

# Management of blast of rice through decontamination of seeds with glow discharge plasma and plasma activated water

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## ABSTRACT

Glow discharge plasma and plasma activated water (PAW) were evaluated for seed treatment (for different time duration) in controlling seed borne *Pyricularia oryzae* causing blast disease of rice. Control (without treatment) and chemical treatment were also used in order to compare the treatments effects. Conidial suspension of *Pyricularia oryzae* treated with O<sub>2</sub>-air glow discharge plasma for 120 s and air-plasma activated water for 12 min successfully reduced the conidial germination ability of *Pyricularia oryzae* on Potato Sucrose Agar (PSA) medium. The plasma seed treatments used in this study showed parallel effect and some cases better effect than chemical seed treatment. The result is very much promising and positive for seed germination, field emergence (plant stand), and seedling health and in reduction of blast severity in the field. Among the plasmas used, O<sub>2</sub>-air glow discharge plasma for 90 s and PAW 9 min showed 14.96 and 20.21% increased plant stand over control treatment. The blast incidence and severity were significantly reduced in the plots which received seed treatment with O<sub>2</sub>-air glow discharge plasma for 120 s and air-plasma activated water (PAW) for 12 min. It can be concluded that the O<sub>2</sub>-air glow discharge plasma for 120 s and PAW for 12 min showed overall better performance with few exceptional cases.

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## Introduction

Rice (*Oryza sativa* L.) is the most important and extensively cultivated grain crop of Bangladesh. It is the second highest consumed cereal next to wheat in the world (Abodolereza and Racionzer, 2009). But the yield of rice is greatly hampered due to different diseases. In

Bangladesh, about 31 diseases are recorded to occur in rice including 10 major diseases (Miah et al., 1985). Major diseases are seedling blight, blast, brown spot, sheath blight, sheath rot, foot rot (bakanae), false smut, bacterial leaf blight (BLB), bacterial leaf streak, ufra and tungro. Most of the devastating diseases of rice are caused by seed borne pathogens. *Pyricularia oryzae* Cavara (anamorph *Magnaporthe grisea*), the causal agent

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of rice blast, is one of the most important pathogen of rice because of its widespread occurrence and destructive in nature (Ou, 1985). It has been reported that the systemic transmission of the fungus takes place from seed to seedling (Manandhar, 1996). This is very destructive pathogen causing significant yield loss of rice in many countries and responsible for 11-15% yield loss annually (Zambryski et al., 1997). Control of seed borne rice diseases in the seed bed is important to avoid epidemics in rice nurseries and paddy field and ensures to produce high-quality healthy nursery plants. Very recently blast of rice has become a serious problem in Bangladesh causing significant yield loss. The disease can be controlled by chemical means but injudicious use of chemical has many negative effects on environment as well as on human health. Now-a-days the demand for more effective and environment friendly technologies (that are not a risk for selecting anti-fungal resistant strains) is increasing. Successful control of *Pyricularia oryzae* infecting the seeds requires fungal inactivation that does not harm the plant or can increase the plant's resistance to infection. Novel therapies such as cold plasma treatments may be an alternative approach that can meet these requirements (Panngom et al., 2014). It is an emerging new technology, which is being currently used to effectively decontaminate the seeds and enhance seed germination and plant growth (Selcuk et al., 2008; Basaran et al., 2008; Jiang et al., 2014). Plasma is considered the fourth condition of matter other than solid, liquid and gas, in other word ionic gas. Use of plasma technology is gaining attention day by day due to its novel applicability. To our knowledge, no research has been done in our country using plasma seed treatment to control *Pyricularia oryzae* that causes blast of rice. The goal of this study was to investigate the potential of atmospheric-pressure glow discharge plasma and plasma activated water to inactivate seed-borne *Pyricularia oryzae*, which causes rice blast, as well as the impact of these treatments on blast occurrence and severity in the field.

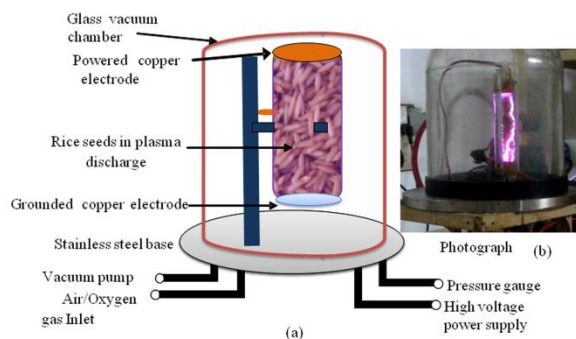
## Materials and Methods

The experiment was conducted at the Plant Pathology Laboratory and Agronomy Experimental field, Department of Agronomy and Agricultural Extension and Plasma Science and

Technology Laboratory, Department of Electrical and Electronic Engineering, University of Rajshahi. Rice variety BRRI dhan 28 and six treatments-  $T_1$  = Seed treatment with  $O_2$ -air glow discharge plasma for 90 seconds,  $T_2$  = Seed treatment with  $O_2$ -air glow discharge plasma for 120 seconds,  $T_3$  = Seed treatment with air-plasma activated water for 9 minutes,  $T_4$  = Seed treatment with air-plasma activated water for 12 minutes,  $T_5$  = Fungicidal seed treatment (Tricyclazole 75 WP @ 0.2% solution) and  $T_6$  = Control were used in this study. The experiment was laid out following Randomized Complete Block Design (RCBD) with tree replications.

### Plasma device setup and seed treatment

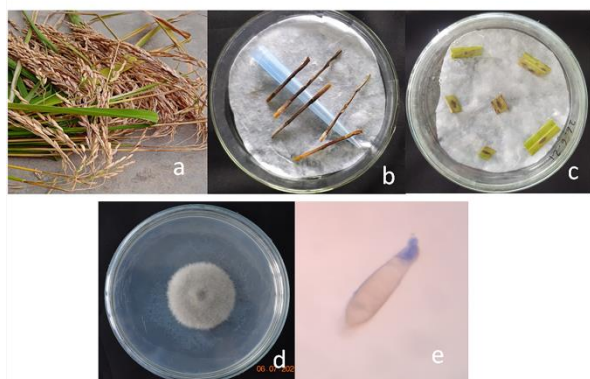
Atmospheric pressures  $O_2$ -air glow discharge plasma and air-plasma activated water (PAW) were prepared in the Plasma Science and Technology Lab, Department of Electrical and Electronic Engineering, Rajshahi University for rice seed treatment. The schematic of the experimental setup (Roy et al., 2017) along with pictorial presentation of rice seed treatment arrangement with  $O_2$ -air plasmas are shown in Fig. 1a and 1b. PAW was prepared with underwater air discharge plasma jet. Flow of air into the jet was controlled with a gas flow controller (KIT 115P)



**Fig. 1.** (a) Schematic diagram of the experimental setup used for rice seed treatment with glow oxygen -air discharge plasmas, (b) photograph of the seed treatment reactor under treatment of seed.

A 1-10kV, 1-10kHz bipolar variable power supply was used for operating the plasma jet. The voltage-current ( $V-I$ ) characteristics were measure with a high voltage probe (HVP-08) and current probe (CP-07C) in combination with a 4-channel digital storage oscilloscope (RIGOL DS1104). The optical emission spectrum (OES) of

O<sub>2</sub>-air discharge plasma were acquired using a spectrophotometer (Ocean Optics USB2000 +XR1: detector wavelength range 180–1100 nm, slit width 25 µm, grating 500 lines/mm, optical resolution 1.7 nm) for the identifications of plasma species produced in O<sub>2</sub>-air discharge. Air was flown through the glass tube into the water treatment reactor at a flow rate of 1 L.min<sup>-1</sup> for 9 min and 12 min. At a time 250 mL of distilled water was used for plasma treatment under different duration (9 and 12 minutes). 1000-seeds of were taken in a 500 mL beaker and according to the treatment specification of plasma activated water (PAW) was added on it and allowed to immerse for 10 minutes in PAW. The PAW treated rice seeds were dried under laminar air hood at room temperature (300C). Likewise, rice seeds were treated with Tricyclazole 75 WP @ 0.2% solution. Isolation of *Pyricularia oryzae* from naturally infected plant parts and inoculum preparation



**Fig. 2.** Induction of sporulation of *Pyricularia* species, a-c) infected panicle and leaves tissues d) pure culture of *Pyricularia oryzae*, photograph was taken by camera model Nikon d3400 and e) conidium of *Pyricularia oryzae*, microscopic image was taken by Olympus BH2 (40X).

Diseased tissue samples (panicle and leaves) of BRRI dhan 28 were collected from farmer's field of Naogaon and Joypurhat districts, Bangladesh. The samples were tested for the isolation of *P. oryzae* following the method Jia (2009). Diseased leaves and nodes were placed on wet filter papers (Fig. 2a-c) in a tissue culture container (12"× 8") to encourage sporulation. The container was covered with lid and placed on a culture rack at 25-28oC. Regular observation was made on whether the fungus grown on the plant tissues was isolated. The isolated fungi were

recultured on Potato Sucrose Agar (PSA) medium in order to get pure culture (Fig. 2d). The fungus was identified based on their color, spore morphology and mycelia following standard mycological books and manuals (Barnett and Hunter, 1972; Singh, 1982; Raper and Fennell, 1965) (Fig. 2e). Conidia of *Pyricularia oryzae* were collected by suspending the mycelia mate in sterile distilled water (SDW). The suspension was filtered through a sterile cheese cloth to remove the hyphae or clumps. Appropriate dilutions of the suspension were performed in order to get a concentration of 1.0×10<sup>5</sup> spores (cfu) mL<sup>-1</sup> by microscopic counting with a cell-counting haemocytometer (Neubauer chamber; Merck S.A., Madrid, Spain).

### Plasma treatment on spore germination of *Pyricularia oryzae*

Spores were treated with different plasmas as per treatment specification. Tricyclazole 75 WP was added to an autoclaved PSA medium at a concentration of 0.2% (Borum and Sinclair, 1968). The plate method was used for spore viability and count. PSA plates were inoculated with three microlitres of treated and untreated (control) spore suspension (1×10<sup>4</sup> cfu mL<sup>-1</sup>) of *P. oryzae* produced from stock solution (1×10<sup>5</sup> cfu mL<sup>-1</sup>). Observation was started after 2 days of incubation, and the number of spores that germinated on PSA was counted (Fig. 3).



**Fig. 3.** Photograph showing the developing individual colony of *Pyricularia oryzae* on PSA medium.

### Effect of plasma treatment of seed on germination

The seeds were placed onto the Petri dishes lined with 2-layers moisten blotter paper to allow the germination of seeds. For germination test, 400 rice seeds of each treatment groups were used. Twenty five seeds were placed onto the each Petri dish separately following ISTA (1996). Petri dishes were incubated at  $28 \pm 1^\circ\text{C}$  for 7 days in alternating cycles of 12 hours darkness and 12 hours light. The observation was started after 3 days of incubation and continued up to 7 days. The germination percentage was estimated by the following formula:

$$\% \text{ Germination} = \frac{\text{Number of seeds produced normal seedlings}}{\text{Number of seeds set for germination}} \times 100$$

### Field experiment

Treated seeds were soaked in water for 24 hours. Excess water was drained out and the seeds were kept covered with gunny bags for 48 hours. The seeds were germinated and sown in the plots of dry nursery bed. The field experiment was laid out following randomized complete block design RCBD with three replications. Based on treatment specifications, 18 plots of 1 m<sup>2</sup> were prepared including control treatment for raising seedlings. After spreading the sprouted seeds onto the dry seedbed, seeds were covered with friable soil and necessary amount of water was added to keep the adequate moisture to soil. A piece of polythene sheet was used to cover the seedbed. Adequate water was added and the land was ploughed and cross ploughed three times with the help of a power tiller followed by laddering to obtain a desirable puddle condition. Necessary fertilizers were applied as per recommendation guide of BIRRI (2016). Thirty five days old seedlings were transplanted onto the plots according to the experimental design. Experimental plots were irrigated as and when necessary.

### Effect of plasma seed treatment on plant stand and seedling health of BIRRI dhan 28 at the nursery bed

During uprooting of seedlings data on total number of established seedlings, normal and abnormal seedlings and number of blighted seedlings were recorded at nursery bed.

The plant stand (%) at nursery bed was recorded by the following formula:

$$\text{Plant stand (\%)} = \frac{\text{Number of seedlings}}{\text{Number of total seeds sown}} \times 100$$

### Determination of disease incidence

The incidence of blast was recorded at 45, 60 and 75 DAT in the field of the experimental plots and it was estimated by the following formula (Teng and James, 2002):

$$\text{Incidence (\%)} = \frac{\text{Number of infected seedlings/plant}}{\text{Total number of seedlings/plant}} \times 100$$

### Determination of disease severity index

The severity of blast in the experimental plots was recorded by regular inspection of the plots at 15 days interval viz. 45, 60 and 75 DAT. For scoring of the disease, standard disease rating scale (0-9) (Table 1) of IRRI (2002) was used. Percentage of disease severity index was estimated using the following formula (Mckinney, 1923):

$$\text{Disease severity index (DSI)} = \frac{\text{Total rating}}{\text{Observation} \times \text{maximum grade}} \times 100$$

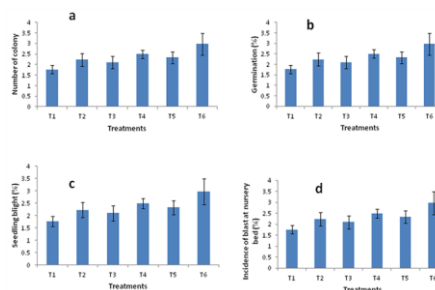
### Statistical analysis

The recorded data for various parameters was collected and compiled in the appropriate format for statistical analysis. Duncan's Multiple Range Test (DMRT) was used to compare the mean differences, and the analysis of variance was performed using the computer program SPSS (version 22).

## Results and Discussion

### Effect of plasma irradiation on the suppression of germination ability of *Pyricularia oryzae*

Inhibition of germination ability of the plasma irradiated conidia of *P. oryzae* was tested and found to be significantly varied under different treatments (Fig. 4a).



**Fig.4.** (a) Effect of plasma irradiation on the suppression of conidial germination of *Pyricularia oryzae* on PSA medium, (b) Effect of plasma irradiation



on seed germination of BRRI dhan 28, (c) Effect of plasma irradiation on seedling blight of BRRI dhan 28 at nursery bed, (d) Effect of plasma irradiation on blast incidence of BRRI dhan 28 at nursery bed. T<sub>1</sub>= O<sub>2</sub>-air glow discharge plasma for 90s, T<sub>2</sub>= O<sub>2</sub>-air glow discharge plasma for 120s, T<sub>3</sub>= Air-plasma activated water for 9min, T<sub>4</sub>= Air-plasma activated water for 12min, T<sub>5</sub>= Chemical (Tricyclazole 75 WP @ 0.2% solution) and T<sub>6</sub>= Control (without treatment). Capped bar indicate standard errors of three replications.

The conidial suspension of *P. oryzae* (1×10<sup>4</sup> cfu mL<sup>-1</sup>) treated with air-PAW plasmas for 9 and 12 min showed the lowest colony formation (7 and 4) on PSA. Next to this, O<sub>2</sub>-air glow discharge plasmas for 90 and 120 s exhibited 11 and 10.2 number of *P. oryzae* colony compared to control treatment (23.67). Statistically similar result was recorded by chemical treatment (12). Suhem et al. (2013) and Jo et al. (2014) discovered that an atmospheric non-thermal plasma treatment stopped *Aspergillus flavus* and *Gibberella fujikuroi* from growing on agar media. Cold atmospheric plasma-generated reactive nitrogen and oxygen species (NO, OH, superoxide), as well as strong oxidizing elements (H<sub>2</sub>O<sub>2</sub>, O<sub>3</sub>), may enter into the microorganisms, oxidize the cytoplasmic membrane, alter cellular systems, and inactivate them (Klampfl et al., 2012). From these reports it can be assumed that plasma treatment can inactivate the spore of *P. oryzae* and causes germination failure on PSA medium.

### Effect of plasma treatment on seed germination

The plasma irradiation of seeds showed significant result on seed germination. From the Fig. 4b, it can be observed that seed treatment with glow discharge O<sub>2</sub>-air plasma for 90 and 120 s and chemical yielded the highest germination

(90.67, 88 and 92.0%) followed by air-PAW for 9 and 12 min, while the lowest germination was recorded from control treatment (80%). Plasma treatment has been demonstrated to promote seed germination and plant growth, which is consistent with the findings of Roy et al. (2017). Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are present in low-temperature atmospheric glow discharge plasma and plasma activated water, which may lead to a considerable germination boost when compared to the control group (Kumar et al., 2021). Jiang et al. (2014) found that cold plasma greatly increased wheat seed germination potential and germination efficiency.

### Effect of plasma seed treatment on plant stand and seedling health of BRRI dhan 28 at nursery bed

Plant stand on seed bed did not vary significantly due to seed treatment with different plasmas although it was numerically varied from 80.0 to 96.17 (Table 2). From the Table it is revealed that the highest plant stand was provided by air-PAW for 9 min which indicated 17.28% increased plant stand over control. Conversely, the lowest plant stand (80.0%) was obtained from T<sub>0</sub> (control). The percentage of normal seedlings in the seed bed was not significantly varied under different treatments even though it was ranged from 77.46 to 94.93, where the highest percentage of normal seedlings was produced by pre-treatment of seeds with air-PAW for 9 min. After then, O<sub>2</sub>-air plasma for 90 s recorded 90.70% normal seedlings, while the control treatment showed only 77.46 % normal seedlings on the nursery bed. On the other hand,

**Table 1.** Rating scale (0-9scale) of blast of rice (IRRI, 2002)

Scale	Description
0	No lesion observed
1	Small brown specks of pin point size
2	Small roundish to slightly elongated, necrotic gray spots, about 1-2 mm in diameter with a distinct brown margin. Lesions are mostly found on the lower leaves
3	Lesion type same as in 2 but significant number of lesions on the upper leaves
4	Typical susceptible blast lesions, 3 mm or longer infecting less than 4% of leaf area
5	Typical susceptible blast lesions, 3 mm or longer infecting 4-10% of the leaf area
6	Typical susceptible blast lesions, 3 mm or longer infecting 11-25% of the leaf area
7	Typical susceptible blast lesions, 3 mm or longer infecting 26-50% of the leaf area
8	Typical susceptible blast lesions, 3 mm or longer infecting 51-75% of the leaf area many leaves are dead
9	Typical susceptible blast lesions, 3 mm or longer infecting more than 75% leaf area affected

the percentage of abnormal seedlings varied significantly between the treated and non-treated

seeds. The lowest value for abnormal seedlings (1.27 and 1.23%) was recorded by the treatments of seeds with O<sub>2</sub>-air plasma for 90 s and air-PAW for 9 min respectively, which was followed by O<sub>2</sub>-air plasma (1.67%) for 120 s. Conversely, the highest abnormal seedlings (2.53%) were counted from the plots where untreated seeds were sown. The chemical seed treatment also resulted in

1.97% abnormal seedlings. According to the findings, plasma treatments had an antifungal impact on the pathogen, resulting in the formation of more normal seedlings and fewer abnormal seedlings. This finding is consistent with Gomez-Ramirez et al. (2017), who studied Quinoa seedlings that had been exposed to air plasma treatments at atmospheric and low pressure and discovered good number of healthy seedlings.

**Table 2.** Effect of plasma seed treatment on plant stand, normal and abnormal seedlings of BRR1 dhan 28 at nursery bed

Treatment	Plant stand (%)	Plant stand increased (+) or decrease (-) over control (%)	Normal seedlings (%)	Abnormal seedlings (%)
T <sub>1</sub>	91.97b±1.90	14.96	90.70±2.10	1.27c±0.12
T <sub>2</sub>	88.33b±2.88	10.41	86.73±2.83	1.60bc±0.20
T <sub>3</sub>	96.17a±1.92	20.46	94.93±1.97	1.23c±0.12
T <sub>4</sub>	81.97b±10.78	2.46	80.23±10.81	1.73b±0.06
T <sub>5</sub>	82.24b±12.91	2.6	80.27±13.07	1.97b±0.14
T <sub>0</sub>	80.0b±5.72	-	77.46±5.79	2.53a±0.14
Level of significance	*		NS	***

T<sub>1</sub>= O<sub>2</sub>-air glow discharge plasma for 90s, T<sub>2</sub>= O<sub>2</sub>-air glow discharge plasma for 120s, T<sub>3</sub>= Air-plasma activated water for 9min, T<sub>4</sub>= Air-plasma activated water for 12min, T<sub>5</sub>= Chemical and T<sub>6</sub>= Control. In column, according to DMRT, figures with similar letter (s) are identical, whereas those with dissimilar letter(s) differ significantly. The symbol ± represents the standard errors of three replications. \*\*\*=Significant at 0.1% level of probability and \*= Significant at 5% level of probability. NS= Non-significant.

Seedling blight was found to differ significantly under different treatment application at nursery bed (Fig. 4c). The lowest percentage of blighted seedlings (1.7%) was observed in the plots which received pre-treatment of seeds with O<sub>2</sub>-air plasma for 90 s followed by rest of the treatments. Nevertheless, the highest percentage of blighted seedlings was recorded from control treatment (2.97%). Plasma treatment may have antimicrobial effect which leads to the production of healthy seedlings. On the other hand, non-treated seeds contain more inoculums load on it and thereby produced more number of infected and blighted seedlings. According to Klampfl et al. (2012), CAP (cold atmospheric plasma) derived reactive oxygen and nitrogen species (NO, OH, superoxide) or strong oxidizing agents (H<sub>2</sub>O<sub>2</sub>, O<sub>3</sub>) may penetrate into microorganisms and alter cell processes, thereby inactivating them, which could lead to phytopathogen decontamination of seed surfaces. As a result, plasma-treated seeds generated healthier, disease-free seedlings in the nursery bed than control seeds.

Blast incidence on seedlings at nursery bed (35 DAS) significantly varied under different treatment application (Fig. 4d). The lowest percentage of seedling with blast symptom (2.60%) was observed in the nursery bed where chemically treated seeds were sown and the result is statistically similar with O<sub>2</sub>-air plasma for 90 s (3.70%) and 120 s (3.60%), and air-PAW for 9 min (3.80%) and 12 min (3.06%), respectively. Nonetheless, the control treatment showed the highest number of seedlings with blast symptoms (5.77%). The result is also supported by Kang et al. (2015). They found that ozone and arc discharge plasma could be used to sterilize seeds, and that plasma-derived ozone inactivated *Fusarium fujikuroi* (the fungus that causes rice bakanae disease) spores more effectively when submerged in water. In comparison to the control group, plasma treated seeds produced healthier and disease-free seedlings in the nursery bed, and disease occurrences were found to be lower.

### Effect of plasma seed treatment on the incidence of blast of BRRI dhan 28 under field condition

Blast incidence in the field under treated and untreated group at 45, 60 and 75 DAT varied significantly. The disease was found to confine within lower limit in those plots which received plasma treatment (Table 3). The plants with the lowest disease incidence at 60 and 75 DAT were given O<sub>2</sub>-air for 120 seconds plasma (16.66%), while control plots had the highest disease incidence (30.0 and 36.55 %). Next to this, the plasma produced from Air-PAWs for 12 min showed good effect in reducing (10.0, 22.33 and 25.0%) the disease incidence at all sampling dates.

**Table 3.** Effect of plasma seed treatment on the incidence of blast of BRRI dhan 28 under field condition

Treatment	Diseases Incidence of blast at different days		
	45DAT	60 DAT	75 DAT
T <sub>1</sub>	10.0 c± 0.57	20.66 cd± 1.76	24.0 ±1.15
T <sub>2</sub>	16.00 b±1.15	16.66 d±1.15	16.66 c± 0.86
T <sub>3</sub>	16.66 b± 0.57	26.66 ab±1.73	27.33 b± 0.38
T <sub>4</sub>	10.0 c± 1.15	22.3300bc ± 0.60	25.0 b± 1.15
T <sub>5</sub>	15.33 b± 1.15	23.33 bc± 1.73	26.33 b± 0.76
T <sub>0</sub>	23.33 a± 1.73	30.0 a± 1.15	36.55 a± 2.02
Level of significance	***	***	***

T<sub>1</sub>= O<sub>2</sub>-air glow discharge plasma for 90s, T<sub>2</sub>= O<sub>2</sub>-air glow discharge plasma for 120s, T<sub>3</sub>= Air-plasma activated water for 9min, T<sub>4</sub>= Air-plasma activated water for 12min, T<sub>5</sub>= Chemical and T<sub>6</sub>= Control. In column, according to DMRT, figures with similar letter (s) are identical, whereas those with dissimilar letter(s) differ significantly. The symbol ± represents the

standard errors of three replications. \*\*\*=Significant at 0.1% level of probability.

In comparison to the control group, chemical seed treatment had a reasonable effect on limiting illness (15.33, 23.33 and 26.33%). According to the data, the use of O<sub>2</sub>-air plasma for 120 s reduced the incidence of blast by almost half. However, the disease incidence was lowered as a result of plasma treatment. The use of plasma therapy is one of the most likely causes for the decrease in disease incidence. It has been observed that ozone and arc discharge plasmas are effective in inactivating *Fusarium fujikuroi* spores and are a possible method for seed sterilization (Kang et al., 2015). In their work, Hasan et al. (2021) found that seed treatment with atmospheric O<sub>2</sub>-air glow discharge plasma for 90 s reduced the incidence of brown spots in BRRI dhan 28 in the field.

### Effect of plasma seed treatment on the severity of blast of BRRI dhan 28 under field condition

In the field experiment, effect of seed treatment with atmospheric cold plasma on blast severity index was assessed following 0-9 scale at 45, 60 and 75 DAT and the findings are presented in Table 4. Seed treatment with different plasmas and chemical had significant influence on Disease Severity Index (DSI). The lowest DSI was recorded from chemical seed a treatment (7.5, 10.4 and 13.75) at all sampling dates which was statistically alike with plasma seed treatments. The highest disease severity index was recorded as 21.42, 29.0 and 29.51, respectively in the control plots. Piza et al.(2018) investigated on the effect of plasma operating at atmospheric pressure air on *Diaporthe/Phomopsis* (D/P), a complex of seed-borne fungi severely affecting soybean (*Glycine max* L.).

**Table 4.** Effect of plasma seed treatment on the severity of blast of BRRI dhan 28 under field condition

Treatment	Severity of blast at different days		
	45DAT	60DAT	75DAT
T1	10.58b ±0.22	15.4b ±3.70	17.91b ±3.25
T2	8.75b ±0.38	12.50b ±1.25	14.58b ±1.10
T3	11.42b ±0.04	14.33b ±0.95	18.57b ±0.91
T4	9.04b ±0.00	13.32b ±0.78	14.83b ±0.00
T5	7.50b ± 0.50	10.41b ±1.66	13.75b ±1.90
T6	21.42a ±0.00	29.04a ±3.72	29.51a ±2.65
Level of significance	***	**	**

T<sub>1</sub>= O<sub>2</sub>-air glow discharge plasma for 90s, T<sub>2</sub>= O<sub>2</sub>-air glow discharge plasma for 120s, T<sub>3</sub>= Air-plasma activated water for 9min, T<sub>4</sub>= Air-plasma activated water for 12min, T<sub>5</sub>= Chemical and T<sub>6</sub>= Control. In column, according to DMRT, figures with similar letter (s) are identical, whereas those with dissimilar letter(s) differ significantly. The symbol ± represents the standard errors of three replications. \*\*\*=Significant at 0.1% level of probability and \*\* = Significant at a 1% level of probability.

As plasma treatment can successfully control seed borne fungi from infected seeds, the seedlings produced from plasma treated seeds grow to be comparatively disease free which may show lower severity under field condition. The findings are comparable to those of Aktar et al. (2021), who investigated the effect of plasma seed treatment on potato late blight disease control and discovered that applying H<sub>2</sub>O/O<sub>2</sub> plasma for 4 and 6 minutes in the field significantly reduced disease severity. Seed treatment with atmospheric glow discharge plasma produced by O<sub>2</sub>-air for 90 s has also been reported to be very effective in reducing brown spot disease in the field and improving the growth and yield of BRRI dhan 28 (Hasan et al., 2021).

## Conclusion

The plasma seed treatments utilized in this investigation had a similar effect to chemical seed treatments, and in some cases were even better. The outcome is highly encouraging and positive in terms of seed germination, field emergence (plant stand), seedling health, and blast severity reduction in the field. Based on the findings, it can be concluded that the O<sub>2</sub>-air glow discharge plasma for 120 seconds and PAW for 12 minutes performed better on average, with a few exceptions. As a result, rice growers can be advised to treat their rice seeds with O<sub>2</sub>-air glow discharge plasma for 120 seconds and PAW for 12 minutes to reduce blast disease in the nursery bed and in the field.

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## Author contributions

Conceptualization, MMH and MRT; Methodology, MRT and MMH; Investigation, MRT

and MMH; Data collection and analysis, HR and PR; Draft preparation, HR and P; Review and editing, MMH. All authors have read and agreed to the published version of the manuscript.

**Conflict of interest:** The authors declare no conflict of interests.

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