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COLD PLASMA: TRANSFORMING SOYBEAN SEED HEALTH PARAMETERS

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ABSTRACT

Cold plasma treatments have demonstrated significant effectiveness in reducing fungal pathogens on soybean seeds. Various treatment conditions, including 10 kV, 20 kV, and 25 kV at durations of 10, 15 and 20 minutes were compared with a control group to assess their impact on seed infection, mycoflora, fungal colonies and field emergence. The 10 kV treatment for 15 minutes proved most effective, reducing seed infection to 15.56%, which represents a 37.11% decrease compared to the control infection rate of 20.67%. This treatment also significantly reduced fungal colonies on soybean seeds, though *Macrophomina* sp. and *Fusarium* sp. were exceptions. Specifically, the 10 kV treatment was most effective against *Colletotrichum* sp. (0.89 colonies) and *Rhizopus* sp. (2.33 colonies), while the 25 kV treatment resulted in the fewest colonies for *Alternaria* sp. (0.89 colonies) and *Curvularia* sp. (0.22 colonies). *Aspergillus* sp. exhibited the lowest colony count with the 20 kV treatment. Despite these reductions, *Aspergillus flavus* had the highest fungal infection rates in both treated (5.48%) and control seeds (6.00%). Cold plasma was notably effective against *Aspergillus niger* but less so for *Curvularia* sp. Field emergence was also significantly improved with cold plasma treatments. The 10 kV for 15 minutes achieved the highest emergence rate of 85 per cent, which was 11.76 per cent higher than the control group's 77 per cent. This improvement in field emergence was attributed to enhanced water uptake, which promoted quicker and more uniform germination, leading to better seedling establishment. The antimicrobial effects of reactive oxygen and nitrogen species generated during cold plasma treatment disrupt pathogen cell membranes. This approach provides an optimal balance between pathogen control and seed viability. These results suggest that cold plasma treatment is a promising, eco-friendly alternative to chemical fungicides, enhancing both seed health and germination without the risk of harmful residues.

Key words : Cold plasma, Fungal infection, Soybean seed, Seed health, *Aspergillus*.

Introduction

Soybean [*Glycine max* (L.) Merrill] is one of the most important legumes and oilseed crops in the world. It is highly valued because it is adaptable, has unique chemical properties, and is rich in nutrients. It also has many uses in food, animal feed and other products. However, fungal contamination is a big problem for soybeans, as it affects seed health and germination rates, ultimately lowering crop yields. Although, traditional chemical fungicides are effective, they can cause environmental and health issues due to harmful residues, pollution, and the possibility of fungi developing resistance, which increases costs and makes the problem worse over time (Mravlje *et al.*, 2021).

To address these challenges, cold plasma treatment has emerged as a promising eco-friendly alternative. Cold plasma is a partially ionized gas rich in reactive species, and it offers a non-chemical method for seed disinfection that avoids harmful residues (Dhayal *et al.*, 2006). This technology disrupts the cell structures of fungi and other microorganisms, leading to their death and reducing the risk of resistance development (Hahn, 2014). Additionally, cold plasma treatment is gentle on seeds, preserving their viability and potentially enhancing plant health by inducing systemic resistance to pathogens (Jiang *et al*., 2014).

Previous studies have demonstrated the effectiveness of cold plasma treatment in inactivating fungal pathogens, such as *Aspergillus parasiticus* and *Penicillium* sp., on various seeds, including common beans (Runtzel *et al*., 2019), chickpeas, lentils and soybeans, without compromising the quality (Selcuk *et al*., 2008). The efficacy of plasma treatment in eliminating *Aspergillus* sp. and *Penicillium* sp. depends on factors such as the type of plasma gas, exposure duration, type and surface of the contaminated seeds. Other research reported significant reductions in microbial loads on seeds like chickpeas (Mitra *et al*., 2014).

Despite its potential, cold plasma treatment still faces a challenge due to the lack of standardized protocols in each crop which limits its effective application across different seed types. This study aimed to standardize cold plasma treatment for soybean seeds to improve seed health and field emergence by analyzing various seed health parameters by exposing seeds to various voltages and durations.

Materials and Methods

Freshly harvested soybean seeds of the JS-335 variety were sourced from the Agricultural Research Station in Adilabad, Telangana, for health assessment. The analysis was conducted jointly at the Department of Seed Science and Technology and the Central Instrumentation Cell (CIC) in Rajendranagar, Telangana. An experiment utilizing an open air multipin plasma reactor, designed by Ingenium Naturae Private Limited and housed at the MFPI-Quality Control Laboratory was employed. This setup featured a high voltage step-up transformer, an electronic control panel, and a plasma reactor with 88 pins arranged in an 11 × 8 grid, allowing adjustable spacing between the pin tips and the plane electrode. Operating at a peak voltage of 35 kV and power levels below 200 W, the reactor functioned without the need for expensive noble gases.

The experiment comprised 10 treatments designed in a Complete Randomized Design (CRD) with three replications. These treatments included nine cold plasma applications using different voltages (10, 20 and 25 kV) and durations (10, 15 and 20 minutes), alongside an untreated control group. Parameters evaluated included percent seed discoloration, seed infection (%), type of fungal flora, number of fungal colonies and field emergence (%).

Percent discoloration of seed

It was observed visually by inspecting a sample of seeds and categorizing them based on their colour difference. The percentage of discoloured seeds was then calculated by dividing the number of discoloured seeds by the total number of seeds in the sample and multiplying by 100.

Seed infection (%)

Seed infection (%) of soybean seeds was determined using the Blotter method in accordance with the International Rules for Seed Testing Association (ISTA, 2022). Seeds were placed on moist filter paper in Petri dishes and incubated at 25ºC with 12-hour light/dark cycles for seven days. After incubation, seeds were examined under a microscope for fungal infections.

$$\text{Seed infection (\%)} = \frac{\text{No. of seeds infected}}{\text{Total no. of seed in each plate}} \times 100$$

Number of fungal colonies and type of mycoflora

Number of fungal colonies was identified by first surface-sterilizing the seeds to remove external impurities and then subjecting them to the blotter method. After incubation, the fungal colonies grown from the seeds were counted. Then they were examined under a stereo binocular microscope at 10x and 40x magnification using water mount slides to observe the type of mycoflora present on the seeds.

$$\text{Total fungal colonies (\%)} = \frac{\text{No. of seeds colonized in each plate by particular species}}{\text{Total no. of seed in each plate}} \times 100$$

Field emergence (%)

Field emergence (%) was determined by sowing one hundred seeds from each treatment in four replications and evaluated on eighth day (ISTA, 2022).

$$\text{Field emergence (\%)} = \frac{\text{No. of seeds germinated on eight day}}{\text{Total no. of seeds sown}} \times 100$$

The above parameters were analyzed using SPSS software for the interpretation of results.

Fig. 1 : Field emergence of control seed and cold plasma treated seed.

Results and Discussion

Percent discoloration of seed

Cold plasma treatment did not significantly affect the seed discoloration percent. As cold plasma was a non-thermal, gentle treatment, its reactive species (such as ions, radicals and UV photons) were not potent enough to significantly alter the pigments or chemical structures responsible for seed discoloration. Since the exposure time to cold plasma was brief, it did not have sufficient interaction time to induce noticeable changes in seed appearance. These results aligned with the observations made by Jo *et al*. (2014) in wheat seeds. The absence of visual damage or discoloration on the seed coats shortly after treatment indicated that non-thermal plasma had effectively treated the seeds without compromising their colour.

Seed infection (%)

Cold plasma treatments resulted in a lower percentage of seed infection (16.52%) compared to the control (20.67%). The 10 kV treatment resulted in lower seed infection (15.56%) followed by 20 kV (16.67%) and 25 kV (17.33%). Among different durations, seeds treated for 15 minutes consistently showed lower infection rates, especially with 10 kV showing a 37.11 per cent reduction compared to the control. This demonstrated the effectiveness of cold plasma in reducing soybean seed infection, likely due to its antimicrobial properties. However, longer durations, such as 20 minutes, led to slightly increased infection rates at higher voltages, indicating that optimal exposure time varies with treatment voltage. Overall, increasing the duration from 10 to 15 minutes at 10 kV decreased infection rates, but further increasing the duration did not always yield better outcomes, suggesting that a combination of voltage and duration must be optimized for maximum efficacy.

Similarly, cold plasma treatments have been shown to reduce fungal infections in various crops, as reported by Jo *et al*. (2014) in rice, Rusu *et al*. (2018) in wheat, Selcuk *et al*. (2008) in wheat and beans and Ahmad *et al*. (2022) in pepper. Studies suggest that the radicals and excited molecules produced by plasma can erode microbial cell surfaces through etching, leading to their inactivation (Moisan *et al*., 2001).

Type of mycoflora (%)

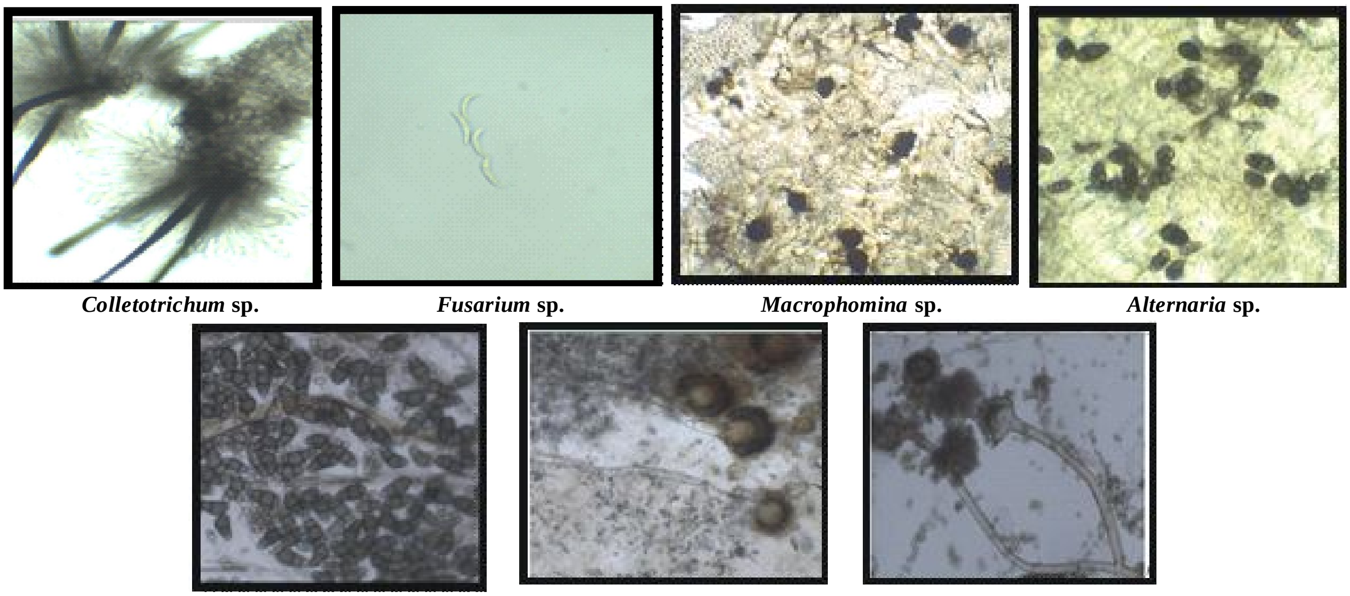
The study revealed the presence of eight fungal species across seven genera: *Colletotrichum* sp., *Fusarium* sp., *Macrophomina* sp., *Alternaria* sp., *Curvularia* sp., *Aspergillus flavus*, *Aspergillus niger* and *Rhizopus* sp., in both the treatment group and the control. Similar fungal genera were reported by Shovan *et al*. (2008) in soybean seed. The presence of both saprophytic and non-saprophytic fungi in the treatment and control groups highlights the diverse fungal community associated with soybean seeds. The saprophytic fungi, while beneficial for nutrient cycling, can also pose a risk if they produce mycotoxins, which can affect seed quality and safety. On the other hand, the non-saprophytic fungi are of particular concern due to their pathogenic nature, which can lead to significant crop losses.

Fig. 2 : Detection of Seed borne mycoflora by Standard Blotter method.

Number of fungal colonies

Cold plasma treatments significantly reduced fungal colonies on soybean seeds, except for *Macrophomina* sp. and *Fusarium* sp. The 10 kV treatment was most effective for *Colletotrichum* sp. and *Rhizopus* sp., while the 25 kV treatment resulted in the fewest colonies for *Alternaria* sp. and *Curvularia* sp., *Aspergillus* sp. had the lowest colony count with the 20 kV treatment. The highest fungal infection rate in both treated (5.48) and control seeds (6.00) was of *Aspergillus flavus*, while *Curvularia* sp. (0.63) and *Macrophomina* sp. (0.67) had the lowest frequencies. Plasma treatment was most effective against *Aspergillus niger* colonies, resulting in a 46 per cent reduction, demonstrating its effectiveness against this fungal species. However, it was less effective against *Curvularia* sp. colonies, with a reduction of only 20 per cent. This suggests that different fungi have varying susceptibilities to plasma treatments, and selecting the appropriate voltage is crucial for maximizing antimicrobial efficacy.

These results align with previous studies on wheat (Zahoranova *et al*., 2016), rice (Jo *et al*., 2014) and common beans (Runtzel *et al*., 2019), which demonstrated that cold plasma treatment inhibits pathogen growth and decreases fungal colonies. The plasma treatment works by generating reactive species that damage fungal cells, disrupt metabolic processes and oxidize cellular components, effectively sterilizing the seed surface and lowering fungal load. Sterilization of *Aspergillus flavus* and *Fusarium solani* after cold



***Colletotrichum* sp.** ***Fusarium* sp.** ***Macrophomina* sp.** ***Alternaria* sp.**

Curvularia* sp.** ***Aspergillus flavus ***Rhizopus* sp.**

Fig. 3 : Microphotographs of mycoflora on soybean.

Table 1 : Effect of cold plasma treatment on percent discoloration of seeds, seed infection % and field emergence %.

Treatments	Percent discoloration of seeds	Seed infection %	Field emergence %
10kV 10min	11.00	15.00^{de}	82^{b}
10kV 15min	12.33	13.00^{e}	85^{a}
10kV 20min	14.33	18.67^{ab}	78^{d}
20kV 10min	13.33	17.33^{bc}	80^{bc}
20kV 15min	13.00	15.67^{cd}	81^{b}
20kV 20min	14.00	17.00^{bcd}	78^{cd}
25kV 10min	13.67	18.00^{b}	80^{bc}
25kV 15min	13.00	18.33^{b}	82^{b}
25kV 20min	13.00	15.67^{cd}	82^{b}
Mean	**13.07**	**16.52**	**81**
Control	12.67	20.67^{a}	75^{e}
CD @ 5%	NS	2.101**	2.077**
SE(m)	0.624	0.707	0.699

Note: **- significant at 1%, *-significant at 5%, kV – kilo Volts Values with same alphabets indicate that treatments are on par with each other.

plasma treatment (180 S) was also reported by Sayahi *et al*. (2024). Cold plasma was not only effective on *Aspergillus*, but also worked against *Alternaria*, *Pencillium*, *Trichoderma* and *Fusarium* in onion (Kopacki *et al*., 2017) showcasing its potential for organic farming as a chemical free alternative.

Field emergence (%)

The cold plasma treatments significantly improved field emergence across nine treatments, with emergence percent ranging from 78 to 85 per cent. The most effective treatment was found to be 10 kV 15 min (85%) followed by 10 kV 10 min (82%), 25 kV 15 min (82%) and 25 kV 20 min (82%). The lowest field emergence was observed in control (75%). The 10 kV 15 min treatment led to an increase of 11.76 per cent over control. The increase in field germination percentage can be attributed to the plasma treatment's ability to improve seed coat permeability, enhance water uptake and activate enzymatic processes. These changes promote quicker and more uniform germination, resulting in better seedling establishment and higher emergence rates in field

Table 2 : Effect of cold plasma treatments on Type of mycoflora and No. of fungal colonies in Soybean.

Treatments	*Colletotrichum*	*Fusarium*	*Macrophomina*	*Alternaria*	*Curvularia*	*Rhizopus*	*Aspergillus flavus*	*Aspergillus niger*
10 kV	0.89	0.89	1.67	1.11	0.78	2.33	5.11	2.78
20 kV	1.56	1.00	1.67	2.11	0.89	3.11	4.78	1.56
25 kV	1.33	0.44	1.00	0.89	0.22	3.11	6.56	3.78
Mean	**1.26**	**0.78**	**1.44**	**1.37**	**0.63**	**2.85**	**5.48**	**2.70**
Control	1.67	1.00	0.67	1.00	1.00	4.33	6.00	5.00
CD @ 5%	0.879*	NS	NS	0.898*	0.879*	1.204*	0.967**	1.583**
SE (m)	0.266	0.404	0.236	0.397	0.096	0.416	0.561	0.478

conditions. These findings align with Filatova *et al.* (2011), who reported that plasma treatment improves the germination of stable and rapid seedlings in the field. However, they contrast with Ivankov *et al.* (2021), who observed that cold plasma treatments slightly reduced the percentage of emerged seedlings under field conditions.

Conclusion

Based on the standardization of cold plasma treatment for seed health parameters, it can be concluded that 10 kV treatment for 15 minutes significantly reduced seed infection and improved field emergence. Cold plasma treatments effectively reduced fungal colonies on soybean seeds, except for *Macrophomina* sp. and *Fusarium* sp. The 10 kV treatment was most effective against *Colletotrichum* sp. and *Rhizopus* sp., while the 25 kV treatment yielded the fewest colonies for *Alternaria* sp. and *Curvularia* sp., *Aspergillus* sp. had the lowest colony count with the 20 kV treatment. The highest fungal infection rate in both treated and control seeds were due to *Aspergillus flavus*, while *Curvularia* sp. and *Macrophomina* sp. had the lowest frequencies. Plasma treatment was most effective against *Aspergillus niger*, but was less effective against *Curvularia* sp. colonies. Therefore, the 10 kV for 15 minutes treatment was recommended as the optimal protocol for enhancing overall seed health and germination, while the 20 kV and 25 kV treatment can be utilized for targeted antifungal control. Managing fungal infection and contamination is crucial for seed production and storage. Therefore, further research is needed to understand how cold plasma treatment inactivates microbial cells, which is essential for maintaining seed quality and viability.

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