attention for its potential to enhance seed germination, plant growth, and secondary metabolite accumulation. Therefore, the present study was designed to comparatively evaluate the influence of CAP on growth performance and phytochemical characteristics of these medicinally and economically significant plants.

All seeds of ashwagandha (*witaniasomnifera*) (figure. 1a) and capsicum (*capsicum annuum*) (figure. 1b) were observed to be uniform in size, shape and colour indicating good seed quality. No visible signs of physical damage, discoloration or infection were detected. The uniformity of seeds ensured reliability and consistency in subsequent experimental observations.

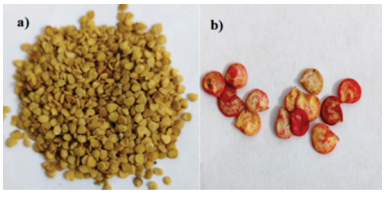


Figure 1. Seeds of a) Ashwagandha and b) Capsicum

3.2. Cold Atmospheric Plasma (CAP) Treatment

Cold atmospheric plasma (CAP) treatment applied to seeds of ashwagandha (*withaniasomnifera*) and capsicum (*capsicum annuum*) (figure. 2a) using a dielectric barrier discharge (DBD) system (figure. 2b) under controlled conditions (80Hz, 17.4- 18kV). Seeds were exposed to plasma for different durations (0.5, 5, 15 and 30 minutes) while untreated seeds served as control.

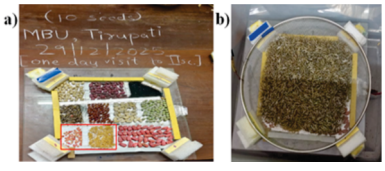


Figure 2 a. Preparation of Seeds for CAP Treatment b. Volume Discharge Plasma

3.3. Seed Sowing Under Tray and Soil Condition

Following plasma treatment, seeds of ashwagandha (*withania-somnifera*) and capsicum (*capsicum annuum*) were successfully established under both controlled germination tray with cocopeat and natural soil conditions to enable a comparative evaluation of growth performance of treated and untreated seeds. The use of two distinct sowing environments allowed for a better understanding of how plasma treated seeds and control seeds respond under both optimized and field like conditions.

3.4. Germination Assay

Germination was monitored daily, and the emergence of the radicle (≥ 2 mm) was considered as successful germination. Germination percentage was calculated based on the number of seeds germinated out of 20 seeds sown per treatment. The results revealed distinct variations in germination behaviour between control and plasma-treated seeds in both *Withaniasomnifera* and *Capsicum annuum*.

In *W. somnifera*, control seeds exhibited a germination rate of 45%. A reduction in germination (20%) was observed at 0.5 min of plasma exposure, indicating initial stress effects. However, with increasing treatment duration, germination improved markedly, reaching 60% at 5 min and 80% at 15 min, with a maximum of 95% recorded at 30 min (Table 1).

In *C. annuum*, control seeds showed a germination percentage of 75%. Plasma treatment resulted in a gradual enhancement, with germination increasing to 80% at 0.5 min, 85% at 5 min, and peaking at 90% at 15 min. However, prolonged exposure (30 min) led to a sharp decline in germination (20%), suggesting detrimental effects on seed viability at higher treatment durations (Table 1).

Germination percentage was calculated in accordance with standard procedures recommended by the International Seed Testing Association (ISTA, 2015) [23].

$$\text{Germination Percentage (\%)} = \frac{\text{Number of seeds germinated}}{\text{Total number of seeds sown}} \times 100$$

Table 1.Comparative Analysis of Germination Percentage in Control and Plasma-treated Seeds of *Withaniasomnifera* and *Capsicum Annuum*

Parameter	Control	0.5 min	5 min	15 min	30 min
Seeds Sown (n)	20	20	20	20	20
Capsicum Seeds Germinated (n)	15	16	17	18	4
Capsicum Germination (%)	75	80	85	90	20
Ashwagandha Seeds Germinated (n)	9	4	12	16	19
Ashwagandha Germination (%)	45	20	60	80	95

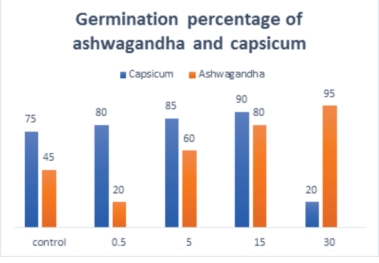


Table 2. Seedling Vigour Index of (SVI) Ashwagandha and Capsicum

	Ashwagandha					Capsicum				
Treatment (min)	Root length (cm)	Shoot length (cm)	Total seedling length (cm)	Germination percentage (%)	SVI	Root length (cm)	Shoot length (cm)	Total seedling length (cm)	Germination percentage (%)	SVI
Control	3.5	4.5	8.0	45	360	4.2	5.8	10.0	75	750
0.5	2.8	3.7	6.5	20	130	4.6	6.2	10.8	80	864
5	4.2	5.3	9.5	60	570	5.6	6.8	12.4	85	1054
15	5.0	6.5	11.5	80	920	6.2	7.5	13.7	90	1233
30	6.0	7.2	13.2	95	1254	-	-	-	-	-

3.5. Qualitative Phytochemical Analysis for Ashwagandha and Capsicum

Qualitative phytochemical screening of methanolic leaf extract of ashwagandha (*withaniasomnifera*) (Table 3) and capsicum (*capsicum annuum*) (Table 4) revealed the presence of several bioactive compounds with noticeable variations in intensity across different plasma treatment durations.

Table. 3 Phytochemical Analysis of Ashwagandha (+) Indicates Presence, (++) Indicates Moderate Presence, (+++) Indicates Strong Presence, and (–) Indicates Absence of Phytochemicals Based on Intensity of Colour Reaction.

S. No	Bioactive	Test	Observation	Time (Min)				
				Control	0.5	5	15	30
1	Alkaloids	Wanger's test	Reddish brown colour	+	+	++	++	++
2	Flavonoids	Alkaline reagent test	Yellow colour	+	++	++	++	++
3	Phenols	Lead acetate test	White precipitate	+	++	+++	++	++
4	Terpenoids	Salkowski test	Reddish – brown colourization	+	+	+	++	++
5	Tannins	Lead acetate test	White precipitate	+	+	++	++	++
6	withanolids	Salowski test	Reddish - brown colourization	+	+	+	+	+
7	Amino acid	Ninhydrin test	No colour change	-	-	-	-	-
8	Quinones	Sulfuric acis test	No nocolour change	-	-	-	-	-
9	Carbohydrates	Benedit's test	Green colourization	+	-	+	++	++

Figure 3. Effect of Treatment Duration on Germination Percentage of Ashwagandha and Capsicum

X-axis: Treatment duration (minutes)
Y-axis: Germination percentage (%)

Seedling Growth and Vigour Index

Seedling growth parameters, including root length, shoot length and total seedling length were measured after 20 days of sowing. Noticeable variations were observed among different plasma treatments in both ashwagandha ashwagandha (*withaniasomnifera*) and capsicum (*capsicum annuum*). All observations were recorded in triplicates (x_1, x_2, x_3) to ensure accuracy and reliability and the mean values were calculated for further analysis.

In ashwagandha, a gradual improvement in seedling growth was observed with increasing plasma exposure. While the control and 0.5 minutes treatments showed relatively lower growth, seedlings from 15, 30 minutes treatments exhibited increased root and shoot length. The highest growth was recorded at 30 minutes, indicating better adaptation of ashwagandha to longer plasma exposure (Table 2).

In capsicum, plasma treatment significantly influenced seedling growth. Seeds treated for moderate durations (5, 15 minutes) exhibited enhanced root and shoot development compared to the control. The highest total seedling length was observed at 15 minutes of treatment, indicating optimal growth conditions. However, seedling from seeds treated for 30 minutes showed reduced growth, suggesting that prolonged plasma exposure may have a negative effect (Table 4).

The seedling vigour index (SVI) was calculated as per the method described by Abdul-Baki and Anderson (1973)[24]. The mean values were calculated using the standard arithmetic mean formula.

Mean Formula

$$\bar{x} = \frac{x_1 + x_2 + x_3}{3}$$

Where x_1, x_2, x_3 represent individual observations and $\bar{x}$represents the mean value.

$$\text{SV1} = \text{Germination percentage (\%)X Total Seedling Length(cm)}$$

Seedling vigour index (SVI) formula

Where:
Germination percentage (%) = number of seeds germinated / total seeds × 100

Total seedling length = root length + shoot length

Table. 4 Phytochemical Analysis of Capsicum

S. No	Bioactive	Test	Observation	Time (Min)				
				Control	0.5	5	15	30
1.	Alkaloids	Wanger's test	Reddish brown colour	+	+	+	+	+
2.	Flavonoids	Alkaline reagent test	Yellow colour	+	+	+	+	+
3.	Terpenoids	Salkowski test	Reddish – brown colourization	+	++	++	++	++
4.	Phenols	Lead acetate test	White precipitate	+	+	+	+	+
5.	Tannins	Lead acetate tesst	White precipitate	+	+	+	+	+
6.	Glycosides	Molish test	Brown – reddish color	+	+	+	+	+
7.	quinones	Alkaline test	Red colorization	+	+	+	+	+
8.	Carbohydrates	Benedict's test	No colour change	-	-	-	-	-
9.	Protein	Biuret test	No colour change	-	-	-	-	-
10.	Resins	Acetate water test	No precipitate found	-	-	-	-	-
11.	Cellulose	Iodine sulfuric acid test	Purple colour formed	+	+	+	+	+

4. DISCUSSION

The present study was designed to explore how cold atmospheric plasma (CAP) treatment influences seed germination, early seedling growth and phytochemical characteristics in two biologically and functionally different plants ashwagandha (withaniasomnifera) and capsicum (capsicum annuum). Although both belong to the same family solanaceae their responses to plasma treatment showed clear differences which makes the findings of this study particularly interesting. One of the first observations that stands out is the effect of plasma treatment on seed germination. In capsicum, germination improved noticeable with increasing exposure time upto 15 minutes, suggesting that moderate plasma treatment creates favourable conditions for seed activation. However, when the exposure was extended to 30 minutes, a clear decline in germination was observed. This indicates that while plasma can stimulate germination, excessive exposure may become stressful or even damaging to the seed. In contrast ashwagandha behaved differently its germination gradually increased with longer exposure time and reached the highest level at 30 minutes. This difference suggests that ashwagandha seeds are more tolerant to prolonged plasma exposure possibly due to differences in seed coat structure or internet biochemical composition.

This variation between the two plants highlights an important point plasma treatment does not have a one size fits all effect. Instead, its impact depends on the biological characteristics of the plant. Earlier studies have also reported similar behaviour, where plasma improves germination by modifying the seed surface, increasing permeability and allowing better water uptake [6, 7]. Plasma seems to activate the selected ashwagandha and capsicum seeds by making it more responsive to its environment but only up to a certain limit. Moving to seedling growth, the results clearly show that plasma treatment influences root and shoot development. In capsicum, the best growth was observed at 15 minutes which aligns with the peak germination results. Both root and shoot length increased, leading to higher total seedling length and vigour. However, at 30 minutes, growth declined, reinforcing the idea that too much plasma exposure can negatively affect plant development. This could be due to excessive generation of reactive species which may start damaging cellular components instead of stimulating them. On the other hand, ashwagandha showed a steady and consistent improvement in growth with increasing that this plant not only tolerates longer exposure but may actually benefit from it. This suggests that ashwagandha may have stronger internal defence mechanisms or better adaptability to oxidative stress conditions induced by plasma treatment.

The seedling vigour index (SVI), which combines both germination and growth performance, further strengthens these observations. Capsicum showed maximum vigour at 15 minutes while ashwagandha reached its highest vigour at 30 minutes. This clearly indicates that optimal plasma exposure time is different for each species. Such findings are important because they emphasize the need to optimize treatment conditions rather than applying a uniform approach to all crops.

Another key part of this study was the qualitative phytochemical analysis. Both plants ashwagandha and capsicum showed the presence of important secondary metabolites such as alkaloids, flavonoids, phenols, tannins and terpenoids. Interestingly, while most compounds remained consistently present across treatments, variations in colour intensity were observed. In capsicum, terpenoids showed increased intensity in plasma treated samples, suggesting that plasma may enhance their accumulation. In ashwagandha, variations were observed in multiple compounds including phenols and flavonoids. Even though this study didn't include qualitative analysis, the changes in colour intensity provide useful preliminary insights. These variations suggest that plasma treatment may influence metabolic pathways involved in the synthesis of secondary metabolites. This is important because these compounds are responsible for many medicinal and nutritional properties of plants. Previous studies have also reported that plasma induced mild oxidative stress can act as a signal, triggering the production of bioactive compounds [30, 31].

From a mechanistic point of view, the effects of plasma can be explained by its interaction with the seed surface and internal plant systems. Plasma generates reactive oxygen and nitrogen species (RONS), which interact with the seed coat, making it more permeable and improving water absorption. At the same time, these reactive species can enter the seed and act as signalling molecules, activating enzymes and growthrelated pathways. However, if their concentration becomes too high, they may cause oxidative damage, which explains the reduced performance at higher exposure levels in some cases [32, 33].

This study demonstrates that cold atmospheric plasma is a powerful and ecofriendly tool with the potential to enhance seed performance and plant growth of selected ashwagandha and capsicum plants. At the same time, it clearly shows that its effects are highly dependent on treatment duration and plant type. While capsicum responds best to moderate exposure, ashwagandha benefits from longer treatment. This difference highlights the importance of tailoring plasma treatment conditions to specific crops.

5. CONCLUSION

The present study demonstrated that cold atmospheric plasma (CAP) treatment positively influences seed germination, seedling growth and phytochemical characteristics in both ashwagandha (*withania-somnifera*) and capsicum (*capsicum annuum*). CAP treated seeds exhibited improved germination percentage, rootlength and seedling vigour index compared to untreated controls. The optimal treatment duration was species dependent, with capsicum showing the best response at 15 minutes and ashwagandha at 30 minutes of plasma exposure. Qualitative phytochemical analysis also indicated potential changes in the accumulation of bioactive compounds following plasma treatment. These findings suggests that CAP is promising, ecofriendly and sustainable technology for enhancing seed performance and plant growth. Further studies involving quantitative phytochemical analysis and field scale validation are recommended to fully explore its agricultural and medicinal applications.

Disclosure Statement

No potential conflict of interest was reported by the author.

Competing Interests

All authors declared that they have no conflicts of interest.

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