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Antifungal effect of glow discharge plasma and plasma activated water against brown spot disease of rice

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ABSTRACT

The impact of atmospheric glow discharge plasma and plasma activated water on reducing rice seed-borne brown spot disease was investigated. It has been shown that the generation of reactive oxygen and nitrogen species by plasma containing electrons, positive or negative ions, and neutral species has antimicrobial activity. In this study, four plasmas were used for seed treatment *viz*., O_2-air glow discharge plasma for 90 s, 120 s, air-plasma activated water for 9 and 12 min. Control (without treatment) and chemical treatment were used in order to compare the treatment effect. The plasma treatments used for this study showed a promising positive effect on seed germination and plant growth, and reduction of brown spot severity in the field. Among the plasmas, O_2-air glow discharge plasma for 90 s significantly increased seed germination (94.67%) compared to control (84.0%). Conidial suspension of *Bipolaris oryzae* treated with O_2-air glow discharge plasma for 90 s and air-plasma activated water for 9 min successfully reduced the conidial germination ability of *Bipolaris oryzae* on Potato Dextrose Agar (PDA). The brown spot incidence and severity were significantly reduced in the plot which received seed treatment with O_2-air glow discharge plasma for 90 s and air-plasma activated water for 9 min. Interestingly, the same treatment remarkably enhances the plant growth parameters and yield of BRRI dhan28 compared to control treatment.

Keywords: O_2-air glow discharge plasma, air-plasma activated water, brown spot disease, *Bipolaris oryzae*

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1 Introduction

Rice (*Oryza sativa*) is the most important and comprehensively cultivated grain crop of Bangladesh. Next to wheat, it is the second highest consumed cereal in the world (Abodolereza and Racionzer, 2009). But the yield of rice is greatly hampered due to diseases. In Bangladesh, 31 diseases have been identified as affecting rice, including 10 severe diseases (Miah et al., 1985). The most common diseases of rice include seedling blight, brown spot, blast, sheath rot, sheath blight, bakanae, false smut, bacterial leaf blight, bacterial leaf streak, ufra and tungro. Most of the devastating diseases of rice are caused by seed borne pathogens. Seed borne fungal pathogen *Bipolaris oryzae* causing brown spot of rice is transmitted to seedling from naturally infected seed. It is a destructive pathogen causing significant yield loss upto 90% particularly when leaf spotting phase is assumed epiphytotic proportions (Ghose et al., 1960). For producing high-quality healthy nursery seedlings, it is

necessary to control the seed borne disease at the early stage in the seedlings of nursery. Fungicides are effective to manage this seed borne disease but chemical resistant strains have been reported (Gupta and Kumar, 2020). Besides this, it is costly and hazardous to our environment and human health.

Cold plasma is an emerging cutting-edge technology which is being currently used in agriculture for enhancement of seed germination, plant growth and microbial inactivation of seed (Sera et al., 2016; Filatova et al., 2014). Plasma is considered 4th basic states of matter other than liquid, solid and gas, in other word ionic gas. The plasma system is now a novel technique for eradicating all microorganisms (Shintani et al., 2010). Furthermore, plasma irradiation systems are chemical-free. In a short period and at a low temperature, it kills all forms of germs. Because of their novel applicability, atmospheric-pressure non-thermal plasmas are gaining interest. Many experimental studies have found that cold atmospheric plasma (CAP) can significantly improve seed wettability and water absorbing capability, improving seed germination (Li et al., 2018; Amnuaysin et al., 2018; Roy et al., 2018) and disinfecting pathogen-inoculated seed surfaces (Jo et al., 2014; Ochi et al., 2016; Selcuk et al., 2008).Very recently, plasma activated water is being used for the control of many plant diseases (Aktar et al., 2021; Adhikari et al., 2019). Plasma activated water (PAW) is created by treating water with plasmas that alter the pH, electrical conductivity, dissolve oxygen, and other properties of the water while also producing reactive oxygen and nitrogen species. The presence of diverse plasma species has an impact on microbial inactivation (Shintani et al., 2010). Furthermore, electric field and shock waves are two other plasma factors that can have a variety of good impacts on seed treatment (eg. germination rate and growth) (Pietruszewski et al., 2007). During seed germination, plasma is also known to cause an improve in seed reserve consumption and α-amylase activity (Islam et al., 2019). As a result, plasma may play a key role in improving plant growth and development, resulting in a healthy and disease-free crop production and yield (Filatova et al., 2014). To our knowledge, no research has been done in our country using plasma on seed to control *Bipolaris oryzae*, that causes brown spot of rice. Therefore, the purpose of the study was to adopt the plasma treatment system as new techniques to disinfect rice seed borne pathogen *Bipolaris oryzae* in controlling brown spot disease of rice in the nursery bed and in the field.

2 Materials and Methods

The current study was conducted at the University of Rajshahi's Plant Pathology Laboratory and Agronomy Experimental Field, as well as the Plasma Science and Technology Laboratory of the Department of Electrical and Electronic Engineering. Rice variety BRRI dhan28 and six treatments *viz*., T1 = Seed treatment with O_2-air glow discharge plasma for 90 seconds, T2 = Seed treatment with O_2-air glow discharge plasma for 120 seconds, T3 = Seed treatment with air-plasma activated water for 9 minutes, T4 = Seed treatment with air-plasma activated water for 12 minutes, T5 = Chemical seed treatment with Provax 200 WP @ 0.4% of seed weight, and T6 = Control (without treatment) were used in this study.

2.1 Experimental setup for plasma seed treatment

For rice seed treatment, atmospheric pressure O_2-air glow discharge plasma and plasma activated water (PAW) were used. The plasmas were produced at Plasma Science and Technology Lab, Department of Electrical and Electronic Engineering, Rajshahi University. A schematic of the experimental setup, as well as a graphical representation of the rice seed treatment arrangement employing O_2-air plasmas is shown in Fig. 1. To make plasma activated water, an underwater air discharge plasma jet was employed. A gas flow controller was used to control the flow of air into the jet (KIT 115P). The plasma jet was powered by a bipolar variable power source with a voltage range of 1-10 kV and a frequency range of 1-10 kHz. The voltage-current (V-I) properties shown in Fig. 1(c) were obtained using a combination of a high voltage probe (HVP-08) and a current probe (CP-07C). For the identification of plasma species produced in O_2-air discharges, the optical emission spectrum (OES) of O_2-air discharge plasma was acquired using a spectrophotometer (Ocean Optics USB2000 +XR1: detector wavelength range180-1100 nm, slit width25 μm, grating500 lines/mm, optical resolution 1.7 nm) (Fig. 1d). For 9 and 12 min, air was pumped through the glass tube into the water treatment reactor at a rate of 1 L min^{-1}. For plasma treatment, a volume of 250 mL distilled water was used at a time for different duration (9 and 12 min). One thousand rice seeds were taken in a 250 mL beaker and according to the treatment specification of plasma activated water (PAW) was added on it and allowed to immerse for 10 min in PAW. The PAW-treated rice seeds were dried at room temperature in a laminar air hood. Rice seeds were placed in a beaker and treated with chemical fungicide according to the treatment specification. Seed treatment was done with Provax 200 WP @ 0.4% of seed weight.

2.2 Isolation and development of pure culture of *B. oryzae*

Bipolaris oryzae was isolated on PDA from naturally infected rice seed. The infected seeds were placed

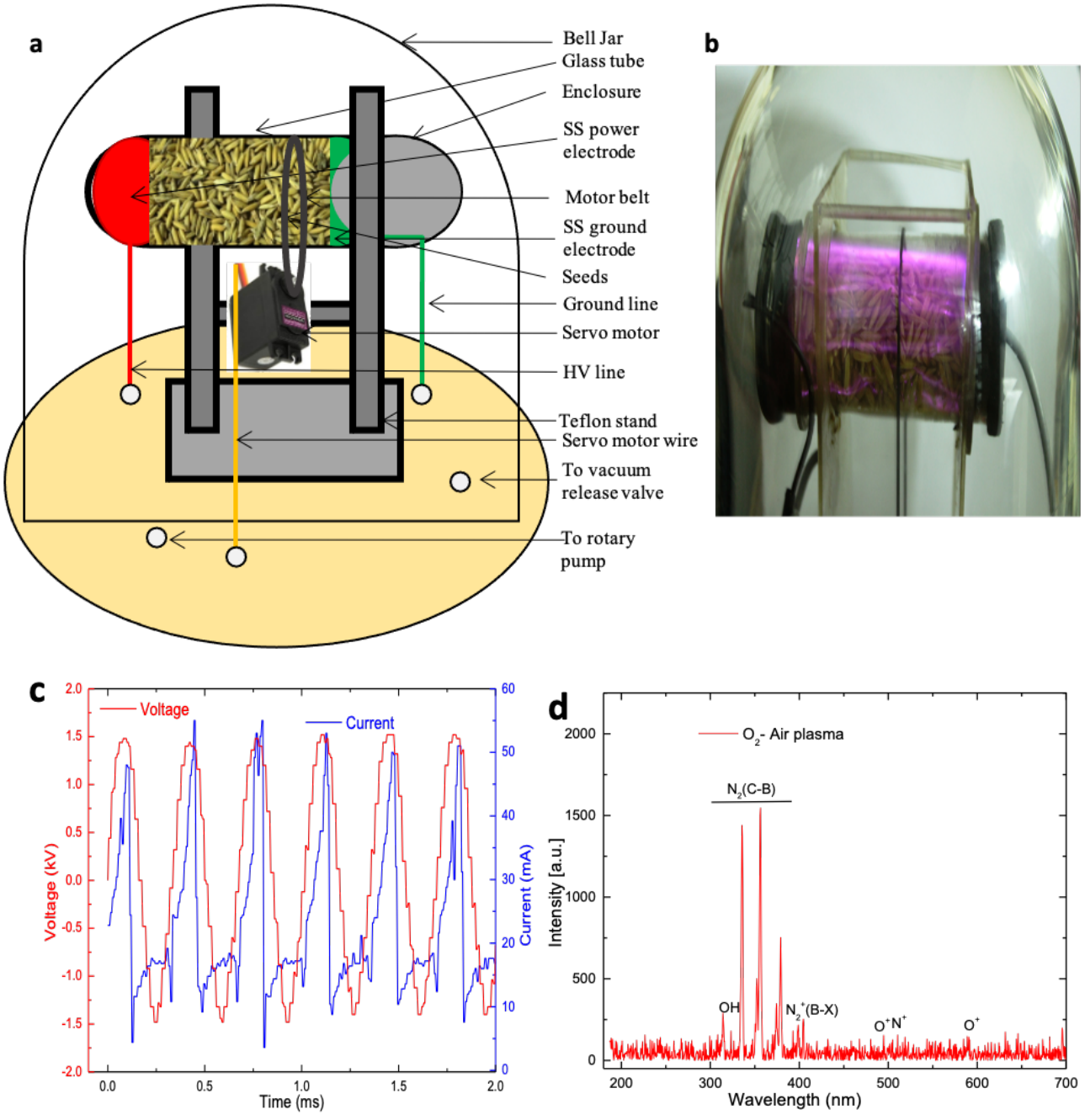

Figure 1. (a) Schematic of the low pressure (100 torr) glow oxygen-air discharge (GOAD) plasma production setup for the treatment of paddy seeds, (b) image of the seed treatment reactor under treatment of seeds, (c) voltage-current characteristics of GOAD plasma seed treatment reactor measured under loaded with seeds, and (d) optical emission spectrum (OES) acquired from the GOAD plasma seed treatment reactor loaded with seeds.

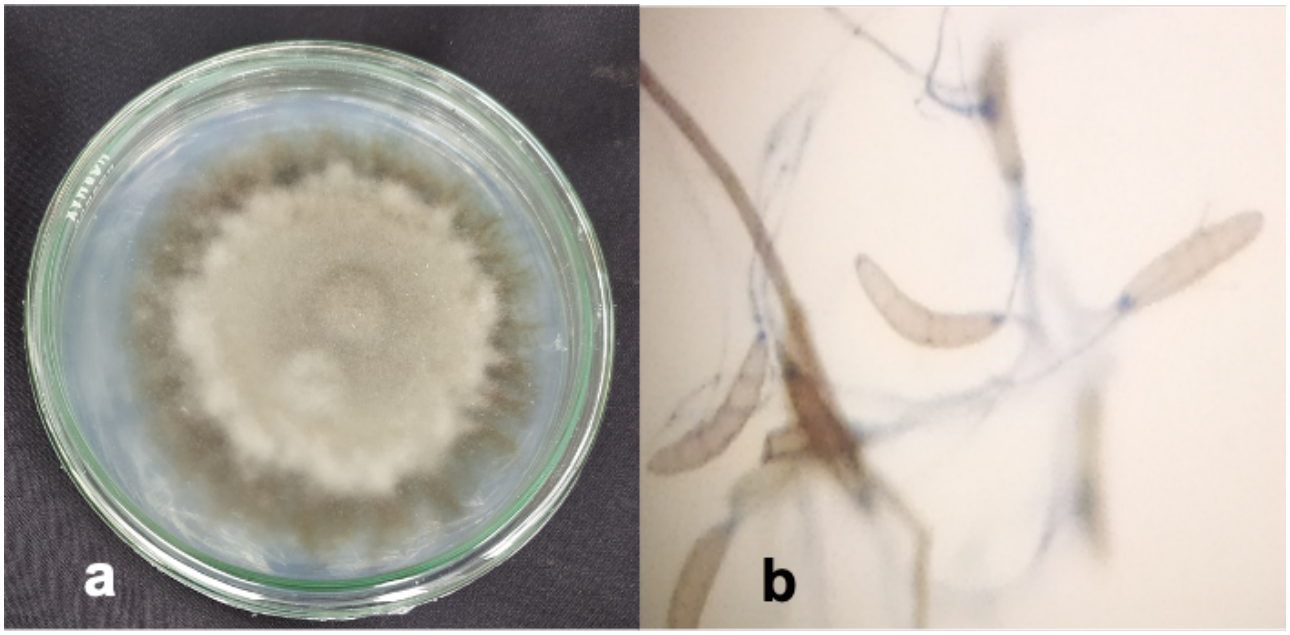

Figure 2. Photographs of (a) culture plate of *Bipolaris oryzae*, taken by camera model Nikon d3400 and (b) microscopic image of *Bipolaris oryzae* taken by Olympus BH2 (40X).

onto PDA plate following 16 seeds in the outer ring, 8 in the middle and one in the center (ISTA, 1996). Then the dishes were incubated at 28 ± 1 °C for 7 days in alternating cycles of 12 hours darkness and 12 hours light for growth of fungal structures on infected seeds. After 2 days of incubation, observation was started. The associated seed borne fungi grown on PDA were isolated. The isolated fungi were recultured further on PDA medium in order to get pure culture. Standard mycological literature and manuals were used to identify the fungus based on its color, spore shape, and mycelia Barnett and Hunter (1972); Singh (1982); Raper and Fennell (1965) (Fig. 2).

2.3 Preparation of inoculum

The isolate of *Bipolaris oryzae* was cultured on PDA solid medium at 28 ± 1 °C for 10 days in the dark. By suspending the mycelia mate in sterilized distilled water (SDW), conidia of *Bipolaris oryzae* were obtained. The suspension was filtered through a sterile cheese cloth to eliminate any hyphae or aggregates. Appropriate dilutions of the conidial solution were made using SDW to obtain a concentration of 1×10^5 spores (cfu) mL^{-1}. Microscopic enumeration was done with a haemocytometer (Neubauer chamber; Merck S.A., Madrid, Spain).

2.4 Germination ability of *B. oryzae* on PDA

For plasma treatment, conidial suspension was irradiated with differnt plasmas as per treatment specifications. In case of chemical treatment, the Provax 200 was added to an autoclaved PDA medium at a concentration of 0.4% (Borum and Sinclair, 1968). As a control, the conidial suspension was irradiated with air. To ensure spore viability and count, the plate method was used. Following treatment, each PDA plate was infected with three microlitres of conidial suspension (1×10^4 cfu mL^{-1}) of *B. oryzae* produced from stock solution (1×10^5 cfu mL^{-1}). For 7 days, the plates were incubated at 28 ± 1 °C for 24 hours, with twelve hours of darkness and twelve hours of light. The individual colonies developed on the PDA plates were counted (Fig. 3).

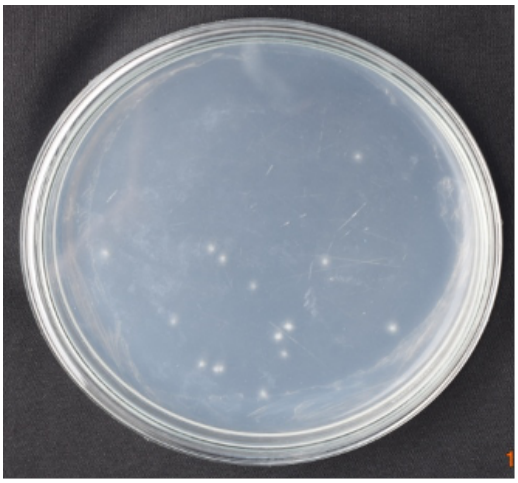

Figure 3. Photograph showing the developing individual colony of *Bipolaris oryzae* on PDA

2.5 Seed germination and seed-borne *B. oryzae*

The blotter method was used to conduct this research. Both treated and untreated rice seeds were placed on a moistened 2-layer blotter paper in this method to allow seed germination. For conducting germination test, 100 seeds of each treatment groups were used. Twenty five seeds were placed on each petridish separately following ISTA (1996). Then the dishes were incubated at 28 ± 1 °C for 7 days in alternating cycles of 12 hours darkness and 12 hours light. The observation started after 3 days of incubation and continued for 7 days. During the investigation, seed-borne *B. oryzae* was identified using standard mycological literature and manuals (Barnett and Hunter, 1972; Singh, 1982; Raper and Fennell, 1965) and presented as a percentage of total *B. oryzae* (Mathur and Kongsdal, 2003). The % germination was estimated by the following formula:

$$G_n\ (\%) = \frac{N}{S} \times 100 \tag{1}$$

where G_n = germination (%), N = number of seeds produced normal seedlings, and S = total number of seeds set for germination.

2.6 Field experiment

For 24 hours, the treated seeds were soaked in water. After that, the seeds were removed from the water and covered with gunny bags. After 48 hours, the seeds were starting germination and sown in the dry nursery bed plots. The field experiment was carried out using a three-replication randomized complete block design (RCBD). According to treatment specifications, 18 plots were prepared including control treatment. Size of the each plot was 1 m^2. After spreading the sprouted seeds onto the dry seedbed, seeds were covered with friable soil; necessary amount of water was added to keep the soil moist. A piece of polythene sheet was used to cover the seedbed. After adding enough water, the soil was ploughed and cross ploughed three times using a power tiller, then laddered to get the desired puddle state. Necessary fertilizers were applied as per suggestion of BRRI (2016). As per treatment specifications 18 plots of 5 m^2 were prepared. According to the experimental design, 35-day-old 3 seedlings $hill^{-1}$ was transplanted on the well-puddled plots after final land preparation. When there was a higher level of weed population infestation, weeding was done twice. Irrigation was applied to the experimental plots as needed.

2.7 Determination of disease incidence

Brown spot disease was observed in the experimental pots at 45, 60, and 75 days after transplanting (DAT). For each treatment, fifteen plants were chosen to collect data on disease incidence. The percent of infected plants was determined using the following formula (Teng and James, 2002):

$$I\ (\%) = \frac{NI}{TP} \times 100 \tag{2}$$

where I = infection (%), NI = number of infected plants, and TP = total number of plants.

2.8 Determination of disease severity

Brown spot disease severity in the experimental plots was measured by regular inspections at 15-day intervals (45, 60, and 75 DAT). For each treatment, fifteen plants were chosen to collect disease rating data. The IRRI standard (IRRI, 2002) disease rating scale was used to score the diseases (Table 1). The following formula was used to calculate the percentage of disease severity (Mckinney, 1923):

$$DSI = \frac{R_T}{(O \times M)} \times 100 \tag{3}$$

where DSI = disease severity index, R_T = total rating, O = observation, and M = maximum grade.

Table 1. Standard rating scale (1-9 scale) of brown spot of rice (IRRI, 2002)

Scale	Description
1	Small brown specks of pin point size on lower leaves
2	Small roundish necrotic brown spots, about 1-2 mm in diameter with a distinct brown margin. Spots are mostly focused on the lower leaves
3	Spot type same as in 2 but significant number of spots on the upper leaves
4	Typical susceptible brown spot, 3 mm or larger infecting less than 4% of leaf area
5	Typical susceptible brown spot,3 mm or larger infecting 4-10% of the leaf area
6	Typical susceptible brown spot, 3 mm or larger infecting 11-25% of the leaf area
7	Typical susceptible brown spot, 3 mm or larger infecting 26-50% of the leaf area
8	Typical susceptible brown spot, 3 mm or larger infecting 51-75% of the leaf area many leaves are dead
9	Typical susceptible brown spot, 3 mm or larger infecting more than 75% of the leaf area

2.9 Collection of plant data

The following data was collected: stem diameter, total dry matter content, plant height (cm), length of panicle (cm), grain panicle^{-1}, filled grain panicle^{-1}, unfilled grain panicle^{-1}, 1000-grain weight (g), and grain yield (t ha^{-1}). For each treatment, fifteen plants were chosen to collect data on growth and yield contributing characteristics. Grain yield was measured by harvesting the crops grown in one square meter of each plot. After that, the samples were threshed, dried, and weighed with a balance, and the results were stated in t ha^{-1}.

2.10 Statistical analysis

For statistical analysis, the recorded data for various parameters were collected and tabulated in the right format. The mean differences were adjusted using Duncan's Multiple Range Test (DMRT) and the analysis of variance was performed using the computer package program SPSS (version 22).

3 Results and Discussion

Low-temperature atmospheric glow discharge plasmas and plasma activated water were applied as seed disinfectant in order to investigate their effects on spore survival of *Bipolaris oryzae*, seed germination, plant growth, disease incidence, disease severity and yield and yield attributes of rice. The study revealed that plasma treatments had noteworthy effect on the above mentioned parameters with few exceptional cases.

3.1 Germination ability of *B. oryzae*

According to Zhang et al. (2014), the germination ability of plasma-treated *B. oryzae* conidia was investigated. Each PDA plate was inoculated with three microlitres of plasma treated conidial suspension (1×10^4 cfu mL^{-1}) of *B. oryzae*. The conidial suspension of *B. oryzae* treated with different plasmas exhibited tremendous effect on the inhibition of germination. Fig. 4(a) shows the lowest number of *B. oryzae* colony (5.33) grown on PDA plate was counted from the treatment of O_2-air glow discharge plasma for 120s followed by Air-PAW for 12 min (6.0) and 9 min (6.44), respectively. But the untreated control showed the maximum number of colony grown on PDA in compare to other treatments. The results exhibited that a longer plasma treatment time was more effective at inhibiting fungal growth. However, from this experiment it has been found that the longer plasma irradiation caused reduction in spore germination percentage and growth. This could be related to cellular damage caused by high dose irradiation. Cell death is caused by direct physical injury and plasma-mediated ROS buildup across the treated tissues (bin Seol et al., 2017). Therefore, to adapt the plasma treatment system to direct inhibition of seed-borne pathogens consequently seed borne diseases, optimization of irradiation time and dose has to be known in order to use it successfully. This is in line with the results of Suhem et al. (2013) and Jo et al. (2014), respectively. They discovered that an atmospheric non-thermal plasma treatment stopped *Aspergillus flavus* and *Gibberella fujikuroi* from growing on agar media. Kranner et al. (2010) found that H_2O_2 and O_2 play an important role in pathogen protection in seeds. Cold atmospheric plasma-generated reactive nitrogen and oxygen species (NO, OH, superoxide), as well as strong oxidizing elements (H_2O_2, O_3), may enter microorganisms, oxidize the cytoplasmic membrane, alter cellular systems, and inactivate them (Klampfl et al., 2012), potentially leading to phytopathogen decontamination of seed surfaces.

3.2 Seed germination and seed-borne *B. oryzae*

The difference in seed germination among plasma irradiated and non-irradiated rice seeds was noteworthy. Furthermore, no abnormal seedlings were seen in the plasma-treated sample (data not shown). From the Fig. 4(b) , it can be observed that seed treatment with glow discharge O_2-air plasma for 90s and Air-PAWs for 9 min showed the highest germination rate (94.67 and 92.0%), whereas the minimum germination was obtained from control treatment (84%). On the other hand chemical seed treatment provided 88% seed germination. In comparison to the control group, due to seed treatment with atmospheric glow discharge plasma and PAWs that contains reactive oxygen and nitrogen species, which may result in a significant germination boost Kumar et al. (2021). This finding is in line with Jiang et al. (2014), who discovered that cold plasma boosted wheat seed germination rate and germination potential significantly when compared to a control group. RNS assimilation on the seed surface has been found to improve seed germination potential (Roy et al., 2018), which agrees with our findings. No seed-borne *B. oryzae* was detected on the seeds which were irradiated with O_2-air glow discharge plasma for 90 and 120 s and air-plasma activated water for 120 s (Fig. 4(c)). On the other hand chemical treatment of seed also showed significant effect in controlling seed-borne *B. oryzae*. The above result is revealed that plasma treatments have antifungal effect against the pathogen. Plasma-derived ROS and RNS have been shown to be powerful oxidizing agents that can penetrate microorganisms and modify their cell processes, rendering them inactive and perhaps leading to the decontamination of seed-borne pathogens (Klampfl et al., 2012).

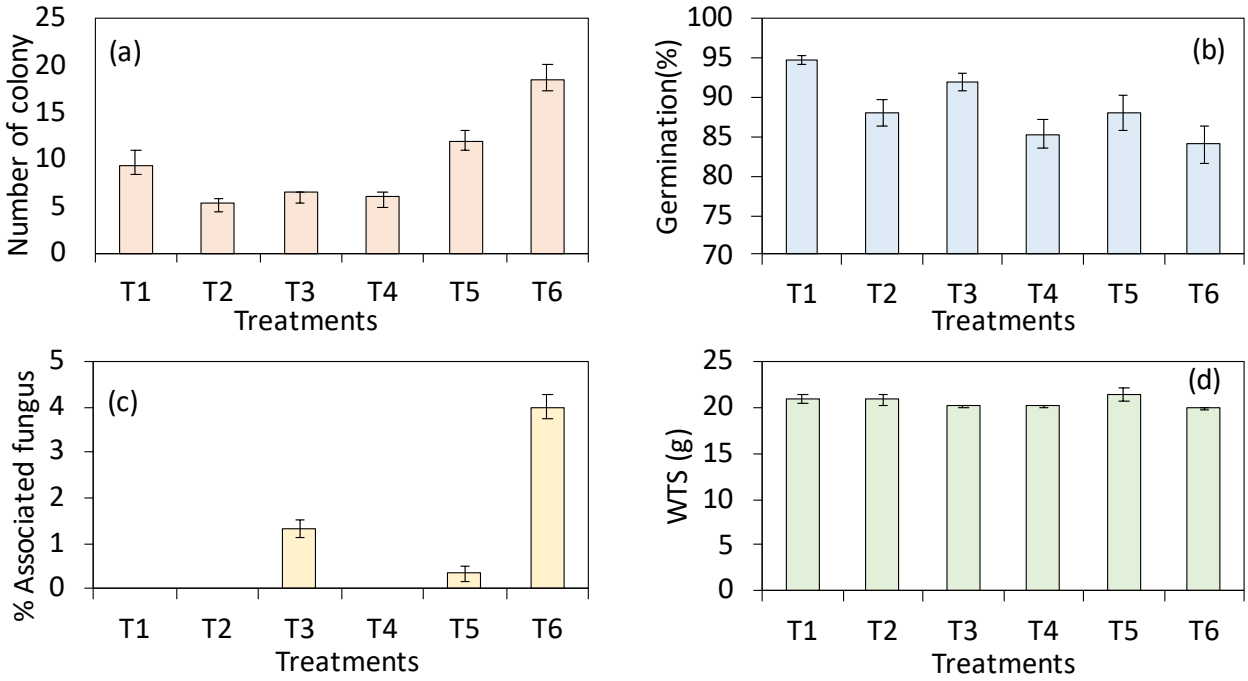

Figure 4. Effect of plasma irradiation on (a) the suppression of conidial germination of *Bipolaris oryzae* on PDA, (b) germination of rice seeds, (c) % associated fungus in rice seed, and (d) weight of 1000 grains. T1 = O_2-air glow discharge plasma for 90s, T2 = O_2-air glow discharge plasma for 120s, T3 = Air-plasma activated water for 9 min, T4 = Air-plasma activated water for 12 min, T5 = Chemical, and T6 = Control

3.3 Brown spot incidence in the field

Disease incidence was found to confine within lower limit in those plots which received plasma treatment (Table 2). The plants with minimum disease incidence at 45, 60 and 75 DAT were recorded with atmospheric cold plasma by O_2-air for 90 s (16.67, 20.0, and 23.11%) while the maximum disease incidence was noticed from control plots (33.33, 40.0, and 50%). Next to this, the plasma produced from O_2-air for 120 s showed 20.0, 23.33, and 25.67% incidence of the disease. Air-PAW for 9 min also exhibited 21.0, 23.33, and 25.0% incidence of the disease. From the findings it was revealed that the incidence of brown spot was reduced to 50% due the application of O_2-air plasma for 90 s and Air-PAW for 9 min. Other treatments also showed significantly similar effect as of chemical seed treatment in reducing disease incidence. Many environmental elements, such as plant growth stage, soil conditions, and other factors, influence the occurrence of disease under field circumstances, and it is impossible to manage all of them. However, due to application of plasma treatment the disease incidence was reduced. 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).

As a result, plasma-treated rice seeds developed healthier and disease-free seedlings in the nursery bed, and disease occurrences in the field were found to be lower than the control. Plasma treatment has been demonstrated to promote seed germination and plant growth in the past, which is consistent with the findings of Roy et al. (2018). As a result, plants' growing state aids in the development of disease resistance by shifting their state of preparation to combat pathogenic attack.

3.4 Brown spot severity in the field

The impact of plasma seed treatment on rice brown spot severity was assessed in the field at 45, 60, and 75 days after transplanting (Table 2). The IRRI standard disease rating scale (1-9) (IRRI, 2002) was used to score the diseases (Table 1). The Mckinney (1923) formula was used to determine the Disease Severity Index (DSI). The disease severity index was significantly reduced due to pre-treatment of seed with different plasmas compared to control. The lowest Disease Severity Index (DSI) (12.91, 15.41, and 19.58) was recorded with pre-treatment of seed with glow discharge O_2-air plasma for 90 s and statistically identical results were recorded from air-PAWs for 9 min, respectively. Chemical seed treatment also significantly reduced brown spot severity upto 60 DAT but the result was similar when evaluated in

Table 2. Effect of plasma irradiation on incidence, and severity index of brown spot disease, and growth characters of BRRI dhan 28 at different days after transplanting

Treatment	45DAT	60DAT	75DAT
	Diseases incidence (%)		
T1	16.67c ± 0.77	20.0c ± 1.15	23.11b ± 1.15
T2	20.0b ± 1.15	23.33bc ± 0.96	25.67.0b ± 1.45
T3	21.0b ± 1.15	23.33bc ± 0.77	25.0b ± 0.19
T4	22.0b ± 0.50	25.0b ± 0.00	26.0b ± 2.30
T5	23.0b ± 0.57	24.33b ± 1.15	28.0b ± 1.82
T6	33.33a ± 1.73	40.0a ± 2.30	50.0a ± 2.88
Sig. level	***	**	***
	Disease severity index		
T1	12.92b ± 2.31	15.42b ± 3.25	19.58b ± 3.00
T2	16.75ab ± 2.98	19.58b ± 3.25	20.00b ± 3.60
T3	14.33b ± 0.78	19.51b ± 1.26	20.95b ± 0.47
T4	16.66ab ± 1.71	21.90ab ± 3.72	19.58b ± 1.81
T5	10.42b ± 1.50	14.17b ± 1.81	28.57a ± 2.18
T6	24.28a ± 3.78	29.52a ± 2.38	35.23a ± 1.90
Sig. level	*	*	**
	Plant height (cm)		
T1	65.33 a ± 0.88	77.00 a ± 2.18	100.67ab ± 2.51
T2	62.66 ab ± 1.45	73.66ab 1.76	96.67ab ± 0.88
T3	65.0 a ± 1.52	76.67 a ± 2.72	101.00ab ± 4.04
T4	62.00ab ± 0.57	72.33 ab ± 0.88	98.66ab ± 0.88
T5	63.66 a ± 0.66	76.00 ab ± 1.52	102.33 a ± 1.76
T6	60.00 b ± 0.00	71.67 b ± 1.20	95.00b ± 2.08
Sig. level	*	*	*
	Stem diameter (cm)		
T1	3.34 a ± 0.08	4.25 a ± 0.07	5.29 a ± 0.09
T2	3.17 bc ± 0.027	4.21 ab ± 0.04	4.94 ab ± 0.15
T3	3.27 b ± 0.02	4.13 ab ± 0.01	4.96 ab ± 0.042
T4	3.22 bc ± 0.03	4.09 bc ± 0.02	4.90 b ± 0.08
T5	3.27 b ± 0.005	4.17 ab ± 0.06	5.10 ab ± 0.03
T6	3.12 c ± 0.02	3.96 c ± 0.03	4.82 b ± 0.18
Sig. level	**	**	*
	Total dry matter (g plant^{-1})		
T1	5.00 a ± 0.23	10.73 ab ± 0.68	15.65a ± 1.15
T2	4.33 ab ± 0.33	10.02 b ± 0.49	13.59ab ± 0.57
T3	4.33 ab ± 0.33	10.71ab ± 0.64	13.68ab ± 1.73
T4	4.06 ab ± 0.54	9.87 b ± 0.63	11.98b ± 0.577
T5	4.83 ab ± 0.22	11.97a ± 0.29	16.00a ± 1.16
T6	3.73 b ± 0.15	9.87b ± 0.63	10.89 ± b0.58
Sig. level	*	*	*

T1 = O2-air glow discharge plasma for 90s, T2 = O2-air glow discharge plasma for 120s, T3 = Air-plasma activated water for 9 min, T4 = Air-plasma activated water for 12 min, T5 = Chemical and T6 = 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. * , **, and *** designate significant at 5%, 1%, and 0.1% levels of probability.

Table 3. Effect of plasma irradiation on yield contributing characters of BRRI dhan 28

Treatment	Plant height (cm)	Ear length (cm)	No. of filled grains panicle^{-1}	No. of unfilled grains panicle^{-1}
T1	117.33a ± 1.20	25.0a ± 0.58	114.67a ± 12.44	45.67 ± 3.38
T2	111.33 b ± 1.45	23.67ab ± 0.67	89.67abc ± 5.48	39.33 ± 8.25
T3	111.67b ± 2.33	23.67ab ± 0.33	87.0bc ± 3.51	38.0 ± 5.29
T4	109.67b ± 1.45	23.33ab ± 0.33	85.67bc ± 2.72	36.0 ± 3.46
T5	118.67a ± 1.66	24.67a ± 0.33	107.0ab ± 14.46	40.33 ± 2.18
T6	108.33b ± 0.33	22.67b ± 0.67	72.33c ± 0.88	39.33 ± 6.96
Sig. level	**	*	*	NS

T1 = O2-air glow discharge plasma for 90s, T2 = O2-air glow discharge plasma for 120s, T3 = Air-plasma activated water for 9 min, T4 = Air-plasma activated water for 12 min, T5 = Chemical and T6 = 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. * and ** designate significant at 5% and 1% levels of probability.

75 DAT in compare to untreated control treatment. Nevertheless, the highest severity index of the disease (24.28, 29.52, and 35.23) at all the sampling dates was recorded from control treatment. As a result, the findings suggest that the reduced disease severity observed in field plants was partially attributable to their better healthy growth after being pre-treated with atmospheric cold plasma. Lower percentage of disease incidences occurred at the early growth stages of the seedlings due to pre-treatment of seeds with cold plasma thereby promoting later stages of crops with lower disease severity. As plasma treatment can successfully control the seed borne fungi, the seedlings produced from plasma treated seeds grown to be comparatively disease free which may show lower disease severity under field condition. The findings are similar to those of Aktar et al. (2021), who looked into the effect of plasma seed treatment on potato late blight disease control and discovered that H_2O/O_2 plasma for 4 and 6 minutes considerably reduced disease severity in the field.

3.5 Growth, yield attributing characters, and yield of rice

The plant height was recorded at 35, 45, and 55 DAT and found to be significantly varied under different treatment applications (Table 2). Seed treatment with atmospheric cold plasma by O_2-air for 90 s, air-PAW for 9 min and chemical showed the highest plant height (65.33, 65, and 63.66 cm) at 35 DAT. Likewise at 45DAT, the longest plants were measured (77 and 76.67 cm) with O_2-air for 90 s and Air-PAW for 9 min in comparison with control (71.67 cm). On the other hand, chemical seed treatment at 55 DAT exhibited the highest plant height (102.0 cm) which was followed by rest of the plasma seed treatments. ROS is known to govern a variety of biological activities, including growth, development, and biotic responses, and it is also a component of the signaling network that plants employ to develop (del Río, 2015). ROS and RNS have recently been discovered to play a critical role in cell proliferation, differentiation, and signaling (Meng et al., 2017). In our experiment, air and O_2-air plasmas with varying abundances produced ROS and RNS (Fig. 1(d)), which may aid rice seedling growth (Meng et al., 2017; Sera et al., 2016). Stem diameter was significantly influenced by plasma seed irradiation at 35, 45, and 55 DAT (Table 2). In every sampling date O_2-air glow discharge plasma for 90 s showed the highest stem diameter (3.34, 4.25, and 5.29 cm) in comparison with control group. In some cases O_2-air glow discharge plasma for 120s and Air-PAW for 9 min also provided good results in increasing stem diameter. Plant dry matter content was significantly influenced due to seed treatment with atmospheric cold plasma under field condition which is presented in Table 2. At 35 DAT, the highest accumulation of dry matter (5.0 g plant^{-1}) was recorded from the plants due to seed pre-treatment with O_2-air glow discharge plasma for 90 s whereas the control group showed 3.73 g dry matter plant^{-1}. And at 45 DAT, the highest result in this regard was obtained by chemical seed treatment (11.97 g plant^{-1}) which was followed by O_2-air glow discharge plasma for 90 s (10.73 g plant^{-1}) and Air-PAW for 9 min (10.71 g plant^{-1}). At the end of the sampling date (at 55 DAT), the maximum plant dry weight (16.0 and 15.65 g plant^{-1}) was measured due to seed treatment with chemical and O_2-air plasma for 90 s. This finding is similar to that of Roy et al. (2018), who found that wheat seedlings from atmospheric pressure plasma treated seed grew faster, accumulated more dry matter, and yielded more than control wheat seeds. Alike results were also reported by Li et al. (2018) in oilseed rape (*Brassica napus* L.) treated with low-vacuum helium cold plasma.

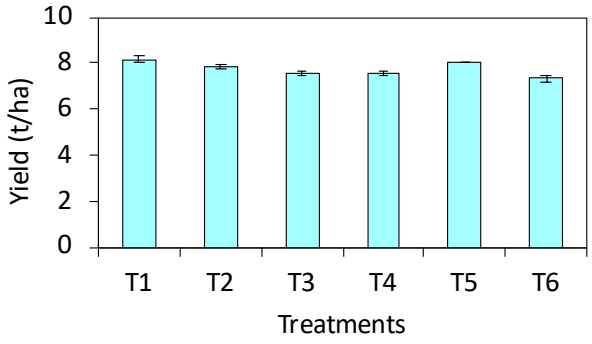

Figure 5. Effect of plasma irradiation on grain yield of BRRI dhan28. T1 = O_2-air glow discharge plasma for 90s, T2 = O_2-air glow discharge plasma for 120s, T3 = Air-plasma activated water for 9 min, T4 = Air-plasma activated water for 12 min, T5 = Chemical, and T6 = Control.

The seed treatment with O_2-air glow discharge plasma for 90 s and chemical provided the highest ear length (25.0 and 24.67 cm) (Table 3). However, O_2-air glow discharge plasma for 120 s Air-PAW for 9 and 12 min also resulted in longer ear length (23.67 and 23.33 cm) in comparison with control treatment (22.67 cm).

This result bears resemblance with the findings of Roy et al. (2018) revealing that longest spikes were obtained from wheat plants where seeds were treated with plasma stimulation. The highest number of filled grains panicle^{-1} (114.66) was counted for 90 s from O_2-air glow discharge plasma, followed by 120 s and chemical treatment (89.67 and 107.0). The number of unfilled grains panicle^{-1} did not differ significantly though it was ranged from 39-45. Similarly, 1000-grain weight did not vary significantly due to different treatment applications but it was numerically varied from 16.99 to 21.38 g, where the maximum value was recorded from chemical seed treatment and the lowest value was measured from control (Fig. 4(d)). The grain yield was found to increase due to seed treatment with different plasmas (Fig. 5). The glow discharge plasma produced from O_2-air for 90 s and chemical showed the highest yield (8.17 and 8.0 t ha^{-1}) followed by O_2-air plasma for 90 s in comparison with control group (7.33 t ha^{-1}). The findings are quite encouraging, and they could have a big impact on agriculture in the future. Jiang et al. (2014) studied the effect of cold helium plasma treatment on wheat seed germination, growth, and yield, finding that the plasma seed treatment promoted wheat plant growth, potentially increasing yield when compared to the yield of the control group. Dobrin et al. (2015) also stated that plasma seed treatment can significantly increase plant height, root and shoot length rendering the improvement in yield of wheat. Overall, the results show that seed treatment with atmospheric glow discharge plasma created by O_2-air for 90 s is very effective in minimizing brown spot disease in the field and enhancing the growth and yield of BRRI dhan28.

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Conflict of Interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

References

Abodolereza A, Racionzer P. 2009. Food Outlook: Global Market Analysis. FAO, Rome, 23-27.

Adhikari B, Adhikari M, Ghimire B, Park G, Choi EH. 2019. Cold atmospheric plasma-activated water irrigation induces defense hormone and gene expression in tomato seedlings. Scientific Reports 9. doi: 10.1038/s41598-019-52646-z.

Aktar A, Sarmin S, Irin UA, Rashid MM, Hasan MM, Talukder MR. 2021. Plasma activated water: Implication as fungicide, growth and yield stimulator of potato (*Solanum Tuberosum* L.). Plasma Medicine 11:31–46. doi: 10.1615/plasmamed.2021038184.

Amnuaysin N, Korakotchakorn H, Chittapun S, Poolyarat N. 2018. Seed germination and seedling growth of rice in response to atmospheric air dielectric-barrier discharge plasma. Songklanakarin Journal of Science & Technology 40:819–823.

Barnett HL, Hunter BB. 1972. Illustrated Genera of Imperfect Fungi, 3rd Edition. Minneapolis, Burgess Publishing Company.

bin Seol Y, Kim J, hong Park S, Chang HY. 2017. Atmospheric pressure pulsed plasma induces cell death in photosynthetic organs via intracellularly generated ROS. Scientific Reports 7. doi: 10.1038/s41598-017-00480-6.

Borum DF, Sinclair JB. 1968. Evidence of systemic fungicides protection against *R. solani* with vitavax in cotton seedling. Phytopathology 58:976–980.

BRRI. 2016. Adhunik dhaner chash, 19th edition. Bangladesh Rice Research Institute, Gazipur, Bangladesh.

del Río LA. 2015. ROS and RNS in plant physiology: an overview. Journal of Experimental Botany 66:2827–2837. doi: 10.1093/jxb/erv099.

Dobrin D, Magureanu M, Mandache NB, Ionita MD. 2015. The effect of non-thermal plasma treatment on wheat germination and early growth. Innovative Food Science & Emerging Technologies Emerging Technologies 29:255–260. doi: 10.1016/j.ifset.2015.02.006.

Filatova II, Azharonok VV, Goncharik SV, Lushkevich VA, Zhukovsky AG, Gadzhieva GI. 2014. Effect of rf plasma treatment on the germination and phytosanitary state of seeds. Journal of Applied Spectroscopy 81:250–256. doi: 10.1007/s10812-014-9918-5.

Ghose RLM, Ghatge MB, Subramanian V. 1960. Rice in India (revised edn). ICAR, New Delhi, India.

Gupta A, Kumar R. 2020. Management of Seed-Borne Diseases: An Integrated Approach. In: Seed-Borne Diseases of Agricultural Crops: Detection, Diagnosis & Management. Springer Singapore. doi: 10.1007/978-981-32-9046-4_25.

IRRI. 2002. Standard Evaluation System for Rice (SES). International Rice Research Institute. Manila, Philippines.

Islam S, Omar FB, Sajib SA, Roy NC, Reza A, Hasan M, Talukder MR, Kabir AH. 2019. Effects of LPDBD plasma and plasma activated water on germination and growth in rapeseed (*Brassica napus*). Gesunde Pflanzen 71:175–185. doi: 10.1007/s10343-019-00463-9.

ISTA. 1996. International Rules for Seed Testing. Seed Science and Technology 4:3-49.

Jiang J, Lu Y, Li J, Li L, He X, Shao H, Dong Y. 2014. Effect of seed treatment by cold plasma on the resistance of tomato to *Ralstonia solanacearum* (bacterial wilt). PLoS ONE 9:e97753. doi: 10.1371/journal.pone.0097753.

Jo YK, Cho J, Tsai TC, Staack D, Kang MH, Roh JH, Shin DB, Cromwell W, Gross D. 2014. A non-thermal plasma seed treatment method for management of a seedborne fungal pathogen on rice seed. Crop Science 54:796–803. doi: 10.2135/cropsci2013.05.0331.

Kang MH, Pengkit A, Choi K, Jeon SS, Choi HW, Shin DB, Choi EH, Uhm HS, Park G. 2015. Differential inactivation of fungal spores in water and on seeds by ozone and arc discharge plasma. PLoS ONE 10:e0139263. doi: 10.1371/journal.pone.0139263.

Klampfl TG, Isbary G, Shimizu T, Li YF, Zimmermann JL, Stolz W, Schlegel J, Morfill GE, Schmidt HU. 2012. Cold atmospheric air plasma sterilization against spores and other microorganisms of clinical interest. Applied Environmental Microbiology 78:5077–5082. doi: 10.1128/aem.00583-12.

Kranner I, Roach T, Beckett RP, Whitaker C, Minibayeva FV. 2010. Extracellular production of reactive oxygen species during seed germination and early seedling growth in *Pisum sativum*. Journal of Plant Physiology 167:805–811. doi: 10.1016/j.jplph.2010.01.019.

Kumar SPJ, Chintagunta AD, Reddy YM, Rajjou L, Garlapati VK, Agarwal DK, Prasad SR, Simal-Gandara J. 2021. Implications of reactive oxygen and nitrogen species in seed physiology for sustainable crop productivity under changing climate conditions. Current Plant Biology 26:100197. doi: 10.1016/j.cpb.2021.100197.

Li L, Li J, Shao H, Dong Y. 2018. Effects of low-vacuum helium cold plasma treatment on seed germination, plant growth and yield of oilseed rape. Plasma Science and Technology 20:095502. doi: 10.1088/2058-6272/aac3d0.

Mathur SB, Kongsdal O. 2003. Common Laboratory Seed Health Testing Method for Detecting Fungi, First Edition. International Seed Testing Association, Bassersdorf, Switzerland.

Mckinney HH. 1923. A new system of grading of plant diseases. Journal of Agriculture Research 26:195–218.

Meng Y, Qu G, Wang T, Sun Q, Liang D, Hu S. 2017. Enhancement of germination and seedling growth of wheat seed using dielectric barrier discharge plasma with various gas sources. Plasma Chemistry and Plasma Processing 37:1105–1119. doi: 10.1007/s11090-017-9799-5.

Miah SA, Shahjahan AKM, Hossain MA, Sharma NR. 1985. A survey of rice diseases in Bangladesh. Tropical Pest Management 31:208–213. doi: 10.1080/09670878509370984.

Ochi A, Konishi H, Ando S, Sato K, Yokoyama K, Tsushima S, Yoshida S, Morikawa T, Kaneko T, Takahashi H. 2016. Management of bakanae and bacterial seedling blight diseases in nurseries by irradiating rice seeds with atmospheric plasma. Plant Pathology 66:67–76. doi: 10.1111/ppa.12555.

Pietruszewski S, Muszynski S, Dziwulska A. 2007. Electromagnetic fields and electromagnetic radiation as non-invasive external stimulants for seeds [selected methods and responses]. International Agrophysics 21:95–100.

Raper KB, Fennell DI. 1965. The Genus *Aspergillus*. The Williams and Wilkins Company, Baltimore.

Roy NC, Hasan MM, Kabir AH, Reza MA, Talukder MR, Chowdhury AN. 2018. Atmospheric pressure gliding arc discharge plasma treatments for improving germination, growth and yield of wheat. Plasma Science and Technology 20:115501. doi: 10.1088/2058-6272/aac647.

Selcuk M, Oksuz L, Basaran P. 2008. Decontamination of grains and legumes infected with *Aspergillus* spp. and *Penicillum* spp. by cold plasma treatment. Bioresource Technology 99:5104–5109. doi: 10.1016/j.biortech.2007.09.076.

Sera B, Sery M, Gavril B, Gajdova I. 2016. Seed germination and early growth responses to seed pretreatment by non-thermal plasma in hemp cultivars (*Cannabis sativa* L.). Plasma Chem Plasma Process 37:207–221. doi: 10.1007/s11090-016-9763-9.

Shintani H, Sakudo A, Burke P, McDonnell G. 2010. Gas plasma sterilization of microorganisms and mechanisms of action. Experimental and Therapeutic Medicine 1:731–738. doi: 10.3892/etm.2010.136.

Singh RS. 1982. Plant Pathogen: The Fungi. Oxford and IBM Publishing Co., New Delhi, India.

Suhem K, Matan N, Nisoa M, Matan N. 2013. Inhibition of *Aspergillus flavus* on agar media and brown rice cereal bars using cold atmospheric plasma treatment. International Journal of Food Microbiology 161:107–111. doi: 10.1016/j.ijfoodmicro.2012.12.002.

Teng PS, James WC. 2002. Disease and yield loss assessment. Monsanto Company Makati city, Philippines and ISAAA americenter. Cornell University, USA.

Zhang X, Liu D, Zhou R, Song Y, Sun Y, Zhang Q, Niu J, Fan H, ze Yang S. 2014. Atmospheric cold plasma jet for plant disease treatment. Applied Physics Letter 104:043702. doi: 10.1063/1.4863204.

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