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Effects of Non-Thermal Plasma on Germination, Root and Shoot Length of Tomato Seeds and Ginger Rhizome

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Key words: ANOVA, Dielectric barrier discharge, Non-thermal plasma, Plant germination, Plant growth

Abstract

This study examines the effects of Dielectric Barrier Discharge using Non-thermal plasma treatment on the germination and growth of tomato (*Solanum lycopersicum*) seeds and ginger (*Zingiber officinale*) rhizomes. Given the environmental concerns over chemical fertilizers, plasma treatment presents a promising, sustainable alternative for enhancing seed germination and growth. Two types of Dielectric Barrier Discharge plasma-Surface Dielectric Barrier Discharge and Volume Dielectric Barrier Discharge were applied to the seeds and rhizomes, with germination rates, root, and shoot lengths assessed through daily observations. Statistical analysis revealed that plasma treatment significantly improved both germination and growth, with Volume Dielectric Barrier Discharge showing superior results compared to Surface Dielectric Barrier Discharge. Additionally, plasma treatment was more effective in breaking dormancy in ginger rhizomes than traditional methods. The findings suggest that plasma treatment can enhance seed germination, growth and dormancy breaking, providing a viable alternative to chemical treatments. Further research on plasma exposure parameters could optimize this method for broader agricultural applications, improving sustainability and crop productivity.

Introduction

India, a prominent global producer of crops, faces challenges in accessing affordable and suitable seeds despite its reliance on domestically sourced ones. Chemical treatments, while common for enhancing seed quality, present environmental and health risks. In response, plasma treatment has emerged as a promising alternative, offering benefits in seed germination and plant growth without the drawbacks associated with chemical

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treatments (Vernooy et al. 2022). Seed treatment is essential for promoting germination, increasing resistance to stress factors and ensuring crop productivity. However, the overuse of chemical treatments can lead to environmental degradation and reduced soil fertility. Plasma treatment offers a sustainable solution by inducing physical and chemical changes on the seed surface through ionized gas application (Lamichhane et al. 2020). This process enhances seed vitality, improves water uptake and boosts resistance to pests and diseases. Moreover, plasma treatment modulates phytohormone balance and stimulates gene expression associated with stress response and plant growth, leading to stronger and more resilient plants.

Plasma is a state of matter with charged gas particles containing positive ions and negative electrons in a 1 : 1 ratio. It exhibits properties like conductivity and response to electromagnetic fields (Ishikawa et al. 2024). Thermal plasma, generated at high pressure and temperature, is vital for waste management, material synthesis, metallurgy, energy generation, sterilization and semiconductor manufacturing. It refines metals, produces nanoparticles and enables surface coating. Non-thermal plasma, with electron temperatures surpassing ion and neutral temperatures, is characterized by vibrational and rotational energy in gas molecules, rendering them highly reactive. While easier to produce at low pressure, atmospheric-pressure discharges require strong electric fields, transitioning rapidly into arcs or sparks. Examples include fluorescent lamps with mercury-vapor gas, where electrons reach high temperatures while the rest remains at room temperature. Both thermal and non-thermal plasma have diverse industrial applications, generating reactive species and high temperatures for various processes (Fig. 1). They offer efficient waste treatment, material synthesis, surface modification, energy generation, sterilization, and semiconductor manufacturing, among others, making them crucial in modern technology and industry (Lin et al. 2022).

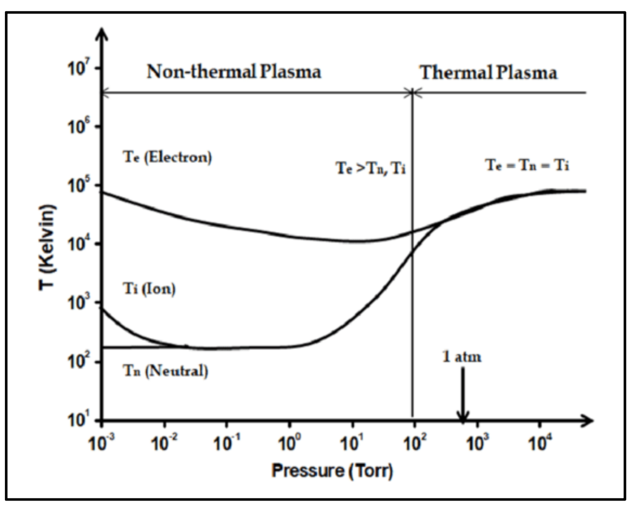

Fig. 1. Types of plasma (Sabat 2021).

Dielectric Barrier Discharge (DBD) employs an insulating substance between electrodes to prevent large electrical current arcs (arcing), creating non-thermal plasma at or near atmospheric pressure. DBD designs offer homogeneous plasma dispersion across electrodes, facilitating a substantial flow of reactive or activated chemicals for material treatment without harmful heat loads. Two recent DBD arrangements include volume discharge (VD) and surface discharge (SD). Numerous microdischarges, or current filaments, occur in DBD as the surrounding gas breaks down, dispersed randomly in space and time depending on electrode voltage. Surface Discharge (SD) treatment modifies material surfaces using ionized gas (Fig. 2) containing reactive species, suitable for heat-sensitive materials due to non-thermal nature. SD moves along the dielectric surface in a non-uniform field, displaying complex patterns in high-voltage oscillating signals, termed forward and backward strokes (Piferi et al. 2021). Volume Discharge (VD) separates electrodes with a dielectric barrier, allowing gas to fill the gap and form plasma, suitable for treating large material quantities with a consistent and wide plasma field. VD plasma treatment finds applications in environmental cleanup, industrial surface treatment and sterilization, offering efficient surface changes for various industries requiring broad plasma environments (Starič et al. 2020). This paper reviews the potential of plasma treatment in agriculture, highlighting its role in sustainable seed enhancement and its broader applications across various fields.

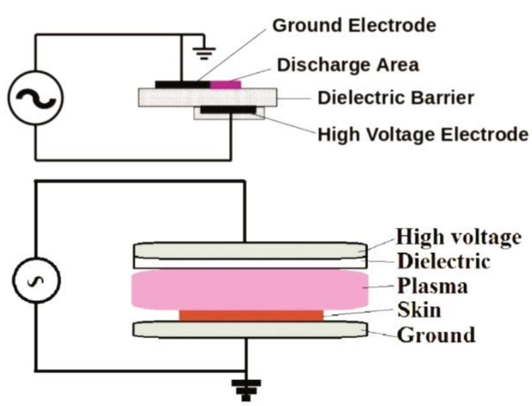

Fig. 2. Surface Dielectric Barrier Discharge (SDBD) and Volume Dielectric Barrier Discharge (VDBD) (Randeniya et al. 2015, Pilla et al. 2005).

Materials and Methods

Tomato seeds were selected due to their susceptibility to diseases like bacterial wilt, prompting research into plasma 12treatment as an alternative to existing management methods. Limited studies on the effects of different dielectric barrier discharge treatments on tomato seeds necessitated a comparative investigation between Surface and Volume DBD treatments. As a major commercial crop, tomatoes require efficient seed processing technologies to maintain seed quality and ensure optimal germination. Similarly, ginger rhizomes were chosen for their susceptibility to various diseases and the lack of research on plasma's effects on ginger growth. Diseases such as soft rot and ginger blight underscore the need for alternative disease management strategies. Additionally, ginger's long dormancy period presents a challenge for farmers and scientists, highlighting the potential of plasma treatment to reduce dormancy and improve rhizome quality. This study aims to explore the efficacy of plasma treatment in enhancing the quality and viability of both tomato seeds and ginger rhizomes (Samal 2017, Shimizu 2017).

The experimental design employs a completely randomized design (CRD) with 3 treatments and 3 replications. It includes untreated seeds as control and two plasma treatments: Surface Dielectric Barrier Discharge (SDBD) and Volume Dielectric Barrier Discharge (VDBD). Randomization within blocks is achieved by varying germination methods (netted pots with cocopeat and germination papers) to mitigate biases. Each sample type undergoes equal seeding and placement protocols to minimize experimental error. Three replications ensure result accuracy, allowing for reliable conclusions. This design's statistical efficiency, unbiased treatment allocation, and versatility make it suitable for various research fields (Cui et al. 2019).

The experimental protocol was to study the effects of different treatments on seeds: control (C), volume discharge plasma (VD), and surface discharge plasma (SD), each with around 100 seeds. For each group, seeds were prepared and sealed in zip-lock bags with aluminum foil preventing the exposure for the seeds to sunlight and air.

For the germination of tomato seeds, the process commenced by soaking the germination papers in water for an hour, followed by semi-drying them for 10 min. Subsequently, seeds were arranged in a 5×5 zig-zag pattern on the germination papers. The seeds were loosely rolled up, ensuring coverage of the seeds. Regular watering of the papers was conducted and daily monitoring was performed. This method facilitated optimal moisture content and provided a conducive environment for seed germination while preventing root penetration through the paper.

The second method utilized netted pots and sterilized coco peat for germinating both tomato seeds and ginger rhizomes. Netted pots, constructed from sturdy polystyrene, offer individual compartments that protect seeds and rhizomes from pests, diseases, and adverse weather. They are lightweight, portable, and suitable for indoor and outdoor settings, optimizing space usage efficiently. The method began with washing and drying the pots thoroughly, followed by filling each pot with sterilized coco peat to a depth of 1 inch. Seeds or rhizomes were then planted in separate pots and covered with an additional layer of coco peat. Regular watering and placement in ambient conditions with adequate sunlight and temperature ensured optimal germination conditions.

The high-voltage circuit for plasma treatment of seeds comprises three primary components: a transformer, capacitor with dielectric and seeds and a 4 Electrode Rotary Spark Gap (Hosseini et al. 2018). The transformer converts low voltage to high voltage, facilitating plasma generation. The capacitor, coupled with a dielectric material containing the seeds, stores this high voltage. Upon discharge, it produces a strong electric field across the dielectric, crucial for initiating and sustaining the plasma discharge. The 4 Electrode Rotary Spark Gap serves as a switching device, periodically discharging the capacitor to create a controlled spark gap. This mechanism charges the capacitor via the transformer, then discharges it across the spark gap, generating a high-energy plasma field. Within this field, seeds placed within the dielectric medium undergo plasma treatment, potentially enhancing their germination and growth properties. This circuit design enables controlled and efficient plasma treatment, offering a promising method for improving seed quality and agricultural productivity.

Tomato seeds and ginger rhizomes underwent plasma treatment using a dielectric barrier discharge (DBD) reactor (Fig. 3) to explore its effects on seed and rhizome germination and growth. The study employed two distinct plasma treatment methods: Surface DBD (SDBD) and Volume DBD (VDBD). SDBD targeted the outer surface of tomato seeds, modifying the seed coat properties to potentially enhance water and nutrient uptake. This approach aimed to improve germination rates and overall germination percentage by facilitating better seed coat interactions with the environment. In contrast, VDBD allowed plasma to permeate deeper into the bulk of tomato seeds, influencing internal physiological processes that could further promote germination and subsequent seedling growth (Sivachandiran et al. 2017). Seeds were uniformly arranged on the sample holder of the reactor for consistent exposure to plasma during treatment.

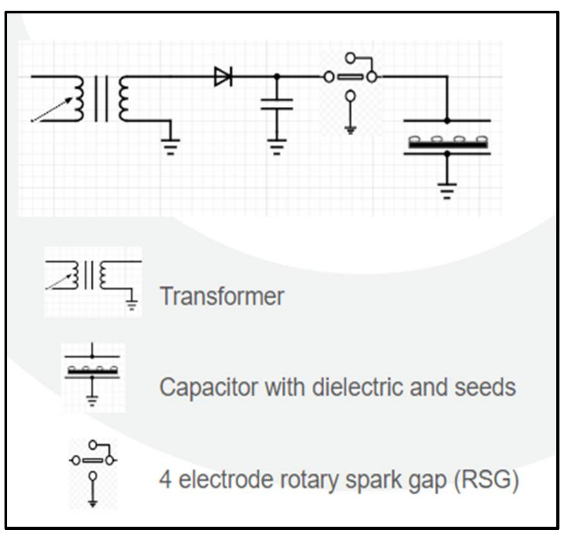

Fig. 3. Circuit Diagram of DBD reactor.

For ginger rhizomes, which were segmented into pieces containing vegetative buds, plasma treatment was applied primarily to the surface area. The close arrangement of rhizome sections in the sample holder ensured that each piece received uniform plasma exposure, targeting the external surfaces where the treatment could potentially stimulate dormancy release and promote initial growth (Attri et al. 2020). Both tomato seeds and ginger rhizomes were subjected to plasma treatment at atmospheric pressure, applying a high voltage of 18kV for a duration of 10 min. This standardized approach facilitated a comparative analysis between SDBD and VDBD treatments, aiming to elucidate the optimal method for enhancing germination rates and growth dynamics in tomato seeds and ginger rhizomes through plasma technology (Fig. 4 and Fig. 5).

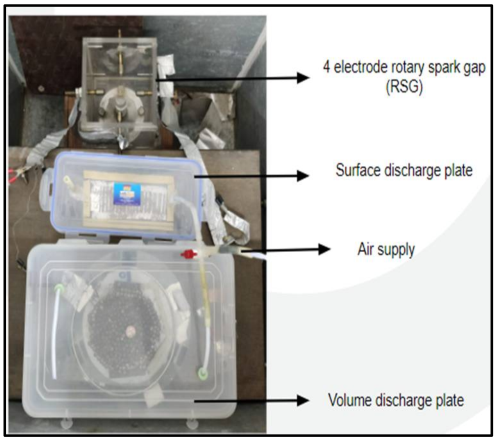

Fig. 4. Experimental setup.

Fig. 5. Surface dielectric barrier discharge for tomato seeds, volume dielectric barrier discharge for tomato seeds, surface dielectric barrier discharge for ginger rhizome.

Results and Discussion

Data was collected from the germination papers as well as from the netted pots in order to analyze various parameters of growth such as germination percentage and root and shoot length. For germination percentage, seeds in germination papers were observed and watered daily, and the number of germinated seeds was counted and photographed for control, surface discharge, and volume discharge treatments. Similarly, in netted pots, each containing five seeds and covered with cocopeat, germination was recorded weekly under natural conditions across ten pots per treatment (Fig. 6).

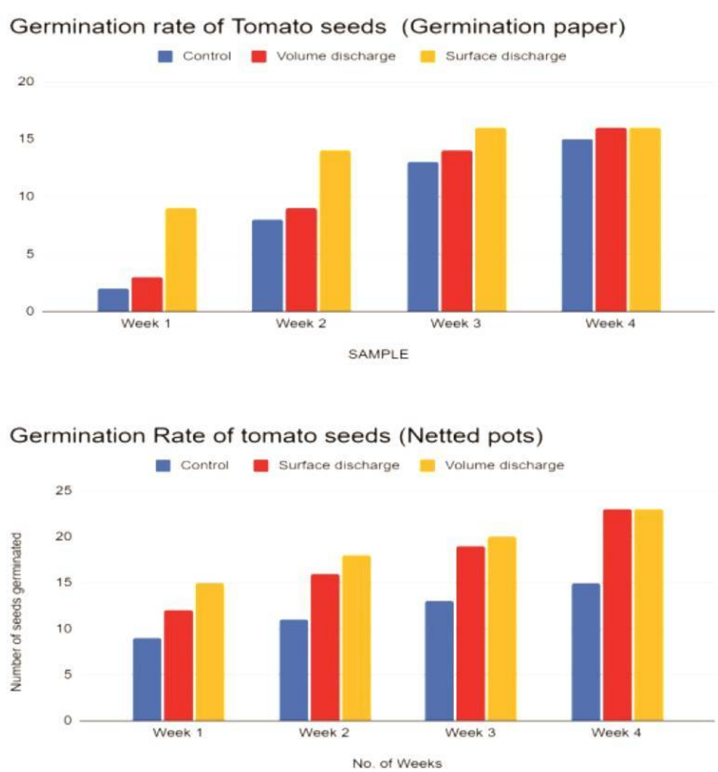

Fig. 6. Germination rate over 4 weeks for germination paper and netted pots.

The p-values obtained are significant at $p < 0.05$. This indicates that there is significant difference in the number of seeds that have germinated in all the three different types of treatments, with surface and volume discharge treated seeds showing higher germination percentage than control seeds.

Root and shoot lengths were measured by removing the germinated seeds from the germination papers and placing them alongside each other against a dark background and a ruler to compare the lengths of each treatment group (Table 1).

Table 1. Analysis of germination percentage for tomato seeds in germination sheets and netted pots.

Analysis Of Germination Percentage						
Germination Paper						
Source	DF	SS	MS	F-Ratio	P-Value	Inference
Between Treatments	2	4.13	2.06	3.779	0.048	Significant
Within Treatments	12	4.8	8.96	-	-	
Netted Pots						
Between Treatments	2	5.89	2.96	3.24	0.045	Significant
Within Treatments	12	4.6	2.06	-	-	

In case of root lengths, the p-values for Weeks 1, 3 and 4 ($p < 0.05$) indicate significant differences in root lengths among the treatments, with surface and volume discharge treated seeds showing longer roots. In Week 2, root lengths were similar across treatments, resulting in insignificant p-values (Fig. 7 and Table 2).

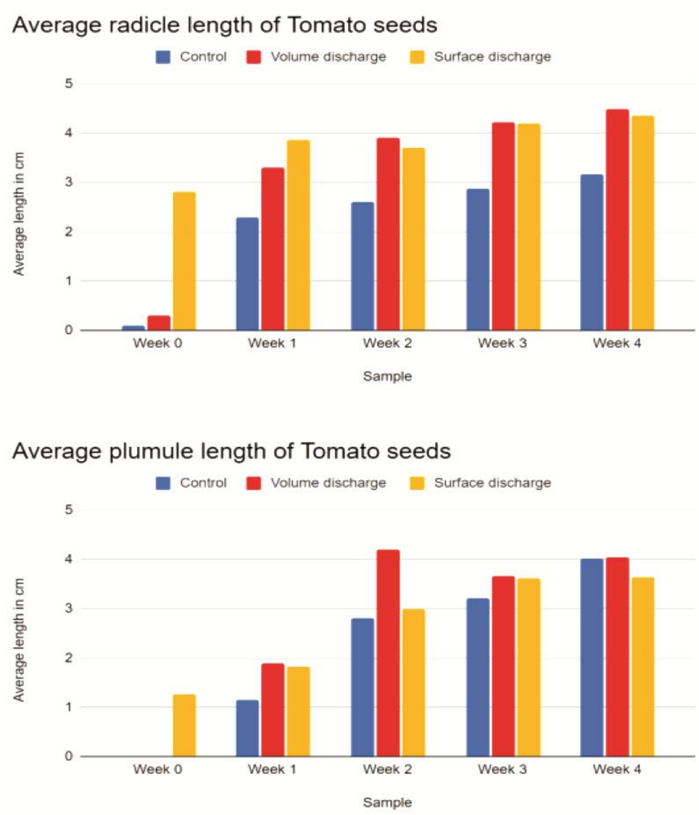

Fig. 7. Graph indicating root and shoot length over 4 weeks.

Table 2. Analysis of radicle lengths.

	Source	DF	SS	MS	F-Ratio	P-Value	Inference
Weak-1	Between Treatments	2	6.24	3.12	5.43	0.02	Significant
	Within Treatments	12	6.89	0.57			
Weak-2	Between Treatments	2	4.90	2.45	3.03	0.08	Insignificant
	Within Treatments	12	9.70	0.80			
Weak-3	Between Treatments	2	5.89	2.94	4.90	0.02	Significant
	Within Treatments	12	7.21	0.60			
Weak-4	Between Treatments	2	5.1613	2.5807	3.75	0.049	Significant
	Within Treatments	12	8.248	0.6837			

In case of shoot lengths, the p-values for Weeks 1 and 2 ($p < 0.05$) reveal significant differences, with surface and volume discharge treated seeds exhibiting longer shoots. In contrast, Weeks 3 and 4 showed insignificant differences, as shoot lengths were similar across all treatments (Table 3).

Table 3. Analysis of plumule lengths.

	Source	DF	SS	MS	F-Ratio	P-Value	Inference
Weak-1	Between Treatments	2	1.64	0.82	8.68	0.004	Significant
	Within Treatments	12	1.14	0.09	-	-	
Weak-2	Between Treatments	2	5.73	2.86	3.24	0.048	Significant
	Within Treatments	12	10.6	0.88	-	-	
Weak-3	Between Treatments	2	0.59	2.9	0.48	0.62	Insignificant
	Within Treatments	12	7.26	0.5	-	-	
Weak-4	Between Treatments	2	0.5	0.25	0.47	0.63	Insignificant
	Within Treatments	12	6.39	0.53	-	-	

In the case of ginger, each pot contained a single ginger rhizome covered loosely with cocopeat. To simulate natural conditions, the pots were watered and exposed to sunlight daily for a specified period. The number of germinated rhizomes was recorded weekly to track germination progress (Table 3 and Fig. 8).

This study examines the impact of plasma treatment on the germination and growth of tomato seeds and ginger rhizomes. Using a dielectric barrier discharge reactor, seeds were subjected to Surface (SDBD) and Volume Dielectric Barrier Discharge (VDBD) plasma treatments. Seeds were cultivated in germination papers and netted pots, and parameters such as germination percentage, root and shoot lengths were recorded daily and analyzed using ANOVA (Mekarun et al. 2022). The results indicated that plasma

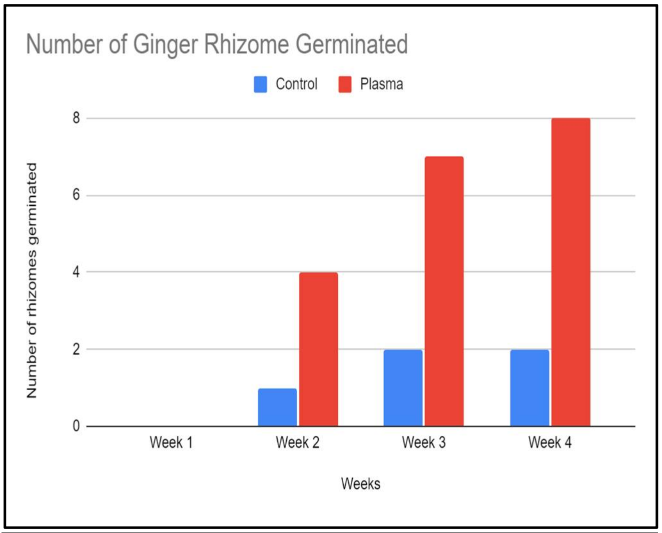

Fig. 8. Number of rhizomes grown over 4 weeks.

treatment effectively broke seed dormancy and enhanced early growth stages, with VDBD showing superior root and shoot development compared to SDBD (Mildaziene et al. 2022). These findings align with existing research, such as Jiang et al.'s work on plasma-treated wheat seeds, demonstrating improved germination and growth (Jiang et al. 2018). Overall, this study highlights plasma technology's potential to boost early plant development in tomato seeds and ginger rhizomes and suggests further biochemical and molecular analyses to understand its long-term effects as an alternative to chemical treatments (Grainge et al. 2022).

This study explored the impact of plasma treatment on tomato (*Solanum lycopersicum*) seeds and ginger (*Zingiber officinale*) rhizomes, two crucial crops in global agriculture. Tomato seeds were subjected to Surface DBD and Volume DBD treatments, whereas Ginger was subjected to Surface DBD using Dielectric Barrier Discharge (DBD) technology (Priatama et al. 2022). The research aimed to determine the most effective treatment method for enhancing seed germination and growth. Results showed that plasma treatment effectively reduced seed dormancy, prompting earlier root and shoot development compared to untreated seeds. Germination percentages were also significantly higher in plasma-treated seeds after four weeks. Among treatments, Volume DBD demonstrated superior outcomes over Surface DBD. These findings suggest that plasma treatment could serve as a viable alternative to chemical methods in agriculture (Waskow et al. 2021). In conclusion, plasma technology shows promise in improving early-stage growth parameters of seeds and rhizomes, with further research recommended to optimize treatment protocols for broader agricultural applications aimed at enhancing crop productivity and sustainability.

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