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COLD PLASMA: AN ECO-FRIENDLY SEED TREATMENT FOR MANAGING *HELMINTHOSPORIUM ORYZAE* AND ENHANCING SEED PERFORMANCE IN RICE

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ABSTRACT

This study assessed the impact of varying cold plasma dosages and exposure durations on seed borne *Helminthosporium oryzae* and rice seed quality on rice cultivar, BPT-5204. The experiment was conducted using a factorial CRD with two factors, seed health conditions (inoculated and healthy) and five plasma treatments (D_1: 0 kV/0 min; D_2: 15 kV/10 min; D_3: 15 kV/15 min; D_4: 20 kV/10 min; D_5: 20 kV/15 min). Results showed significant effects of plasma treatment on most seed quality parameters. Cold plasma notably reduced seed infection and total fungal colonies in pathogen inoculated seeds while improving germination up to 93.33%, seedling vigor and field emergence. It has also reduced seedling mortality and electrical conductivity. Among treatments, 20 kV for 10 minutes was found effective against pathogen control, whereas, 20 kV for 15 minutes further improved emergence but comparatively less effective. Overall, cold plasma proves to be an effective, eco-friendly seed treatment for managing *H. oryzae* and enhancing rice seed quality.
***Keywords*: Cold Plasma, Fungal infection, Seed health condition, *Helminthosporium oryzae*.**

Introduction

Agriculture is the backbone of many economies, particularly in developing countries where it remains the primary source of livelihood. Among agricultural commodities, rice (*Oryza sativa* and *Oryza glaberrima*) holds a pivotal position as a staple food for more than half of the global population, with Asia being the dominant consumer. In 2024–25, global rice production is projected to reach a record 535.8 million tonnes (milled), alongside consumption and ending stocks of 532.1 and 183.2 million tonnes, respectively. In India, rice production is estimated at 1364.37 lakh tonnes, while Rabi paddy sowing increased by 8.05% to 44.27 lakh hectares. Telangana has emerged as a key contributor, expanding its Rabi paddy area to 24.19 lakh hectares and achieving productivity of 6.94 t ha^{-1}, well above the national average of 3.08 t ha^{-1} (Anonymous, 2025).

Being cultivated under diverse agroecological and climatic conditions, rice is vulnerable to several biotic and abiotic stresses (Wilson and Talbot, 2009). Among seed-borne pathogens, *Bipolaris oryzae* (syn. *Helminthosporium oryzae*), *Fusarium spp.*, *Curvularia oryzae*, *Aspergillus spp.*, *Penicillium spp.*, and *Alternaria sp.* are frequently associated with discoloured or deteriorated rice seeds, impairing seed quality and germination (Ibiam *et al.*, 2008; Iwuagwu *et al.*, 2018). Of these, *Bipolaris oryzae* is one of the most destructive, causing brown spot disease that has been reported across India's major rice-growing regions (Arshad *et al.*, 2008; Surendhar et al., 2022). Infected seeds act as primary inoculum, producing necrotic lesions on coleoptiles and leaf sheaths, which later spread through airborne conidia, resulting in severe secondary infections (Sato *et al.*, 2008; Damicone *et al.*, 1992). Characteristic brown spot symptoms include reddish-brown to dark lesions with yellow halos on leaves and glumes, often leading to seedling mortality and a scorched field appearance (Dallagnol *et al*., 2009; Sunder *et al*., 2010). Yield losses ranging from 10% to 50% have been reported, substantially impacting farmer incomes and raising production costs through fungicide use and other management practices (India Stat, 2020-21).

In recent years, cold plasma technology, also known as non-thermal plasma, has emerged as a sustainable alternative for seed treatment and disease management. Plasma, the fourth state of matter, consists of ions, electrons, and neutral molecules generated under ambient conditions. Cold plasma has demonstrated antimicrobial activity against a broad spectrum of pathogens and has been applied successfully in seed sterilization, disease suppression, and soil disinfestation (Bourke *et al*., 2018; Sakudo *et al*., 2019). Studies further revealed its ability to improve seed germination, seedling vigour, and stress tolerance (De groot *et al*., 2018), while reducing the incidence and severity of plant diseases, which annually account for 20–30% crop losses worldwide (Oerke, 2006). Additionally, plasma-activated water has shown promise in controlling several plant pathogens (Aktar *et al*., 2021). Given its eco-friendly, residue-free and multi-functional potential, cold plasma technology represents a promising approach for managing *H. oryzae* in rice while simultaneously enhancing seed quality.

Materials and Methods

The experiment was conducted during 2024-25 at Department of Seed Science and Technology, Seed Research and Technology Centre, PJTAU. The study was laid out in a Factorial Completely Randomized Design (CRD) with two factors: (i) Seed health condition – SH1: Pathogen-inoculated seeds and SH2: Healthy seeds; and (ii) Cold plasma dosage and duration – D1: 0 kV for 0 min, D2: 15 kV for 10 min, D3: 15 kV for 15 min, D4: 20 kV for 10 min and D5: 20 kV for 15 min. Ten treatment combinations (T_1- pathogen inoculated seed without any exposure of cold plasma; T_2- pathogen inoculated seed exposed to 15 kV for 10 minutes; T_3- pathogen inoculated seed exposed to 15 kV for 15 minutes; T_4- pathogen inoculated seed exposed to 20 kV for 10 minutes; T_5- pathogen inoculated seed exposed to 20 kV for 15 minutes; T_6- healthy seeds without any exposure of cold plasma; T_7- healthy seed exposed to 15 kV for 10 minutes; T_8- healthy seed exposed to 15 kV for 15 minutes; T_9- healthy seed exposed to 20 kV for 10 minutes; T_{10}- healthy seed exposed to 20 kV for 15 minutes) were evaluated with three replications of each treatment. Seeds designated as pathogen-inoculated were artificially inoculated with *Helminthosporium oryzae* using a spore suspension technique. Actively growing cultures of the pathogen were prepared on Potato Dextrose Agar (PDA) and conidial suspensions of required concentration ($1x10^6$ spores/ml) prepared using haemocytometer. Rice seeds were soaked in the spore suspension, air-dried under aseptic conditions and used for treatments. Healthy and pathogen inoculated seeds were subjected to cold plasma treatment at different dosages and durations including control. Post treatment the seed health and seed quality parameters such as seed infection (%), total fungal colonies (No.), seed moisture content (%), seed germination (%), seedling abnormality (%), seedling mortality (%), seedling length (cm), seedling dry weight (mg), seedling vigour index I, seedling vigour index II, electrical conductivity and field emergence (%) were assessed.

Seed Infection (%)

Seed infection percentage of paddy seeds was evaluated using Potato Dextrose Agar (PDA) medium. Ten seeds were placed equidistantly on PDA in each Petri plate using sterilized forceps, with three replications per treatment. The plates were incubated at 25±2°C in BOD incubator for 7 days. Post-incubation examined under microscope to identify the fungal infection of *H. oryzae*.

Total Fungal Colonies (No.)

The total number of fungal colonies was determined on PDA medium under aseptic conditions. Seeds were placed on the medium and incubated in BOD incubator. On the 7th day, the number of seeds colonized by specific fungal species was recorded and the total fungal colonies were calculated using the formula:

Total fungal colonies (%) = (Number of seeds colonized in each plate by a particular species/ Total number of seeds in each plate) × 100

Seed Moisture Content (%)

Seed moisture content was determined following ISTA (2022) guidelines. For each treatment, 3 grams of finely ground paddy seeds were taken in triplicate and placed in aluminium containers. These were dried in a hot-air oven at 130°C for 2 hours, then cooled in desiccators to prevent moisture absorption. The moisture content was calculated using the formula:

$$\text{Moisture content (\%)} = ((W_2 - W_3) / (W_2 - W_1)) \times 100$$

Where:

- W_1 = Weight of the empty container with lid (g)

- W_2 = Weight of the container with lid and ground seed sample before drying (g)
- W_3 = Weight of the container with lid and ground seed sample after drying (g)

Seed Germination (%)

Germination tests were conducted as per ISTA (2022) using the paper towel method, with four replications of 100 seeds each. Seeds were placed on a moist paper towel, covered with another moist layer, and incubated in a germination chamber at 25±1°C, 95% relative humidity. On the 14th day, normal seedlings were counted, and germination percentage was calculated as:

Germination percentage (%) = (Number of normal seedlings / Total number of seeds planted) × 100

Seedling Abnormality (%)

Seedling abnormality was assessed using the formula:

Seedling abnormality (%) = (Number of abnormal seedlings / Total number of seeds evaluated) × 100

Seedling Mortality (%)

Seedling mortality was calculated as:

Seedling mortality (%) = (Number of dead seedlings / Total number of seeds evaluated) ×100

Seedling Length

Seedling length was evaluated using the rolled paper towel method. On the 14th day, ten normal seedlings were randomly selected, and their root and shoot lengths were measured from root tip to shoot tip using a measuring scale, expressed in centimetres (cm).

Seedling Dry Weight (mg)

Ten normal seedlings, selected for length measurement, were placed in butter paper bags and dried in a hot-air oven at 100°C for 24 hours. After cooling in a desiccator for 30 minutes, their dry weight was recorded in milligrams (mg).

Seedling Vigour Index (SVI-I and SVI-II)

Seedling vigour indices were calculated using the formulas by Abdul Baki and Anderson (1973):

SVI-I = Germination (%) × Seedling length (cm)

SVI-II = Germination (%) × Seedling dry weight (mg)

Electrical Conductivity (µS $cm^{-1}g^{-1}$)

Electrical conductivity was measured as per ISTA (2022) protocols. Twenty seeds were weighed to two decimal places and placed in 100 ml conical flasks with 100 ml distilled water. Flasks were sealed with aluminium foil and incubated at 20°C for 24 hours. The water was then transferred to another flask with agitation, and seeds were removed using a nylon sieve. The conductivity of deionized water (control) was measured, followed by the seed-soaked water. The final conductivity was calculated by subtracting the control value and expressed as microSiemens per centimetre per gram of seed.

Field Emergence (%)

Field emergence was tested using 100 seeds per treatment, with three replicates, sown in pots at a depth of 4–5 cm with adequate moisture. On the 14th day, the number of normal seedlings emerged above the soil was counted and the field emergence rate was calculated as:

Field emergence (%) = (Number of seedlings emerged on 14th day / Total number of seeds sown) × 100

Results and Discussion

The experimental results revealed significant differences among seed health conditions, different dosages and durations of cold plasma treatments for seed health and quality parameters in rice.

Seed Infection (%)

Seed infection percentage was significantly influenced by both seed health condition and cold plasma treatment. In pathogen-inoculated seeds, the maximum infection (100%) was recorded in T_1 (SH_1 - 0 kV for 0 min), the untreated control (Table 1). A progressive decrease in infection was observed with increasing voltage and exposure duration. The lowest infection among pathogen-inoculated seeds was in T_4 (SH_1 – 20 kV for 10 min) at 53%, followed by T_5 (56%) and T_3 (73%). In healthy seed treatments, infection levels were lower, ranging from 20% in T_6 (SH_2 – 0 kV for 0 min) and T_7 (SH_2 – 15 kV for 10 min) to a minimum of 10% in T_9 (SH_2 – 20 kV for 10 min) and T_{10} (SH_2 – 20 kV for 15 min) (Fig. 1).

The reduction in seed infection with higher voltage and longer exposure duration of cold plasma treatment can be attributed to plasma-induced disruption of fungal cell walls and membranes, inactivating *H. oryzae* spores. Cold plasma generates reactive oxygen and nitrogen species (RONS) and UV photons, which possess strong antimicrobial properties, degrading microbial proteins, lipids, and nucleic acids. The difference between pathogen-inoculated and healthy seeds supports the role of initial inoculum load in determining infection severity. The treatment at 20 kV for 10 minutes was effective in controlling pathogen load in both seed conditions. These findings align with reports by Jo *et al.* (2014) in rice and Rusu *et al.* (2018) in wheat, where non-thermal plasma treatments reduced seed-borne fungal infections.

Total Fungal Colonies (No)

The data on total fungal colonies showed a significant influence of seed health condition and cold plasma treatment. Among pathogen-inoculated seeds, the highest colony count (3.00) was recorded in T_1 (0 kV for 0 min), the untreated control. Cold plasma exposure reduced fungal colonies, with the lowest count (1.00) in T_4 (20 kV for 10 min), followed by T_2, T_3, and T_5, each recording 2.00 colonies (Table 1). In healthy seeds, a similar trend was observed, with the maximum colony count (3.00) in T_6 (0 kV for 0 min) and the minimum (1.00) in T_9 (20 kV for 10 min). Treatments T_8 and T_{10} recorded 1.33 colonies, while T_7 recorded 2.00 colonies. Statistical analysis confirmed that the interaction of seed health and cold plasma dosage/duration significantly reduced fungal colonies across both seed conditions.

The reduction in fungal colonies with increasing voltage and exposure duration is likely due to the antimicrobial effects of cold plasma, which generates reactive oxygen and nitrogen species that disrupt cell membranes, denature proteins, and damage nucleic acids of fungal pathogens. In pathogen-inoculated seeds, the highest fungal colonies were in the untreated control, significantly suppressed by cold plasma, especially at 20 kV for 10 minutes. In healthy seeds, the initial fungal load was lower, yet cold plasma further minimized residual colonies, demonstrating its potential for maintaining seed health. These results align with studies by Zahoranova *et al.* (2018) in maize and Jo *et al.* (2014) in rice, which showed that cold plasma inhibits pathogen growth and reduces fungal colonies. Sayahi *et al.* (2024) also reported that cold plasma effectively reduced infections caused by *Aspergillus flavus* and *Fusarium solani* in soybean seeds.

Seed Moisture Content (%)

The data indicated that seed moisture content was significantly influenced by seed health status, cold plasma dosage/duration, and their interaction. Pathogen-inoculated seeds had a higher mean moisture content (11.789%) compared to healthy seeds (11.500%) (Table 1). Among cold plasma treatments, the maximum seed moisture content (11.895%) was observed in seeds exposed to 0 kV for 0 min, while the lowest (11.580%) was recorded in seeds treated with 20 kV for 15 min. The highest moisture content (12.13%) was found in pathogen-inoculated seeds without plasma exposure (T_1), while the lowest (11.377%) was in healthy seeds treated at 20 kV for 15 min (T_{10}) (Fig. 2).

The reduction in seed moisture content with cold plasma treatment may be due to the physical and thermal effects of ionized gas, enhancing surface drying and marginally reducing seed moisture. Similar trends were reported by Los *et al*. (2020), where plasma exposure led to slight moisture loss compared to untreated controls. Higher moisture content in pathogen-inoculated seeds could result from fungal mycelium and associated metabolic activity influencing water retention. Optimal moisture reduction through plasma treatment is advantageous for storage, minimizing microbial growth and maintaining seed quality.

Seed Germination (%)

Seed germination percentage was significantly influenced by seed health condition, cold plasma treatment dosage and duration, and their interaction. The lowest germination percentage was recorded in T_1 (pathogen-inoculated seed without cold plasma) at 81.33%, while the highest was in T_9 (healthy seed exposed to 20 kV for 10 minutes) at 93.33%. In pathogen-inoculated seeds, germination improved with increasing plasma voltage and duration, reaching a maximum in T_4 (93.00%), followed by T_5 (90.66%) and T_3 (90.00%) (Table 2). Healthy seeds also showed improved germination with plasma treatment, though the increase was less pronounced compared to pathogen-inoculated seeds (Fig. 1).

Enhanced germination following cold plasma exposure is likely due to pathogen suppression and improved seed coat permeability. In pathogen-inoculated seeds, plasma treatment reduced surface-borne *H. oryzae* inoculum, decreasing infection and promoting germination. Similar findings were reported by Hasan *et al.* (2020) in rice and Zahoranova *et al.* (2018) in maize, where plasma treatment enhanced germination in pathogen-infected seeds due to microbial decontamination. Meng *et al.* (2017) also noted enhanced germination in wheat.

Seedling Abnormality (%)

The results exhibited a significant reduction in seedling abnormality percentage with increasing cold plasma dosage and duration, particularly in pathogen-inoculated treatments. The highest abnormality was recorded in T_1 (pathogen-inoculated seed without cold plasma) at 7.33%. A gradual decrease was observed with cold plasma treatment, with T_4 (20 kV for 10 minutes) showing the lowest value among pathogen-inoculated seeds at 3.66%. In healthy seeds, the highest abnormality (5.66%) was in T_7 (15 kV for 10 minutes), while the lowest (3.00%) was in T_9 (20 kV for 10 minutes) (Table 2). Interaction effects were significant, indicating that both seed health status and plasma dosage influenced the reduction in seedling abnormality (Fig. 2).

The decline in abnormal seedlings with increased cold plasma treatment may result from the plasma's ability to inactivate surface-borne pathogens and improve seed physiological status, reducing deformities during germination. Cold plasma generates reactive oxygen and nitrogen species that break down pathogen cell walls and enhance seed metabolic activity. This effect was more pronounced in pathogen-inoculated seeds, suggesting that cold plasma not only disinfects but also improves seedling morphology. These findings align with Dobrin *et al.* (2015), who reported that non-thermal plasma promotes uniform seedling growth by reducing abnormal germination.

Seedling Mortality (%)

The data on seedling mortality revealed significant differences across treatments. Among pathogen-inoculated seeds, the highest mortality was in T_1 (pathogen-inoculated without cold plasma) at 11.33%, followed by T_2 (15 kV for 10 minutes) at 8.33%. A significant reduction in mortality was observed with increasing voltage and exposure time, with the lowest mortality (3.33%) in T_4 (20 kV for 10 minutes) among pathogen-inoculated seeds. In healthy seeds, the lowest mortality was in T_9 (20 kV for 10 minutes) at 3.66%, while the highest was in T_7 (15 kV for 10 minutes) at 6.33% (Table 2). The interaction effect between seed health condition and cold plasma dosage/duration was significant (Fig. 2).

Higher voltage treatments (20 kV) reduced seedling mortality in both seed conditions, highlighting cold plasma's efficacy in reducing seed-borne pathogens and improving post-germination survival. The significant reduction at higher voltages is likely due to the inactivation of fungal propagules on the seed surface, preventing infection during germination and seedling establishment. These results align with Randeniya and de Groot (2015), who reported that cold plasma treatments reduce microbial contamination, enhancing seedling health.

Seedling Length (cm)

Seedling length varied significantly with seed health condition, cold plasma treatment, and their interaction. Among pathogen-inoculated seeds, the shortest seedlings were in T_1 (20.73 cm), while the maximum length was in T_4 (20 kV for 10 min) at 22.34 cm, followed by T_3 (22.13 cm). In healthy seeds, T_9 (20 kV for 10 min) produced the longest seedlings (22.52 cm), while the shortest was in T_6 (21.43 cm) (Table 3). Overall, higher cold plasma voltage and duration produced longer seedlings compared to untreated controls. T_9 (healthy seeds at 20 kV for 10 min) recorded the highest seedling length, while T_1 (pathogen-inoculated seed at 0 kV for 0 min) showed the minimum.

Improved seedling length with cold plasma treatment may be due to enhanced metabolic activity and improved seed coat permeability, facilitating better water uptake and enzyme activation during germination. Higher voltage levels (15–20 kV) likely promoted physiological and biochemical changes, leading to vigorous growth. Similar findings were reported by El Shaer *et al.* (2023) and Meng *et al.* (2017) in rice, where plasma-treated seeds exhibited increased seedling vigor and elongation. The lower seedling length in pathogen-inoculated untreated seeds is likely due to fungal infection impairing early growth, while plasma treatment reduced pathogen load, enabling better root and shoot elongation.

Seedling Dry Weight (mg)

Seedling dry weight was significantly influenced by seed health status, cold plasma treatment levels, and their interaction. Among pathogen-inoculated seeds, the lowest mean dry weight (10.56 mg) was recorded in T_1 (0 kV for 0 min) and T_2 (15 kV for 10 min), while the highest (10.90 mg) was in T_4 (20 kV for 10 min). In healthy seeds, dry weight ranged from 10.60 mg in T_6 (0 kV for 0 min) to a maximum of 10.96 mg in T_9 (20 kV for 10 min). T_9 exhibited the highest dry weight, followed by T_4, T_{10} and T_8 (Table 3).

Improved seedling dry weight with increased voltage and duration of cold plasma treatment may result from enhanced seed physiological performance and metabolic activity. Cold plasma likely contributed to better nutrient mobilization and reduced microbial load, improving early seedling growth. Similar findings were reported by Ling *et al.* (2014) in soybean and Dobrin *et al.* (2015) in wheat, where plasma treatment enhanced seedling dry weight through increased enzymatic activity and membrane integrity. Healthy seeds consistently recorded slightly higher dry weights than pathogen-inoculated seeds, highlighting the negative impact of seed-borne pathogens on biomass accumulation.

Seedling Vigour Index I

Seedling Vigour Index I (SVI-I) showed significant differences among treatments. SVI-I values ranged from 1685.76 to 2102.09. Among pathogen-inoculated seeds, the lowest vigour index was in T_1 (without cold plasma) at 1685.76, and the highest was in T_4 (20 kV for 10 min) at 2078.35, followed by T_3 (15 kV for 15 min) at 1991.34 (Table 3). In healthy seed treatments, the maximum SVI-I was in T_9 (20 kV for 10 min) at 2102.09, on par with T_4, while the lowest was in T_7 (15 kV for 10 min) at 1899.19 (Fig. 3).

Improved SVI-I with cold plasma exposure, particularly at 20 kV for 10 minutes, is likely due to enhanced physiological activity and metabolic efficiency. Plasma treatment likely facilitated the breakdown of dormancy factors, stimulated enzyme activity, and improved membrane integrity, promoting healthier seedling growth. In pathogen-inoculated seeds, the greater relative increase compared to the untreated control is due to inactivation of *H. oryzae*, which otherwise impairs seedling vigor. Prolonged exposure (20 kV for 15 min) showed a slight decline in SVI-I, possibly due to mild oxidative stress or desiccation effects. These findings align with Amnuaysin *et al.* (2018), who reported enhanced germination and vigour index in rice.

Seedling Vigour Index II

Seedling Vigour Index II (SVI-II) was significantly influenced by seed health condition, cold plasma treatment dosage and duration, and their interaction. The lowest SVI-II was in T_1 (pathogen-inoculated seed without exposure) at 859.4, and the highest was in T_9 (healthy seed at 20 kV for 10 min) at 1023.63. In pathogen-inoculated seeds, SVI-II increased with higher dosage and moderate duration, reaching a maximum in T_4 (1013.6), significantly higher than other pathogen-inoculated treatments. In healthy seeds, T_9 (20 kV for 10 min) also resulted in the maximum SVI-II, on par with T_8 (967.8) and T_{10} (988.33) (Table 4).

Marked improvement in SVI-II with cold plasma treatment, particularly at 20 kV for 10 min, is attributed to enhanced germination and seedling growth due to surface sterilization and physiological stimulation by reactive plasma species. In pathogen-inoculated seeds, plasma reduced infection load, improving metabolic activity and nutrient mobilization, supporting vigorous seedling development. Higher duration (15 min) with higher voltage showed no further benefits and slightly reduced vigour, possibly due to oxidative stress. In healthy seeds, the positive effect was more pronounced at moderate exposure. These results align with results of Amnuaysin *et al.* (2018) in rice and Singh *et al.* (2019) in sweet basil.

Electrical Conductivity (µS cm^{-1}g^{-1})

Electrical conductivity (EC) of seed leachates varied significantly due to seed health condition, cold plasma dosage, and their interaction. Among pathogen-inoculated seeds, the highest EC was in T_1 (180.38 µS cm^{-1}) without cold plasma, indicating maximum membrane leakage. Progressive reductions in EC were observed with increasing voltage and exposure duration, with T_5 (127.75 µS cm^{-1}) showing the lowest value among infected seeds. In healthy seeds, the lowest EC was in T_{10} (107.39 µS cm^{-1}) at 20 kV for 15 min, while the highest was in T_7 (136.74 µS cm^{-1}) at 15 kV for 10 min (Table 4). Overall, higher-intensity and longer-duration cold plasma treatments lowered EC values.

The reduction in EC with increasing voltage and exposure duration is likely due to enhanced membrane stability and reduced solute leakage, resulting from microbial inactivation and physiological strengthening of the seed coat. Pathogen-inoculated seeds showed higher EC values than healthy seeds, indicating membrane damage and solute leakage caused by fungal infection. Similar observations were reported by Sayahi *et al.* (2024) in wheat, where plasma-treated seeds had lower EC than controls.

Field Emergence (%)

Field emergence varied noticeably among treatments under different seed health conditions and cold plasma exposure durations. In pathogen-inoculated seeds, the lowest emergence was in T_1 (without cold plasma) at 79.33%. A progressive improvement was observed with increasing voltage and exposure time, with the highest value in T_5 (87.33%) at 20 kV for 15 minutes. In healthy seeds, the minimum emergence was in T_6 (84.67%), and the highest was in T_{10} (90.00%) at 20 kV for 15 minutes, the highest among all treatments (Table 4). Healthy seeds consistently showed higher emergence percentages than pathogen-inoculated seeds under the same treatments (Fig.1).

Improved field emergence in cold plasma-treated seeds, especially at higher voltages and longer durations, is attributed to reduced seed-borne pathogens and enhanced physiological activity. Pathogen-inoculated seeds showed lower emergence due to residual infection and seed damage. These findings are consistent with Filatova *et al.* (2011), who reported that cold plasma enhances seedling establishment by improving seed health and vigor. However, they contrast with Ivankov *et al*. (2021), who noted a slight reduction in emerged seedlings with cold plasma treatments.

Conclusion

The study revealed that seed health condition, dosage and durations of cold plasma treatment significantly influenced seed health and quality parameters in rice. cold plasma treatment effectively reduced seed infection and fungal colonies, improving seed germination, seedling growth and vigour. Higher voltage (20 kV) and exposure duration (10 minutes) was found to be most effective, particularly for pathogen-inoculated seeds. This treatment enhanced seed health by inactivating pathogens, stabilizing seed membranes and promoting better seedling emergence, demonstrating cold plasma's potential for improving seed quality and vigour.

Table 1: Effect of seed health condition, duration and dosage of cold plasma treatment on seed infection (%), total fungal colonies (No.) and seed moisture content (%).

Treatments	Treatment description	Seed infection (%)			Total fungal colonies (No.)			Seed moisture content (%)		
T_1	Pathogen inoculated seed exposed to 0 kV for 0 minutes	100			3			12.13		
T_2	Pathogen inoculated seed exposed to 15 kV for 10 minutes	80			2			11.717		
T_3	Pathogen inoculated seed exposed to 15 kV for 15 minutes	73			2			11.763		
T_4	Pathogen inoculated seed exposed to 20 kV for 10 minutes	53			1			11.55		
T_5	Pathogen inoculated seed exposed to 20 kV for 15 minutes	56			2			11.783		
T_6	Healthy seed exposed to 0 KV for 0 minutes	20			3			11.66		
T_7	Healthy seed exposed to 15 kV for 10 minutes	20			2			11.537		
T_8	Healthy seed exposed to 15 kV for 15 minutes	13.33			1.33			11.48		
T_9	Healthy seed exposed to 20 kV for 10 minutes	10			1			11.447		
T_{10}	Healthy seed exposed to 20 kV for 15 minutes	10			1.33			11.377		
	Factors	**A**	**B**	**AXB**	**A**	**B**	**AXB**	**A**	**B**	**AXB**
	CD@5%	2.781	4.398	6.219	0.197	0.311	0.44	0.067	0.106	0.15
	SE(m)	0.943	1.491	2.108	0.067	0.105	0.149	0.023	0.036	0.051
	CV %	8.492			13.832			0.755		

Note: A- Seed health condition; B – Duration of soaking.

Table 2: Effect of seed health condition, duration and dosage of cold plasma treatment on seed germination (%), seedling abnormality (%) and seedling mortality (%).

Treat-ments	Treatment description	Seedling length (cm)			Seedling dry weight (mg)			Seedling vigor index I		
T_1	Pathogen inoculated seed exposed to 0 kV for 0 minutes	20.73			10.56			1685.76		
T_2	Pathogen inoculated seed exposed to 15 kV for 10 minutes	21.78			10.56			1857.61		
T_3	Pathogen inoculated seed exposed to 15 kV for 15 minutes	22.13			10.73			1991.34		
T_4	Pathogen inoculated seed exposed to 20 kV for 10 minutes	22.34			10.9			2078.35		
T_5	Pathogen inoculated seed exposed to 20 kV for 15 minutes	21.71			10.6			1968.28		
T_6	Healthy seed exposed to 0 KV for 0 minutes	21.43			10.6			1915.35		
T_7	Healthy seed exposed to 15 kV for 10 minutes	21.58			10.7			1899.19		
T_8	Healthy seed exposed to 15 kV for 15 minutes	22.02			10.83			1967.47		
T_9	Healthy seed exposed to 20 kV for 10 minutes	22.52			10.96			2102.09		
T_{10}	Healthy seed exposed to 20 kV for 15 minutes	22.06			10.9			2000.19		
	Factors	**A**	**B**	**AXB**	**A**	**B**	**AXB**	**A**	**B**	**AXB**
	CD@5%	0.093	0.147	0.208	0.112	0.177	0.208	53.841	85.129	120.391
	SE(m)	0.204	0.323	0.457	0.038	0.06	0.085	18.251	28.857	0.085
	CV %	3.627			1.371			3.631		

Note: A- Seed health condition; B – Duration of soaking.

Table 3: Effect of seed health condition, duration and dosage of cold plasma treatment on seedling length (%), seedling dry weight (mg) and seedling vigor index I.

Treat-ments	Treatment description	Seedling length (cm)			Seedling dry weight (mg)			Seedling vigor index I		
T_1	Pathogen inoculated seed exposed to 0 kV for 0 minutes	20.73			10.56			1685.76		
T_2	Pathogen inoculated seed exposed to 15 kV for 10 minutes	21.78			10.56			1857.61		
T_3	Pathogen inoculated seed exposed to 15 kV for 15 minutes	22.13			10.73			1991.34		
T_4	Pathogen inoculated seed exposed to 20 kV for 10 minutes	22.34			10.9			2078.35		
T_5	Pathogen inoculated seed exposed to 20 kV for 15 minutes	21.71			10.6			1968.28		
T_6	Healthy seed exposed to 0 KV for 0 minutes	21.43			10.6			1915.35		
T_7	Healthy seed exposed to 15 kV for 10 minutes	21.58			10.7			1899.19		
T_8	Healthy seed exposed to 15 kV for 15 minutes	22.02			10.83			1967.47		
T_9	Healthy seed exposed to 20 kV for 10 minutes	22.52			10.96			2102.09		
T_{10}	Healthy seed exposed to 20 kV for 15 minutes	22.06			10.9			2000.19		
	Factors	**A**	**B**	**AXB**	**A**	**B**	**AXB**	**A**	**B**	**AXB**
	CD@5%	0.093	0.147	0.208	0.112	0.177	0.208	53.841	85.129	120.391
	SE(m)	0.204	0.323	0.457	0.038	0.06	0.085	18.251	28.857	0.085
	CV %	3.627			1.371			3.631		

Note: A- Seed health condition; B – Duration of soaking.

Table 4: Effect of seed health condition, duration and dosage of cold plasma treatment on seedling length (%), seedling dry weight (mg) and seedling vigor index I.

Treat-ments	Treatment description	Seed vigor index II			Electrical conductivity			Field emergence (%)		
T_1	Pathogen inoculated seed exposed to 0 kV for 0 minutes	859.4			180.37			79.33		
T_2	Pathogen inoculated seed exposed to 15 kV for 10 minutes	901.73			151.10			81.33		
T_3	Pathogen inoculated seed exposed to 15 kV for 15 minutes	965.96			138.254			85.33		
T_4	Pathogen inoculated seed exposed to 20 kV for 10 minutes	1013.6			135.33			85.66		
T_5	Pathogen inoculated seed exposed to 20 kV for 15 minutes	961			127.74			87.33		
T_6	Healthy seed exposed to 0 KV for 0 minutes	946.93			130.03			84.66		
T_7	Healthy seed exposed to 15 kV for 10 minutes	941.66			136.73			86.33		
T_8	Healthy seed exposed to 15 kV for 15 minutes	967.8			126.59			85.00		
T_9	Healthy seed exposed to 20 kV for 10 minutes	1023.63			128.28			87.00		
T_{10}	Healthy seed exposed to 20 kV for 15 minutes	988.33			107.39			90.00		
	Factors	**A**	**B**	**AXB**	**A**	**B**	**AXB**	**A**	**B**	**AXB**
	CD@5%	13.491	21.331	30.166	5.759	9.105	12.877	2.263	3.579	5.062
	SE(m)	4.573	7.231	10.226	1.9952	3.086	4.365	1.099	1.738	2.459
	CV %	1.851			5.552			3.488		

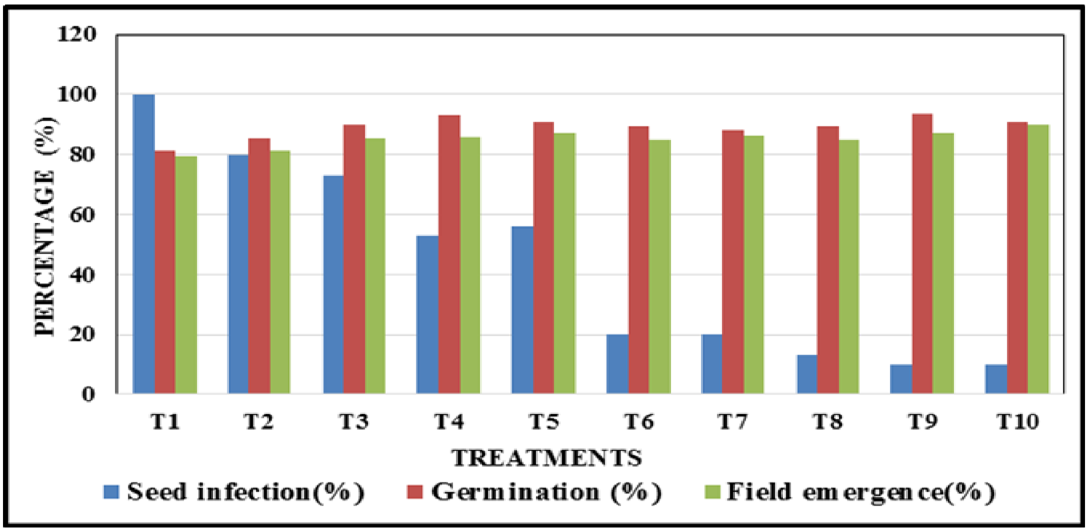

Fig.1: Graph representing seed infection (%), seed germination (%) and field emergence (%).

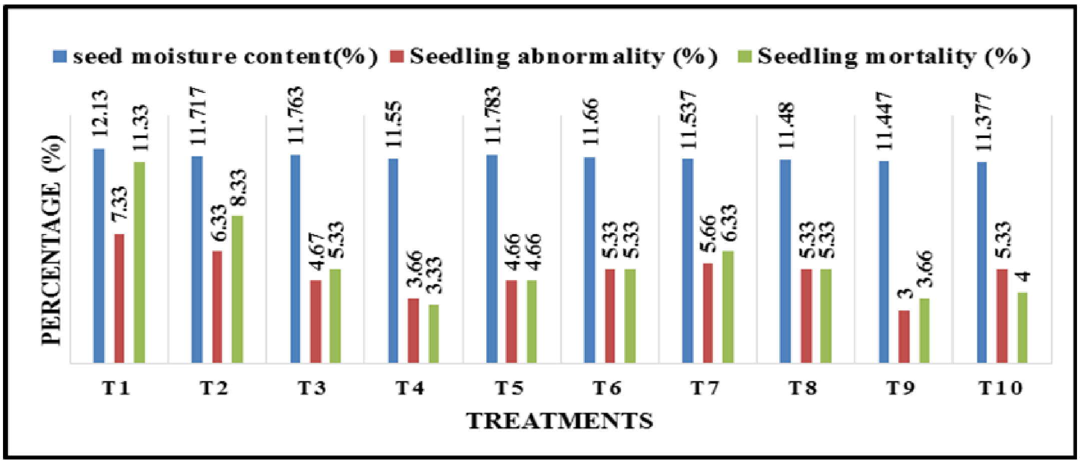

Fig. 2: Graph representing seed moisture content (%), seedling abnormality (%) and seedling mortality (%).

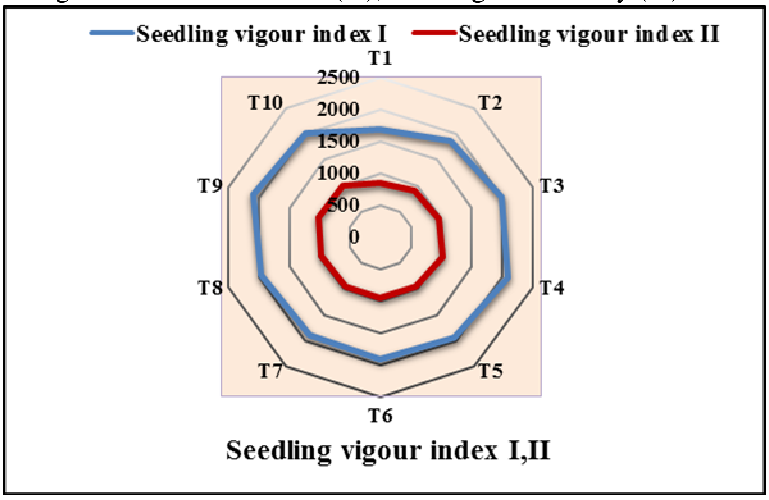

Fig. 3: Graph representing seedling vigour index I and seedling vigour index II.

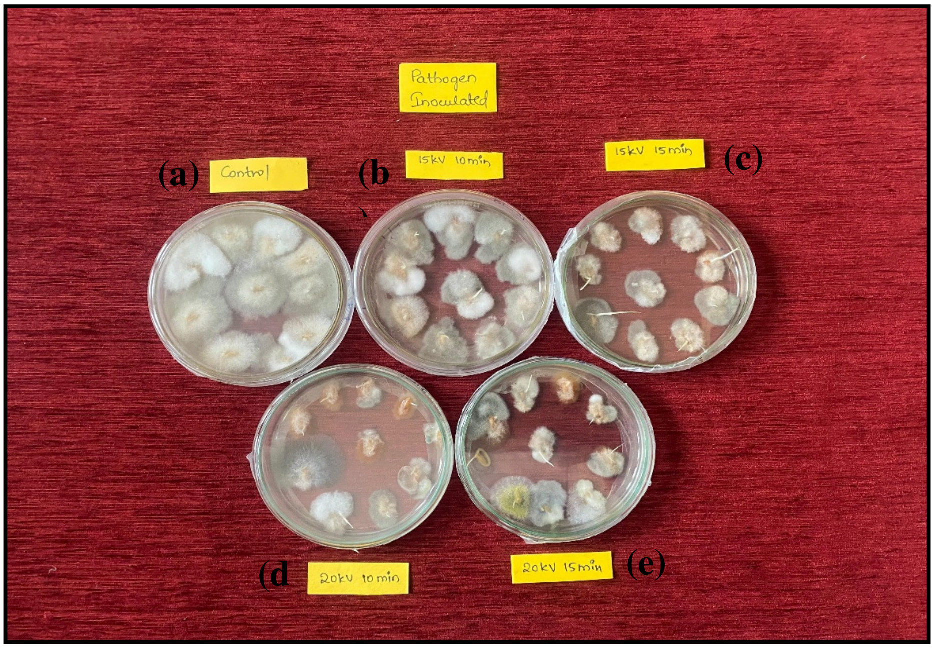

Fig. 4: Seed infection in pathogen inoculated seeds (a) 0 kV/0 min (b) 15 kV/10 min (c) 15 kV/15 min (d) 20 kV/10 min (e) 20 kV/15min

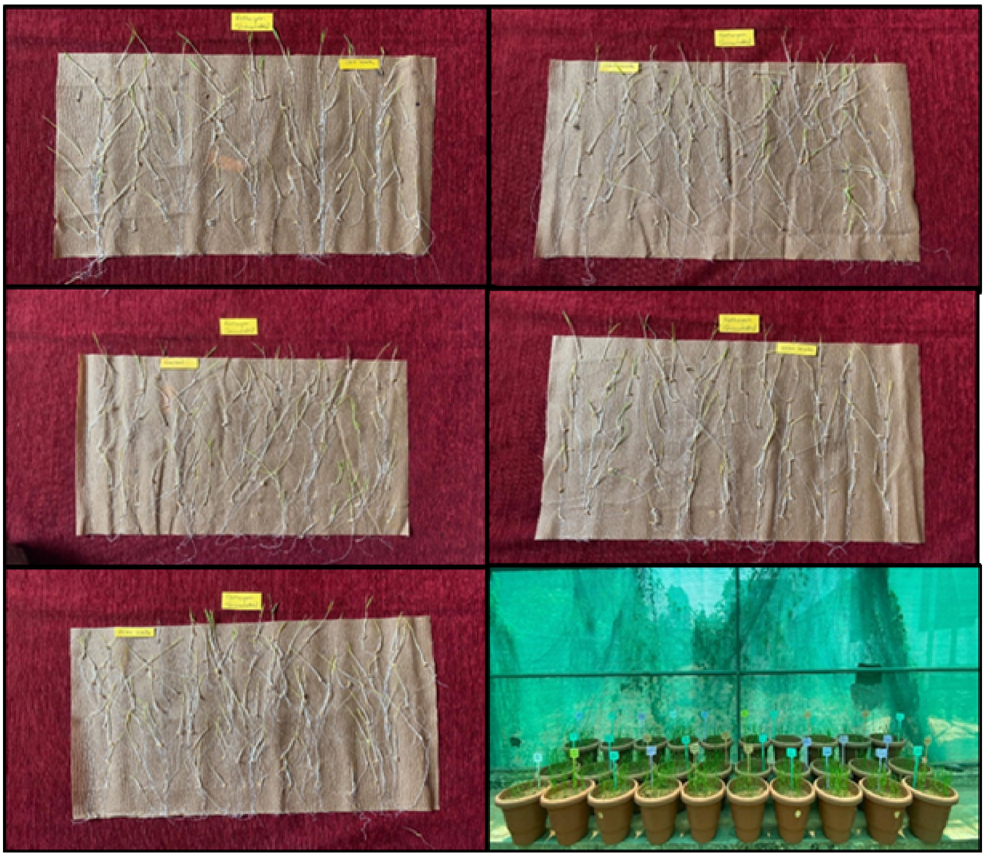

Fig. 5: Effect of cold plasma treatment on seed germination and field emergence

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Competing Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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