

Plasma Activated Water: Implication as Fungicide, Growth and Yield Stimulator of Potato (*Solanum Tuberosum* L.)

A. Aktar,^a S. Sarmin,^a U.A. Irin,^a M.M. Rashid,^b M.M. Hasan,^a & M.R. Talukder^{b,*}

^aPlant Pathology Laboratory, Department of Agronomy and Agricultural Extension, University of Rajshahi, Rajshahi, Bangladesh; ^bPlasma Science and Technology Laboratory, Department of Electrical and Electronic Engineering, University of Rajshahi, Rajshahi, Bangladesh

*Address all correspondence to: M.R. Talukder, Plasma Science and Technology Laboratory, Department of Electrical and Electronic Engineering, University of Rajshahi, Rajshahi, Bangladesh; Tel.: +880721711572; Fax: +880721750064, E-mail: mrtalukder@ru.ac.bd

ABSTRACT: Seed germination rate, plant growth, and yield of crops are increased due to treatment of seeds with atmospheric pressure cold plasma (ACP). Seed treatment and foliar spray with ACP-treated water (PAWs) may play important roles in the control of plant disease because of the presence of highly reactive oxygen species in water. PAWs were prepared with atmospheric pressure discharges in waters with different treatment durations. The present experiment explored the effects of seed treatment and foliar spray by PAWs to control late blight disease and the yield of potato (*Solanum tuberosum* L.). The study reveals that the late blight incidence, disease severity, and the number of leaf infections were significantly reduced, with a few exceptions, in comparison with control, due to the seed treatments and foliar spray of PAWs. The plant height, tuber number, and tuber yield were increased. The results obtained in this field experiment are promising. Thus, this investigation reveals that the PAWs can be an effective alternative of fungicides to control the late blight disease and to stimulate the enhancement of yield of potato as well. Further investigations have to be conducted in order to draw definite conclusion.

KEY WORDS: plasma activated water, fungicide, potato, growth enhancer, O₃, H₂O₂

I. INTRODUCTION

Potato (*Solanum tuberosum* L.) is one of the most used vegetables and cash crop in Bangladesh,¹ is a source of low-cost energy to the human diet, and contributes to food and nutrition worldwide. Depending on food consumption, it has gained a third position² after rice and wheat. Potato contains 79% water, 17% carbohydrates (of which 88% is starch), 2% protein with negligible amount of fat but rich³ with vitamin B6; 100 g potato provides 77 kcal of energy. The rank of Bangladesh is seventh in terms of potato production in the world.⁴ There are many constraints to lower yield of potato in Bangladesh such as disease, lack of quality seeds, and improper use of fertilizers. Fifty-four diseases (both biotic and abiotic) of potato are identified and recorded⁵ in Bangladesh. Among them, late blight caused by the plant pathogen *Phytophthora infestans* is one of the most devastating diseases worldwide.

In 2017, a late blight outbreak of potato occurred in the northern region of Bangladesh in the districts of Panchagarh, Thakurgaon, Naogaon, Joypurhat, and Bogura. The potato growers of these areas were severely worried due to the attack of late blight disease. They failed to control the disease using fungicides available in the market. Outbreaks of late blight is a new issue concerning agricultural production of potato. Thus, one may assume that the climate change may alter the growth rate and pathogenicity of the pathogen due to the development of resistance to some fungicides. Impact of climate change on potato late blight resurgence indicates the potential increase of risk in the crop production in Bangladesh. The consequences of climate change for the alteration of crop disease scenarios are drawing much attention for the severity of the disease in field conditions. Thus, the conventional disease management strategies should be reoriented in order to overcome the consequences of climate change, as considered due to the development of resistance of fungicides, to ensure sustainable food production.

Chemical treatment of seeds (tubers) is effective in reducing seed-borne infectious diseases, but the use of chemicals is costly, and hazardous to the eco-system. Planting of tubers treated with chemicals in general result in accumulation of harmful chemical residues in soil as well as in the plant products that are hazardous to animal and eco-system in the long run.

Treatments of water with plasmas are termed plasma-activated water (PAW), modify the properties^{6–8} of water such as pH, electrical conductivity (EC), and concentration of dissolve oxygen (DO), and produce reactive oxygen (ROS) and nitrogen species (RNS). As a result, PAW contains different chemical compositions that serve as antimicrobial⁹ as well as plant growth promoting agents. Thus, PAW is an alternative for microbial disinfectant^{10,11} instead of fungicides. Furthermore, PAW and nonthermal plasma treatments of seeds have important roles in the development and physiological processes in plants.^{6,7,12–16} These include the reduction of microbes bearing rate of seeds, modification of seed coat structures, enhancement of permeability of seed coats, stimulation of seed germination rate, and seedling growth.^{12,13,15,17} These phenomena are demonstrated in several plants such as in *Chenopodium album*,¹⁸ *Oryza sativa*,¹⁹ *Triticum aestivum*,²⁰ *Lycopersicon esculentum*,²¹ and *Solanum melongena*.²² In addition, plasma treatments of seeds also could improve¹⁵ the physiological metabolism of the plant such as activity of dehydrogenase, superoxide dismutase, and peroxidase, photosynthetic pigments, photosynthetic efficiency, and nitrate reductase activity.²³ Adhikary et al.²⁴ have shown that PAW irrigation of tomato seedlings and can induce defense hormone and gene expression. Finally, plasma treatments of seeds significantly increased crop yields.^{12,13}

Most of the studies mentioned above, with a few exceptions,^{12,14} are limited to the applications of dry seed treatment plasma technology for the investigation of seed germination rate, breaking seed dormancy, and seedling growth within the laboratory only. To the best of our knowledge, no complete work (i.e., from seed treatments to yields in field condition), has been carried out up to now applying PAWs both as seed treatments and foliar spray on controlling plant disease and on enhancing yield. The present study was designed to develop (a) an effective control approach against *P. infestans* that causes late blight of potato, and (b) enhanced yield of potato, adopting new emerging

ecofriendly plasma technology as an alternative for sustainable disease management strategy as well as chemical fertilizers.

II. MATERIALS AND METHODS

The present work was conducted at the Plant Pathology Laboratory, Agronomy Field Laboratory, Department of Agronomy and Agricultural Extension, and Plasma Science and Technology Laboratory, Department of Electrical and Electronic Engineering, University of Rajshahi, Bangladesh. The potato variety BARI Alu-7 was collected from the local seed market and used as the planting materials. Apparent healthy, mature, and disease-free potato tubers were extracted and kept in a ventilated room and allowed to sprout under diffused sunlight. The damaged tubers were discarded, and well-sprouted tubers were used for seed treatments and planting. Potato tubers were cut into pieces so that each piece contained at least one sprouted bud.

An atmospheric pressure nonthermal submerged plasma jet reactor was designed for the treatment of water using O_2 gas for the productions of ROS in water. The plasma reactor was made of a Pyrex glass bottle (height and inner diameter were 180 mm and 90 mm, respectively). A tungsten wire (length and diameter were 160 mm and 0.5 mm, respectively) was inserted through a Pyrex glass tube (length and inner diameter were 170 mm and 3 mm, respectively) and used as power electrode, as shown in Fig. 1(a). This glass tube was also used as gas flow channel into the water treatment reactor. A 10 mm gap between the head of the glass tubes and power electrode was maintained in order to produce discharges within this gap and to avoid arcing. Another electrically insulated tungsten wire (length and diameter were 175 mm and 0.5 mm, respectively) keeping 10 mm bared was

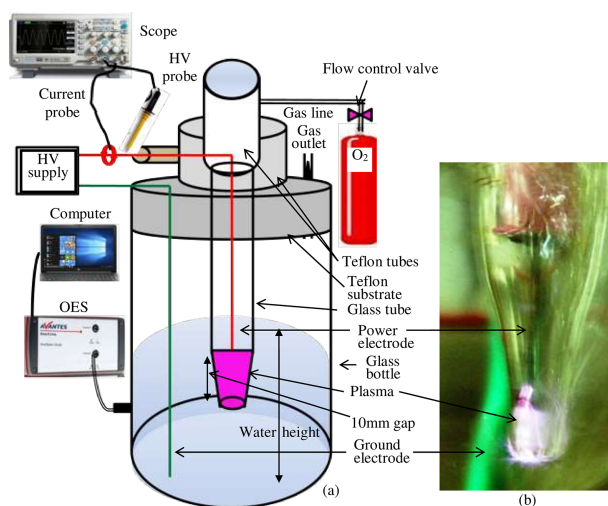


FIG. 1: Experimental setup for the preparation of plasma activated water (PAW) with underwater O_2 discharge plasma jet: (a) schematic diagram and (b) plasma discharge in water

also inserted through another tube. This bared portion of the electrode was submersed in water and used as grounded electrode. A Teflon tube was penetrated through the upper surface used as gas inlet, while an electrical wire was penetrated through the side wall of the Teflon substrate for providing electrical connection with the powered tungsten wire. The Teflon substrate was placed on another air sealing substrate made of nylon in order to keep the glass container air sealed, as shown in Fig. 1(a). A one-way gas outlet valve was also installed at the nylon air sealing substrate. A 1–10 kV, 0.5–10 kHz, sinusoidal power supply was used for the productions of underwater O_2 discharge plasma. O_2 was flown through the glass tube into the water treatment reactor at a flow rate of 1 L/min^{-1} . A volume of 250 mL deionized water was used at a time for plasma treatment under different durations, as shown in Fig. 1(b). No significant temperature change of PAW was observed because of gas flow through the glass tube into the water under treatment.

The current-voltage characteristics from the O_2 discharge are presented in Fig. 2(a). Power absorbed by the plasma was $\sim 9\text{ W}$ determined by integrating the discharge current and voltage over a period. Optical emission spectroscopic (OES) data, shown in Fig. 2(b), reveal the plasma species²⁵ produced in the O_2 discharge plasmas. It is to be mentioned that no traceable amount of nitrogen-related species found in PAWs may be due to using as O_2 working gas. pH of PAW was measured using a pH meter (model: HI 2002-02, pH range: -2.00 to 16.00 , resolution: 0.01 , accuracy: ± 0.01 , Hanna Instrument, USA), while the DO was measured using a DO meter (model: HI 98193, DO range: 0.00 to 50.00 mg/L^{-1} , resolution: 0.01 mg/L^{-1} , accuracy: $\pm 15\%$, Hanna Instrument, USA). On the other hand, the concentrations of H_2O_2 (colorimetric test kit traceable to NIST standard reference material: HI 3844, Hanna Instrument, USA) and O_3 (colorimetric test kit traceable to NIST standard reference material: HI 38054, Hanna Instrument, USA) in PAWs were determined using the assay kits following the methods instructed in their manuals. It is to be noted that there were no significant changes of water temperature (increased from 25°C to 26°C for 15 min treatment duration) was found because of gas flow through the glass tube into water under treatment.

Required quantity of potato seed tubers were taken in different beakers and PAWs were added in each beaker according to the treatment specification of PAWs and allowed to immerse for 10 min. The PAW-treated seed tubers were dried under laminar air hood at room temperature. It is well known that the late blight disease is seed-borne as well as air-borne in field conditions. Pre-treatment was done to disinfect tubers so that it may reduce the primary infection of seedling and in consequence one may expect infection-free seedlings. Further, one may expect the reduction of primary sources of inocula due to pre-treatment and consequently the chance of disease incidence will be reduced in the field. The fungicide Ridomil was also used (at 0.3% solution) for the treatment of seed tubers and foliar spray in the experiment.

Both treated (PAW and Ridomil) and untreated (control) potato tubers were planted, on November 15, 2019, in the assigned plots as per treatment specification at a spacing of $50 \times 50\text{ cm}$. The depth of planting was $\sim 6\text{ cm}$. Tubers were planted in each plot by line planting method. After planting, fine soil mixture was used for covering the tubers. Weeding was done twice to control the weeds in the experimental plots. The first

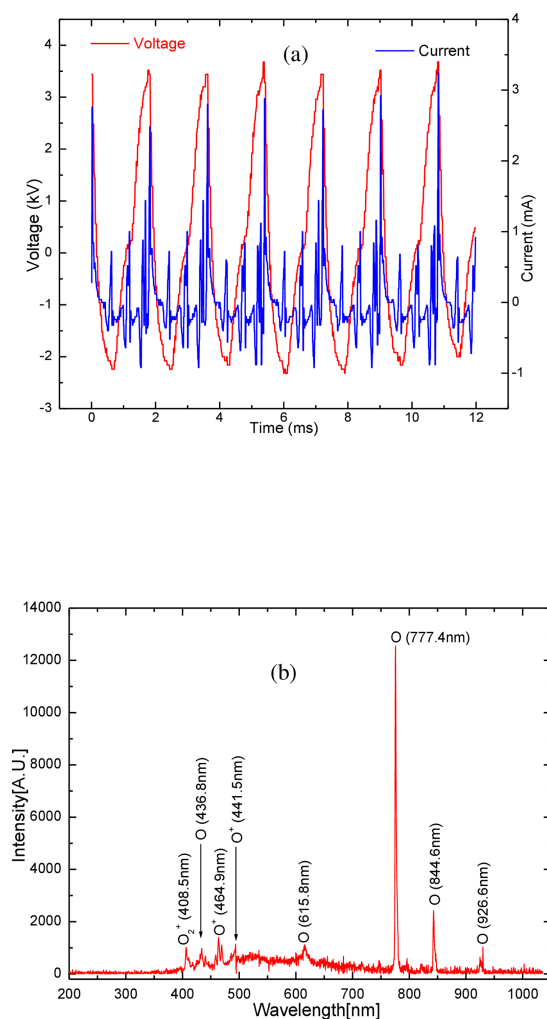


FIG. 2: Discharge voltage-current characteristics (a), and emission spectrum (b) of the underwater O₂ discharge plasma jet

weeding was done on January 15, 2020 and the second weeding was done on February 10, 2020. Earthing up was executed two times throughout the growing period, one at 30 days and another one at 55 days after planting (DAP). The field plots were irrigated twice at 35 and 65 DAP. O₂ treated PAWs (0.5 L/m²) and fungicide (Ridomil: 3 g/L⁻¹) were sprayed thrice to potato plants at 40, 50, and 60 DAP.

Infected leaves or segments of plants were collected in polythene bags from the experimental plots and brought to the Plant Pathology laboratory in order to identify the disease. The samples were then washed in tap water to remove sand and soil. The samples were cut into small pieces (1 cm) and then sterilized with 70% alcohol. After surface disinfection, they were washed thrice with sterilized water and placed on sterile filter paper to

remove water from the surface of the samples. Five pieces of the samples were adhered to the PDA plates aseptically. The plates were incubated at 20°C for 7 days and observations were made regularly to see the growth of fungi. After 3 days of incubation, fungi grew on PDA were purified by a hyphal tip culture method. The pure culture of the fungi was preserved in PDA slants at 4°C in the refrigerator as stock culture. The isolated fungi were identified based on morphology, growth characters, colony color, conidia, and papillae, following a standard method.²⁶ The growth of the fungi was also studied (at 20 ± 1°C) for 5 days. Figure 3 shows the photographs of the culture plate and *Phytophthora infestans*.

Fresh potato leaves were collected from the field and inoculated with 6 mm fungal block of *P. infestans* for pathogenicity test in the laboratory. The inoculated leaves were then placed in a moisture chamber. Temperature (20°C) and humidity (95%) were maintained for the development of fungi on the leaves and regular observations were made to see their development.

The incidences of late blight disease in the experimental plots were recorded through regular inspection of the plots at 10-day intervals viz. 50, 60, and 70 DAP. The percentage of plant infection was estimated by the following formula

$$\text{Plant infection(\%)} = \frac{\text{Number of infected plants}}{\text{Total number of plants}} \times 100 \quad (1)$$

On the other hand, the percentage of severity was calculated employing the following formula

$$\text{Disease severity(\%)} = \frac{\text{Total rating}}{\text{Total observation} \times \text{maximum grade}} \times 100 \quad (2)$$

Evaluation of late blight severity was carried out using 1–9 scale (Table 1) as suggested by Cáceres et al.²⁷

The harvesting time of potato is very important because it is related to the growth and production. The development of tuber continues till the death of vines. The potato can be harvested when the majority of the leaves become yellow-brown, within 75–120

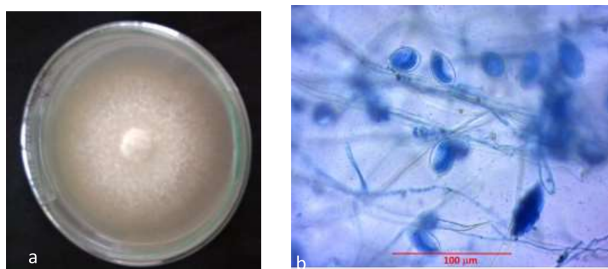



FIG. 3: Photographs of (a) culture plate of *Phytophthora infestans* taken by camera model no. Nikon d3400, and (b) microscopic image of *Phytophthora infestans* taken by Zeiss Axio Lab.A1

TABLE 1: Rating scale (1–9 scale) of late blight disease of potato

		
Score	% foliage affected	Description
1	0	None or a very few lesions on the leaflets
2	3	More than 0% but less than 10%
3	10	More than 10% but less than 25%
4	25	More than 25% but less than 50%
5	50	Half of the foliage destroyed
6	75	More than 50% but less than 75%
7	90	More than 75% but less than 90%
8	97	Only very few green areas on leaf (much less 10%)
9	100	Foliage completely destroyed

days of planting, depending on the area, soil type, and variety planted. The potato tuber was harvested on March 30, 2020 at the age of 90 days. Number of total tubers per plot and weight of the tubers per plot were recorded.

The recorded data on different parameters were subjected to statistical analyses using SPSS software to find out the significance of variance obtained from experimental treatments. The differences among the treatments were judged by Duncan's Multiple Range Test (DMRT).

III. RESULTS AND DISCUSSION

Figure 4 includes the properties of water treated with O_2 plasmas under different treatment durations. It is known that pH indicates whether the PAWs are acidic or alkaline, while O_3 and H_2O_2 play important roles in fungi inactivation.^{28,29} It is seen from Fig. 4 that pH in PAWs is decreasing with treatment duration in O_2 plasmas, maybe due to increasing concentrations of ROS in water, which indicates that the PAW becomes more acidic. The initial concentration of DO in deionized water was 4.10 mg/L^{-1} . The concentrations of DO in PAWs increases sharply in O_2 discharges. The concentration of O_3 is found to increase with treatment duration as observed from the figure. On the other hand, the concentration of H_2O_2 is increasing with treatment duration.

The incidence of late blight of potato was recorded at 50, 60 and 70 DAP (Fig. 5). Initially the disease incidence was lowest at early growth stage and then it increased gradually when the plants attained their maturities. Significant variations in late blight incidences were observed under different treatment conditions and at different sampling dates. At 50 DAP, the lowest (1.0%) incidence was observed after application of $PAW_{O_2}^8$, and $PAW_{O_2}^{10}$ which was statistically similar with $PAW_{O_2}^6$ (1.33%). $PAW_{O_2}^4$ also showed good result in controlling late blight incidence in comparison with control. At 60 DAP,

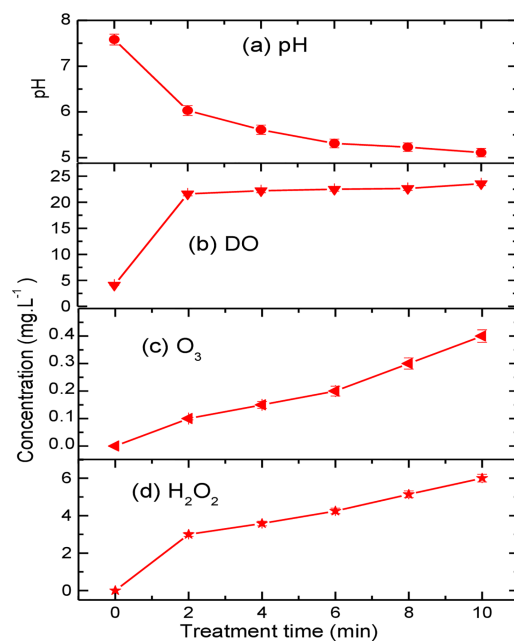


FIG. 4: pH (a), concentrations (mg/L⁻¹) of dissolve oxygen (DO) (b), O₃ (c) and H₂O₂ (d) in deionized and plasma activated water (PAW). Waters were treated with O₂ plasmas for different treatment durations. The capped lines indicate instrumental errors.

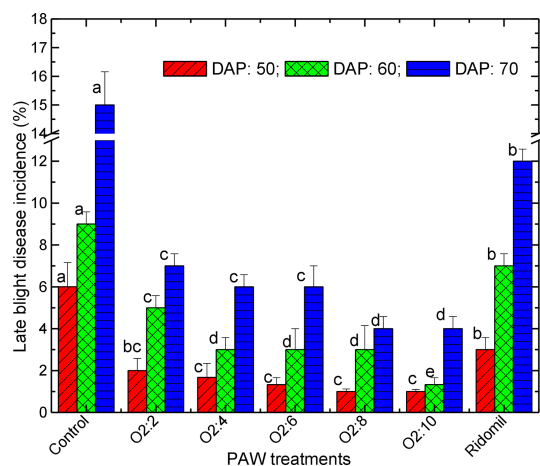


FIG. 5: Effects of PAW and fungicide as foliar spray on late blight incidence (at 5% level of confidence) of potato plants. The disease incidences were counted at different days after planting (DAP). In the horizontal axis, O₂-2 means water treated with O₂ for 2 min, and so on, which is represented as PAW_{O₂} in the text. In column, figure bearing similar letter(s) are identical and those dissimilar letter(s) differed significantly as per DMRT. Capped bars indicate standard errors of three replications.

the disease incidence under different treatments was ranged from 1.33% to 9.00%. The highest (9.00%) disease incidence was recorded in control plants, while the lowest (1.33%) was obtained in the plants treated with $PAW_{O_2}^{10}$. At the end of December, the plants were in vegetative stage and the weather condition was favorable for occurring highest disease incidence. But the plots that received PAW treatments showed minimum disease incidence as compared to control. On the other hand, the treatment with $PAW_{O_2}^4$, $PAW_{O_2}^6$, and $PAW_{O_2}^8$ were exhibited 3.0% disease incidence. At 70 DAP, the late blight incidence was recorded in the range 4%–15.0%, where the highest (15.0%) disease incidence was recorded in control plot and the lowest (4%) was recorded by $PAW_{O_2}^8$ and $PAW_{O_2}^{10}$. Besides, the minimum (6.0%) disease incidence was recorded with $PAW_{O_2}^4$ and $PAW_{O_2}^6$. There are many factors (environmental factors, critical growth stage of plant, soil condition) influence the disease occurrence in the field, therefore it is difficult to control all the factors. However, due to foliar spray of PAWs to potato plants, the disease severity was reduced. The probable reasons for the reduction of disease incidence due to foliar spray of PAWs to plants will be discussed later.

The effects of PAWs on leaf infection of plants (%) were evaluated at 50, 60, and 70 DAP (Fig. 6). A significant variation in leaf-infected plants under different treatment conditions were recorded during this study. Leaf-infected plants (%) at 50 DAP under different treatments varied from 1.0% to 11.33%. The maximum (11.33%) leaf-infected plants were recorded in control plots and the minimum (1.0%) infected leaves were counted with $PAW_{O_2}^{10}$. 1.67% leaf-infected plants were also recorded for $PAW_{O_2}^8$.

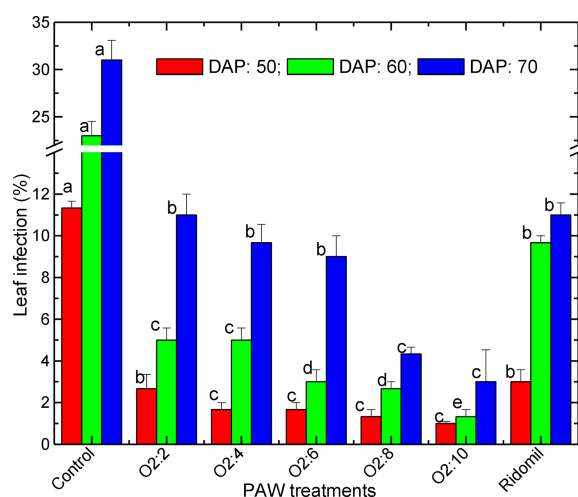


FIG. 6: Effects of PAW and fungicide as foliar spray on leaf infection (at 5% level of confidence) of the potato plants. The leaf infection was counted at different days after planting (DAP). In the horizontal axis, O_{2-2} means water treated with O_2 for 2 min, and so on, which is represented as $PAW_{O_2}^2$ in the text. In column, figure bearing similar letter(s) are identical and those dissimilar letter(s) differed significantly as per DMRT. Capped bars indicate standard errors of three replications.

Leaf-infected plants (%) at 60 DAP under different treatments ranged from 1.33%–23.0%. The plants with the highest (23.0%) leaf infection were recorded in control plants while the minimum (1.33%) infections were recorded for $\text{PAW}_{\text{O}_2}^{10}$. Next to $\text{PAW}_{\text{O}_2}^{10}$, the plants with 3.0% leaf infection were recorded from the plots treated with $\text{PAW}_{\text{O}_2}^8$. Plants with 3.0%–31.0% leaf infection were at 70 DAP. The maximum (31.0%) leaf infection was recorded in control plots while $\text{PAW}_{\text{O}_2}^{10}$ was exhibited 3.0% only. Better results of controlling late blight on leaves were also observed (4.33%) with $\text{PAW}_{\text{O}_2}^8$. Among different treatment durations, PAWs showed the best result (3.00%) in controlling leaf infection especially with $\text{PAW}_{\text{O}_2}^{10}$. The concentrations of O_3 and H_2O_2 were, 0.15 mg/L^{-1} and 3.58 mg/L^{-1} respectively, produced in water with O_2 plasma for the treatment duration of 10 min. ROS (H_2O_2) participate in plant defense through signaling and strengthening cell wall by oxidative cross-linking.^{25,30} ROS are toxic, and reduce fungal growth *in vitro*, and can prohibit infection in leaf disks. ROS (O_3) is a strong oxidant, can oxidize contaminants³¹ in air and has been demonstrated to limit growth of fungi. It is to be mentioned that ROS (H_2O_2 and O_3) in PAWs have both positive and negative effects that depend on their concentrations to plants and also show antifungal activity. Besides, H_2O_2 acts as signal to influence plant defense mechanism. Thus, optimized concentration of ROS in PAWs (i.e., applicable dose level) must be known in order to use it as fungicide to potato plants to control late blight disease. Based on overall observation, it is found that $\text{PAW}_{\text{O}_2}^{10}$ was provided the best result than other treatment conditions.

The effects of s on late blight severity (1–9 scale; Fig. 7 and Table 1) of potato were evaluated at 50, 60, and 70 DAP (Fig. 7). Late blight severity (1–9 scale) was

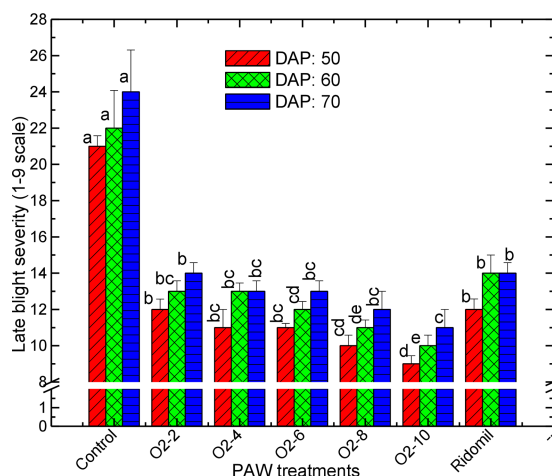


FIG. 7: Effects of PAW and fungicide as foliar spray on late blight severity (1–9 scale) (at 5% level of confidence) of potato plants. The late blight severities were counted at different days after planting (DAP). In the horizontal axis, $\text{O}_{2.2}$ means water treated with O_2 for 2 min, and so on, which is represented as $\text{PAW}_{\text{O}_2}^2$ in the text. In column, figure bearing similar letter(s) are identical and those dissimilar letter(s) differed significantly as per DMRT. Capped bars indicate standard errors of three replications.

significantly reduced in the plants treated with compared to control. The disease severity was lowest at the early stage and then it increased gradually when plants became mature. Disease severity at 50 DAP was ranged from 9.00% to 21.00%, where the lowest disease severity (9.00%) was recorded with $\text{PAW}_{\text{O}_2}^{10}$. In case of longer plasma treatment duration, PAWs became more acidic (Fig. 4), which was more effective for the inhibition of fungal growth. This may help in the reduction of late blight severity. On the other hand, the highest disease severity (21.0%) was recorded in control plant. The range of disease severity (1–9 scale) was 10.0%–22.0% at 60 DAP, where the lowest (10.0%) and highest (22.0%) were recorded in $\text{PAW}_{\text{O}_2}^{10}$ and control plants, respectively. Further, the disease severities (1–9 scale) under different treatments were 11.0%–24.0% at 70 DAP. The lowest (11.0%) severity was observed in the plants where $\text{PAW}_{\text{O}_2}^{10}$ treatment was provided. The highest (24%) disease severity was recorded from the control plants which was almost double with respect to PAW-treated plants. The severity of late blight disease of potato depends on critical weather condition, as well as growth stage of plant. Therefore, the lesser changes of late blight severity were between the sampling dates of 50 and 60 DAP. The distinct changes in late blight severity were observed at 70 DAP. During this study, $\text{PAW}_{\text{O}_2}^8$ exhibited 12.0% disease severity, that is, treatments were reduced almost 50% disease severity compared to control.

The fungicide Ridomil was showed average result in controlling late blight disease in comparison with control. It is to be noted that the treatment provided with $\text{PAW}_{\text{O}_2}^{10}$ showed better antifungal activity of late blight with respect to ridomil. The combined effects of O_3 (0.45 mg/L^{-1}) and H_2O_2 (3 mg/L^{-1}) contained in $\text{PAW}_{\text{O}_2}^{10}$ shows the best performance in controlling late blight disease. ROS provide host-derived oxidative burst both to pathogenic and beneficial fungi during plant infection which is a part of the plant innate immune response and suppresses further triggering of the plant defense. In order to survive, the fungi overcome the stress produced by the plant-derived ROS. ROS produced in hosts is considered as toxic to plant pathogen.^{32–34} O_3 provides the plant defense against pathogen.³⁵ Therefore, this concentration, neither higher nor lower, of O_3 may have antifungal effect without damaging plant tissue.

The probable reasons for the reduction of disease incidence, leaf infection, and disease severity due to foliar spray of PAWs to plants may be explained as follows. Fungal plant pathogens penetrate into the cell for feeding the living cells.²⁹ During the interaction process between fungus and plant cell wall (i.e., when penetration event initiates), the plant cell triggers a signal that activate localized defense system. Mellersh et al. investigated the roles of H_2O_2 in the penetration failure of fungi in plant–fungal interactions. External intrusion of localized hemicellulase sequentially produces localized responses, generation of extracellular H_2O_2 , accumulation of phenolic compounds and cross-linking of proteins in the cell wall. It is inferred³⁰ that extracellular generation of H_2O_2 is solely responsible for the penetration failure of fungi in the cell wall, because H_2O_2 itself shows antifungal activity.²⁸ Due to foliar spray of (as it contains H_2O_2 as presented in Fig. 4) at certain time interval to potato plants, the following events may occur: (a) the fungi may directly be inactivated if they reside just over the plant surface; (b) H_2O_2 may penetrate and arrive at the cell wall, and inactivate the fungi if they exist there; (c) H_2O_2 may

penetrate inside the cell wall and inactivate the fungi, and enzymatic removal of H_2O_2 may take place within the cell. On the other hand, ozone is a powerful abiotic elicitor and cross inducer. Sometimes ozone responses are localized in the areas of subsequent HR-like (hypersensitivity reaction) lesions, while SAR-like (systemic acquired resistance) responses take place in the adjacent tissue. The ozone postulated for the hypersensitive response induced in incompatible plant–pathogen interactions. In fact, ozone induces defensive and antioxidative reactions of secondary metabolism which are distinguished for plant defense against pathogens.³⁶ Thus, one may conclude from the above discussion that foliar spray of PAWs can effectively be inactivated fungi of potato plants.

The consequences of foliar spray of treatments on plant growth characters such as plant heights were evaluated (Fig. 8). Plant heights at 70 DAP under different treatments were ranged from 28.0 to 36.67 cm. The highest plant height (36.67 cm) was measured from the plants where $PAW_{O_2}^{10}$ treatments were applied, while the control plants produced the lowest plant height (28.0 cm). Identical results were also obtained with $PAW_{O_2}^4$, $PAW_{O_2}^6$, $PAW_{O_2}^8$ and fungicide over control. The plant height at 90 DAT differed significantly under different treatment conditions. The plants that received $PAW_{O_2}^{10}$ showed the highest plant length (63.00 cm) followed by $PAW_{O_2}^6$, $PAW_{O_2}^8$, and fungicide. On the other hand, control treatment showed the lowest plant height. The plant heights at 50 and 90 DAP did not differ significantly though it was varied numerically.

Number of tubers per square meter under different treatments ranged from 75.0 to 110.0 (Fig. 9). The highest (120) number of tubers per square meter was recorded with fungicide treatment. Next to fungicide, $PAW_{O_2}^{10}$ also provided a good number of potato

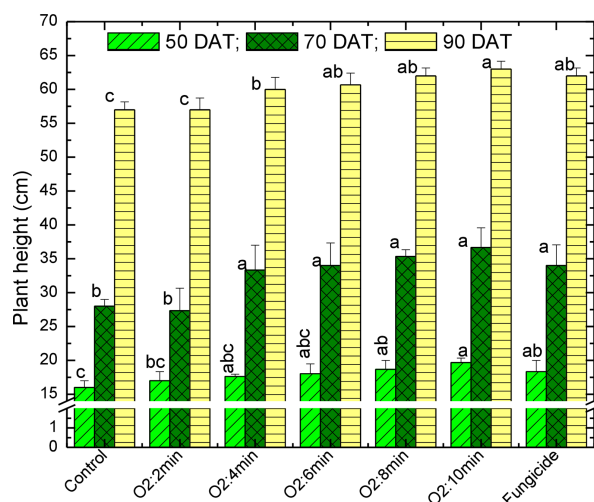


FIG. 8: Effects of PAW and fungicide as foliar spray on plant height of potato at 50, 70, and 90 DAP. In the horizontal axis, O_{2-2} means water treated with O_2 for 2 min, and so on, which is represented as $PAW_{O_2}^2$ in the text. In column, figure bearing similar letter(s) are identical and those dissimilar letter(s) differed significantly as per DMRT. Capped bars indicate standard errors of three replications.

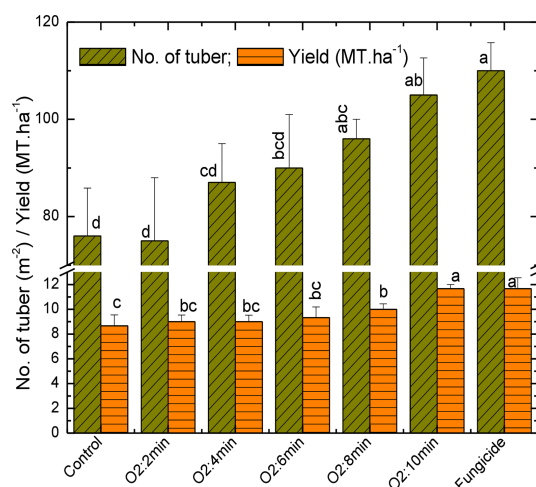


FIG. 9: Effects of PAW and fungicide as foliar spray on the number of tubers per square meter and weight of tuber. In the horizontal axis, O₂₋₂ means water treated with O₂ for 2 min, and so on, which is represented as PAW_{O₂} in the text. In column, figure bearing similar letter(s) are identical and those dissimilar letter(s) differed significantly as per DMRT. Capped bars indicate standard errors of three replications.

tuber (105) over the control. was evaluated for increasing the yield per square meter and the results are presented in (MT/ha⁻¹) (Fig. 9). A significant variation was found among the treatments regarding tuber yield which was ranged from 8.75 to 11.67 MT/ha⁻¹. From the column graph, it is observed that Ridomil and PAW_{O₂}¹⁰ both provided equal (11.67 MT/ha⁻¹) results for increasing tuber yield over the control. PAW treatments were significantly reduced the late blight severity and enhanced the growth of potato plant rendering increased tuber yield. As it is known that the late blight disease damages the leaves of potato plant as a result the photosynthetic area is reduced or destroyed. Healthy leaves retain more photosynthetic area and enhances the tuber yield. Thus, the findings of the present work are consistent with the results obtained by Piikki et al.³⁴ and Emberson et al.³³ Soffer et al.³⁵ studied the consequences of DO concentration on the *Chrysanthemum* and *Ficus* plants growth and found that their growths were increased with the increase of DO concentration in hydroponic media. The plant growth was enhanced due to increased uptake of nitrogen and phosphate from the hydroponic media with the increase of DO. It is observed in Fig. 4 that the concentration of DO in PAW is much higher. The height of plants, number of tubers, and weight of tubers are higher in the O₂-treated PAWs. These enhancements may be due to foliar spray of PAWs to the plants.

IV. CONCLUSION

Waters were treated in the range 2–10 min with atmospheric pressure O₂ plasma and sprayed onto potato plants in order to study the consequences of foliar spray of PAWs.

The study includes leaf infection, disease incidence, disease severity, yield contributing character, and finally the yield of potato. The study reveals that due to foliar spray of PAWs the late blight incidence, disease severity, and number of leaf infections were reduced as compared to control of the potato plants. Besides, the plant height, tuber number, and tuber yield were also enhanced due to foliar spray of PAWs. The results obtained in this investigation are promising. Thus, one may conclude from this field study that especially $PAW_{O_2}^{10}$, can be an effective alternative of fungicides to control the late blight disease and enhanced yield of potato as well. Further investigations have to be conducted in order to draw conclusive remarks.

ACKNOWLEDGMENTS

M.R. Talukder thanks the Ministry of Education (Grant No. LS2017544), Government of the People's Republic of Bangladesh, and the University of Rajshahi (Grant No. 62/5/52/RU/Engg-05/2020-2021), for their partial financial supports to carry out this work. The author would also like to thank Mizanur Rahman, Lab Technician, Plasma Science and Technology Lab, Department of Electrical and Electronic Engineering, University of Rajshahi for his continuous cooperation both in the laboratory and field.

REFERENCES

1. Haque HA, Miah MA, Hossain S, Rahman MM. Profitability of bari released potato (*Solanum tuberosum* L.) varieties in some selected locations of Bangladesh. *Bangladesh J Agril Res.* 2012;37(1): 149–58.
2. Food and Agriculture Organization (FAO) of United Nations. Available from: <http://faostat.fao.org/site/567/desktopdefault.aspx#ancor>. 2014. Accessed 16 December 2020.
3. U.S. National Nutrient Database. 2017. Accessed 16 Dec 2020. Available from: <https://data.nal.usda.gov/dataset/usda-national-nutrient-database-standard-reference-legacy-release>.
4. United Nations, UN Food and Agriculture Organization, Statistics Division (FAOSTAT) Production of potato in 2016; Crops/World Regions/Production Quantity from pick lists. 2017. Accessed 16 December 2020. Available from: <http://www.fao.org/faostat/en/#home>.
5. Dey TK, Ali MS. Pathological research on tuber crops in Bangladesh. In: *Proc. of Workshop on Transf of Tech of CDP Crops under Res Ext Linkage prog*, held on Oct 22–27 BARI Gazipur Bangladesh; 1994. p. 159–65. Available from: http://bari.portal.gov.bd/sites/default/files/files/bari.portal.gov.bd/annual_reports/f301c91f_74ed_4cbe_a414_019cdac83dd8/2020-10-08-17-38-79c02e8bb05d060a94a4508e35a1389c.pdf.
6. Islam S, Omar FB, Sajib SA, Roy NC, Reza A, Hasan M, Talukder MR, Kabir AH. Effects of LPDBD plasma and plasma activated water on germination and growth in rapeseed (*Brassica napus*). *Gesunde Pflanzen.* 2019;71:175–85.
7. Sajib SA, Billah M, Mahmud S, Miah M, Hossain F, Omar F B, Roy NC, Hoque KMF, Talukder MR, Kabir AH, Reza MA. Plasma activated water: The next generation eco-friendly stimulant for enhancing plant seed germination, vigor and increased enzyme activity, a study on black gram (*Vigna mungo* L.). *Plasma Chem Plasma Process.* 2020;40:119–43.
8. Bradu C, Kutasi K, Magureanu M, Puač N, Živković S. Reactive nitrogen species in plasma-activated water: Generation, chemistry and application in agriculture. *J Phys D: Appl Phys.* 2020;53: 223001–22.

9. Zhao YM, Patange A, Sun DW, Tiwari B. Plasma-activated water: Physicochemical properties, microbial inactivation mechanisms, factors influencing antimicrobial effectiveness, and applications in the food industry. *Compr Rev Food Sci Food Saf.* 2020;1–29.
10. Rodriguez C, Wandell RJ, Zhang Z, Neurohr JM, Tang Y, Rhodes R, Kinsey ST, Locke BR. *Escherichia coli* survival in plasma-treated water and in a gas–liquid plasma reactor. *Plasma Process Polym.* 2020; e2000099.
11. Thirumdas R, Lyulukothakota A, Annapure U, Siliverce K, Blundell R, Gatt R, Valdramidis VP. Plasma activated water (PAW): Chemistry, physico-chemical properties, applications in food and agriculture. *Trends in Food Sci Tech.* 2018;77:21–31.
12. Roy NC, Hasan MM, Talukder MR, Hossain MD, Chowdhury AN. Prospective applications of low frequency glow discharge plasmas on enhanced germination, growth and yield of wheat. *Plasma Chem Plasma Process.* 2018;38:13–28.
13. Roy NC, Hasan MM, Kabir AH, Reza MA, Talukder MR, Chowdhury AN. Atmospheric pressure gliding arc discharge plasma treatments for improving germination, growth and yield of wheat. *Plasma Sci Technol.* 2018;20:115501–11.
14. Kabir AH, Rahman MM, Das U, Sarkar U, Roy NC, Reza MA, Talukder MR, Uddin MA. Reduction of cadmium toxicity in wheat through plasma technology. *PLoS One.* 2019;14(4):e0214509–16.
15. Rahman MM, Sajib SA, Sifat MS, Tahura S, Roy NC, Parvez S, Reza MA, Talukder MR, Kabir AH. Mechanisms and signaling associated with LPDBD plasma mediated growth improvement in wheat. *Scientific Report.* 2018;8:10498–11.
16. Billah M, Sajib SA, Roy NC, Rashid MM, Reza MA, Hasan MM, Talukder MR. Effects of DBD air plasma treatment on the enhancement of black gram (*Vigna mungo* L.) seed germination and growth. *Archives Biochem Biophys.* 2020;681:108253–10.
17. Selcuk M, Oksuz L, Basaran P. Decontamination of grains and legumes infected with *Aspergillus* spp. and *Penicillium* spp. by cold plasma treatment. *Bioresour Technol.* 2008;99:5104–9.
18. Sera B, Stranak V, Sery M, Tichy M, Spatenka P. Germination of *Chenopodium album* in response to microwave plasma treatment. *Plasma Sci Technol.* 2008;10(4):506–11.
19. Chen HH, Chen YK, Chang HC. Evaluation of physicochemical properties of plasma treated brown rice. *Food Chem.* 2012;135:74–9.
20. Sera B, Spatenka P, Sery M, Vrchotova N, Hruskova I. Influence of plasma treatment on wheat and oat germination and early growth. *IEEE Trans Plasma Sci.* 2010;38:2963–8.
21. Yin MQ, Huang MJ, Ma BZ, Ma TC. Stimulating effects of seed treatment by magnetized plasma on tomato growth and yield. *Plasma Sci Technol.* 2005;7:3143–247.
22. Jiang JF, Lu YF, Li JG, Li L, He X, Shao HL, Dong YH. Effect of seed treatment by cold plasma on the resistance of tomato to *Ralstonia solanacearum* (Bacterial Wilt). *PLoS One.* 2014;9:1–6.
23. Wu HZ, Chi LH, Bian SF, Xu KZ. Effects of plasma treatment on maize seeding resistance. *Maize Sci.* 2007;15:111–3.
24. Adhikari B, Adhikari M, Ghimire B, Park G, Choi EH. Cold atmospheric plasma-activated water irrigation induces defense hormone and gene expression in tomato seedlings. *Sci Reports.* 2019;9:16080–15.
25. National Institute of Standard and Technology (NIST) database, U.S. Department of Commerce. Available from: https://physics.nist.gov/PhysRefData/ASD/lines_form.html.
26. Singh BP, Singh PH, Gupta J, Singh L. Integrated management of late blight under Shimla hills. *J India Potato Assoc.* 2001;28(1):84–5.
27. Cáceres PA, Pumisacho M, Forbes GA, Andrade-Piedra JL. Learning to control potato late blight. A facilitator's guide. Lima, Perú: International Potato Center (CIP); 2008.
28. Fridman A, Friedman G. *Plasma Medicine*. London: John Wiley & Sons; 2013.
29. Misra NN, Schluter OK, Cullen PJ. *Cold Plasma in Food and Agriculture: Fundamentals and Applications*. London: Academic Press; 2016.
30. Mellersh DG, Foulds IV, Higgins VJ, Heath MC. H₂O₂ plays different roles in determining penetration failure in three diverse plant-fungal interactions. *Plant J.* 2002;29:257–68.

31. Barth MM, Zhou C, Mercier J, Payne FA. Ozone storage effects on anthocyanin content and fungal growth in blackberries. *J Food Sci.* 1995;60:1286–8.
32. Segal LM, Wilson RA. Reactive oxygen species metabolism and plant-fungal interactions. *Fungal Genetics Biol.* 2018;110:1–9.
33. Emberson LD, Pleijel H, Ainsworth EA, et al. Ozone effects on crops and consideration in crop models. *Euro J Agron.* 2018;100:19–34.
34. Piikki K, Selldén G, Pleijel H. The impact of tropospheric O₃ on leaf number duration and tuber yield of the potato (*Solanum tuberosum* L.) cultivars Bintje and Kardal. *Agric Ecosyst Environ.* 2004; 104:483–92.
35. Softer H, Burger DW, Lieth JH. Plant growth and development of Chrysanthemum and Ficus in aero-hydroponics: Response to low dissolved oxygen concentrations. *Scientia Horticulturae.* 1991; 45:287–94.
36. Sandermann H Jr. Ozone and plant health. *Annu. Rev. Phytopathol.* 1996; 34:347–66.